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Additional Information

Short Communication: Drug residues in goat's milk after to the prophylactic use of antibiotics in intravaginal sponges for estrus synchronization

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ABSTRACT

The aim on this study was to determine whether the prophylactic use of antibiotics in the placement of intravaginal sponges for estrus synchronization in caprine may be the cause of the presence of inhibitors in milk and, therefore, of positive results in the microbial screening tests. Ninety-eight Murciano-Granadina goats divided into 7 groups of 14 animals were used. Intravaginal sponges were placed in 6 groups using two concentrations of different commercial antibiotics: doxycycline (Hipradoxi®), oxytetracycline (Terramycin®) and sulfathiazhole-framycetin (Framicas®). The sponges of the control group were placed without antibiotics. Milk samples were collected daily along the 7-day-post-treatment and analyzed by three microbial tests (BRT MRL, Delvotest SP-NT MCS and Eclipse 100). Positive samples were retested by specific receptor-binding assays (SNAP Tetracycline and Sulfasensor tests) to confirm the non-compliant results. The vagina status was evaluated through the visual assessment of the sponge external aspect after removal. Results indicate that the microbial test response was unaffected by neither the day post-treatment nor the dose of antibiotic used, except for oxytetracycline at highest concentration. Moreover, no positive results were obtained using receptor-binding assays suggesting that residues, if present in milk, not exceed the safety levels established for these drugs in the legislation. The occurrence of soiled sponges was higher in the control group. Respect to the dose of antibiotics used no significant differences were obtained for the lowest dose administered. However, a significant increase in the percentage of clean sponges was observed for the highest dose of doxycycline. It can be concluded that the prophylactic use of low amounts of doxycycline, oxitetracycline and sulfathiazole in the synchronization of estrus by intravaginal sponges contributes to reduce the 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.

Key words: goat milk, intravaginal sponge, antibiotic screening methods.

SHORT COMMUNICATION

Intravaginal sponges impregnated with progesterone or synthetic progestogens during 6-16 days before the artificial insemination are usually employed for oestrus synchronization in goats (Menchaca and Rubianes, 2004; Rowe *et al*., 2009). However, this practice has been related to the occurrence of clinical vaginitis in goats (Motlomelo *et al.*, 2004; Penna *et al.*, 2003), which could negatively affect the fertility rates reached on farms (Scudemore, 1988).

To avoid these negative effects, some authors recommend to sprinkle the sponges with antibiotics prior to its insertion into the vagina (Guerra *et al.*, 2002; Suarez *et al*., 2006; Gatti *et al*., 2011), oxytetracycline being the drug commonly used for this purpose in practice (Manes *et al*., 2013).

It should be noted that the prophylactic use of antibiotics in the placement of intravaginal sponges is an unregulated reproductive management on which there are no recommendations approved with regard to the dose and the withdrawal period required to avoid the presence of drug residues in milk. In caprine, the synchronization of estrus by intravaginal sponges takes place during lactation and, therefore, the inclusion of antibiotics to sprinkle them may pose a risk of contamination in milk. Thus, some authors have found antibiotic residues in cow milk 24-48 hours after the intrauterine administration of suppositories, infusions and tablets of penicillin, streptomycin, and tetracycline (Miller and Bergt, 1974; 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 doubtful results were obtained at the time of the first milking and, sometimes, afterwards 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 antibiotics in milk above maximum residue limits (MRLs) established by legislation (Commission Regulation UE Nº 37/2010). The performance of these screening tests (specificity and detection capability) allows the detection of a large number of substances in milk at or below MRLs to guarantee the safety of milk and related products.

Thus, the aim of this study was to evaluate whether the prophylactic use of antibiotics in the synchronization of estrus by intravaginal sponges in caprine may be the cause of 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 prior to the experiment.

Goats were randomly divided into seven groups of 14 animals. A polyurethane sponge containing 30 mg of flugestone acetate (Sincropart, Ceva Salud Animal, Barcelona, Spain) was inserted into the vagina along 11-day period. Prior the insertion, the sponges were impregnated with two concentrations of three 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 (96%) and framicetin 4% (Framicas®, Laboratorios Ovejero S.A., León Spain). In agreement

with the veterinary practice, antibiotics were added into the bag containing 25 sponges and mixed by shaking. In the control group the sponges did not contain any antibiotic. Two different doses of antibiotics were used: Dose 1 equivalent to 1g of commercial product corresponding to a concentration of active principle for doxycycline of 100 mg, for oxytetracycline of 550 mg and for sulfathiazole of 960 mg, and Dose 2= 2 g equivalent to twice the amount of active compound of Dose 1.

According to the estrus synchronization by hormonal treatment, on the 9^{th} day from the placement of 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) were applied. The vagina status was evaluated through the visual assessment of the sponge 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, 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 (07:00 h) in a milking parlour and during the 7 days after the sponge insertion, individual goat milk samples (100 mL) were collected and transported in refrigeration (< 10 ºC) to the laboratories of the Universitat Politècnica de València.

Milk samples were analyzed in triplicate by 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 the manufacturer's instructions. In all cases, negative (antimicrobial-free milk) and positive (milk spiked with 4 μgkg^{-1} of benzylpenicillin) controls were included in each test. Interpretation of the test results

was carried out independently by three trained technicians assessing visually the colour 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 the 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 (DLs) of microbial inhibitor tests for doxycycline, oxytetracycline and sulfathiazole were calculated according to the IDF recommendations (IDF, 2003) and summarized in Table 1. The DLs 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 employed as 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 post-treatment (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); ε_{ijk} = residual error

To evaluate the status of the sponges as indicator of the antibiotic's effectiveness to prevent clinical vaginitis, the χ^2-test was applied. Statistical analyses were performed using SAS (version 9.2. 2001; SAS Institute Inc. Cary, NC).

Table 2 shows the positive results obtained in the microbial inhibitor tests during the first week after treatment. In general, the microbial test response was unaffected by the dose of antibiotic added to intravaginal sponges ($P > 0.05$). Thus, the occurrence of positive results in milk from goats belonging to the control group was similar to that obtained for milk from goats treated with antibiotics. Only for the highest dose of oxytetracycline (2g/25 sponges) a significant increase ($P < 0.05$) of positive results in the BRT MRL test was observed. Nor significant differences along the 7 days-post-treatment were found ($P > 0.05$). The results obtained herein differ from those reported by Berruga *et al*. (2008), who using intravaginal sponges impregnated with a drug that combines benzylpenicillin procaine and DH-streptomycin in ovine, indicated the highest occurrence of positive results in the first milking after treatment.

No positive results were obtained when milk samples were retested by specific receptor-binding assays, suggesting that drug residues, if present in milk, are below the DLs of these tests, not exceeding the MRLs established for these drugs in the legislation. Therefore, positive outcomes in the microbial inhibitor tests herein may be related to the performance of these unspecific tests that can be affected by several factors related to the milk composition leading to non-compliant results in milk free of antibiotics. Thus, the percentage of positive results obtained in this study are similar to the false-positive rate reported by Beltrán *et al*. (2015) for these microbial tests using individual goat's milk samples (Delvotest SP-NT MCS: 3.1 %; Eclipse 100: 0.6 %) except for the BRT MRL test that was higher than that reported by these authors (6.1 *vs* 1.4 %).

Regarding to the status of the vagina evaluated through the presence of bloody, purulent or foul-smelling discharges into sponges after removal (Figure 1), the occurrence of soiled sponges in the control group was higher ($P < 0.05$) than that obtained in the groups treated with antibiotics. Regarding to the dose of antibiotics used no significant

differences were obtained for the lowest dose administered, showing similar percentages of soiled sponges ($P > 0.05$). However, for the highest concentrations, a significant increase in the percentage of clean sponges was observed, being doxycycline (Hipradoxi®) the most effective drug ($P < 0.05$) to protect the vaginal status. These results were in agreement to those reported by Manes *et al*. (2013) and Guerra *et al*. (2002) in goats, who also indicated that the inclusion of antibiotics prevent the vaginal infection caused by the use of intravaginal sponges.

It can be concluded that the prophylactic use of low amounts of doxycycline, oxitetracycline, or sulfathiazole in the synchronization of estrus by intravaginal sponges contributes to reduce the incidence of 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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REFERENCES

Black, W. D., A. L. Mackay, P. A. Doing, and M. J. Claxton. 1979. A study of drugs residues in milk following intrauterine infusion of antibacterial drugs in lactating cows. Can. Vet. J., 20:354–357.

Beltrán, M.C., M.I. Berruga, A. Molina, R.L. Althaus, and M.P. Molina. 2015. Performance of the current microbial tests for screening antibiotic in sheep and goat milk. Int. Dairy J., 41:13–15.

193 Berruga, M. I., A. Rodriguez, R. Rubio, R. Gallego, and A. Molina. 2008. Short
194 communication: Antibiotic residues in milk following the use of intravaginal
195 sponges for estrus synchronization in dairy ewes. J. Dairy Sci., 91:3917–3921.
196 Bishop, J.R., A.B. Bodine, G.D. O'Dell, and J.J. Jazen. 1984. Retention data for
197 antibiotics commonly used for bovine infections. J. Dairy Sci., 67:437–440.
198 Commission Regulation (EU) Nº 37/2010. On pharmacologically active substances and
199 their classification regarding maximum residue limits in foodstuffs of animal origin
200 Off. J. L., 15:1–72.
201 Gatti, M., P. Zunino, and R. Ungerfeld. 2011. Changes in the aerobic vaginal mucous
202 load after treatment with intravaginal sponges in anoestrous ewes: effect of
203 medroxiprogesterone acetate and antibiotic treatment use. Reprod. Domest. Anim.,
204 46:205–208.
205 Guerra, M.M.P., R.A. Mota, F.C.C. Mergualhao, F.R. Lima, A.F. Souza, E.H. Melo,
206 K.P.C. Silva and P.C. Solares. 2002. Study of the microbial flora and evaluation of
207 the effectiveness of Gentocin ® 40 mg in the prevention of vaginal infection in dairy
208 goats submitted to estrous synchronization. A. Hora Vet., 22:13–17.
209 IDF. International Dairy Federation. 2003. Milk and milk products- Guidelines for a
210 standardized description of microbial inhibitor tests. IDF Standard Nº 183. Int.
211 Dairy Fed., Brussels, Belgium.
212 Manes, J.M., A. Fiorentino, F. Hozbor, F. Paolicchi, R. Alberio, and R. Ungerfeld.
213 2013. Changes in the aerobic vaginal bacteria load and antimicrobial susceptibility
214 after different oestrus synchronization treatments in goats. Anim. Prod. Sci.,
215 53:555–559.
216 Menchaca, A., and E. Rubianes. 2004. New treatments associated with timed artificial
217 insemination in small ruminats. Repord. Fert. Develop., 16:403–143.

218 Miller, G. E., and G. P. Bergt. 1974. Oxytetracycline in bovine plasma, milk, urine after
219 intrauterine administration. J. Dairy. Sci., 59:315–317.
220 Motlomelo, K.C., J.P.C. Greyling, and L.M.J. Schwalbach. 2002. Synchronisation of
221 oestrus in goats: The use of different progestogen treatments. Small Rumin. Res.,
222 45:45–49.
223 Penna, B., H. Libonati, A. Director, A.C. Sarzedas, G. Martins, F.Z. Brandão, J.
224 Fonseca, and W. Lilenbaum. 2013. Progestin-impregnated intravaginal sponges for
225 estrus induction and synchronozation influences on goats vaginal flora and
226 antimicrobial susceptibility. Anim. Reprod. Sci., 142:71–74.
227 Rowe, J.D., L.A. Tell, and D.C. Wagner. 2009. Animal safety report on intravaginal
228 progesterone controlled internal drug releasing devices in sheep and goats. J. Vet.
229 Pharmacol. Ther., 32:303–305.
230 Scudamore, C.L. 1988. Intravaginal sponge insertion technique. Vet. Rec., 123:554.
231 Suárez, G., P. Zunino, H. Carol, and R. Ungerfeld. 2006. Changes in the aerobic vaginal
232 bacterial mucous load and assessment of the susceptibility to antibiotics after
233 treatment with intravaginal sponges in anoestrous ewes. Small Rumin. Res., 63:39–
234 43.

Table 1. Detection capability of microbial inhibitor tests and receptor-binding assays for the detection of antimicrobials in goat's milk

Methods	Detection Limits (µg/kg)		
	Doxycycline[1]	**Oxytetracycline[2]**	**Sulfathiazole[3]**
Microbial inhibitor tests			
BRT MRL	135	124	48
Delvotest SP-NT MCS	106	297	39
Eclipse 100	113	134	47
Receptor-binding assays[4]			
SNAP Tetracycline Test	30[5]	≥ 50	-
Sulfasensor test	-	-	5-8

[1]MRL not stablished, since it cannot use in animals from which milk is produced for human consumption; [2]MRL 100 µg/kg; [3]MRL 100 µg/kg; [4]data provided by manufacturers; [5]Cross reactivity 100% of positive results at 30 µg/kg.

252 **Table 2.** Positive results in microbial inhibitor tests during the first week after the

253 intravaginal sponge insertion in dairy goats

Test	Treatment		Days after sponge insertion							Total (%)
			1	2	3	4	5	6	7	
BRT MRL	Control		0	2	1	1	2	0	0	6.1
	Doxycycline	Dose 1	0	3	1	0	1	2	0	7.1
		Dose 2	0	2	2	0	1	2	0	7.1
	Oxitetracycline	Dose 1	1	1	0	4	0	0	0	6.1
		Dose 2	3	2	3	3	3	0	0	14.3
	Sulfathiazole	Dose 1	0	1	1	1	1	0	0	4.1
		Dose 2	1	1	0	0	0	0	1	3.1
Delvotest SP-NT MCS	Control		0	0	0	0	1	0	0	1
	Doxycycline	Dose 1	0	0	0	0	0	0	0	0
		Dose 2	0	0	0	1	0	0	0	1
	Oxitetracycline	Dose 1	0	1	0	0	0	0	0	1
		Dose 2	0	0	1	0	0	2	0	3.1
	Sulfathiazole	Dose 1	0	0	1	0	0	0	0	1
		Dose 2	0	1	0	0	0	0	0	1
Eclipse 100	Control		0	0	1	0	0	0	0	1
	Doxycycline	Dose 1	0	0	0	0	0	0	0	0
		Dose 2	0	0	0	0	0	0	0	0
	Oxitetracycline	Dose 1	0	0	0	1	0	0	0	1
		Dose 2	0	0	0	1	0	0	0	1
	Sulfathiazole	Dose 1	0	0	0	1	0	0	0	1
		Dose 2	0	0	0	0	0	0	0	0

254

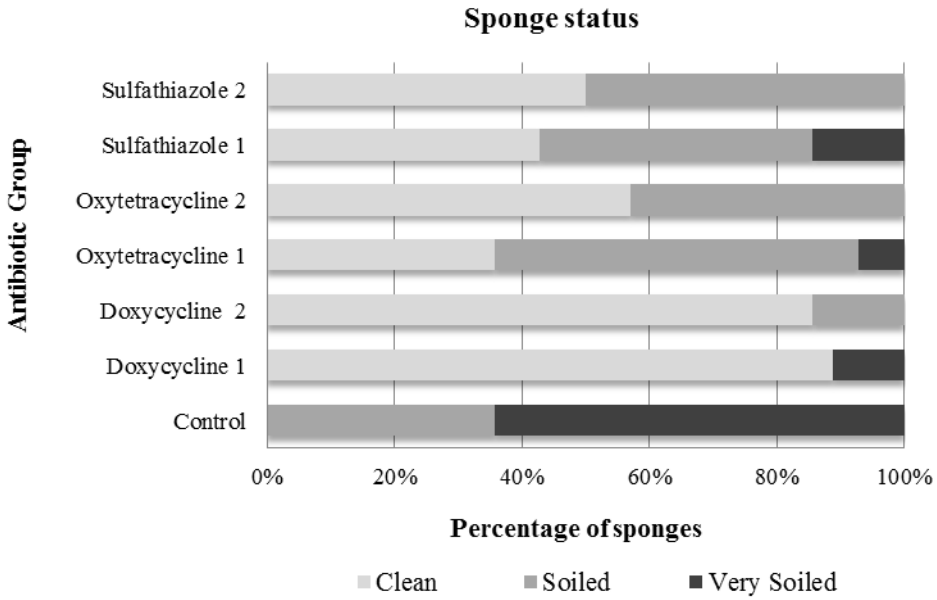

Figure 1. Visual classification of the sponge status after removal according to the effectiveness of the different antibiotics and doses used (1 and 2)