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Progestin-impregnated intravaginal sponges for estrus induction and synchronization influences on goats vaginal flora and antimicrobial susceptibility

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ABSTRACT

The objective was to characterize vaginal bacteria, their antimicrobial sensitivity, and the incidence of vaginitis, in goats before and after insertion of intravaginal sponges containing progesterone. Sponges were inserted in 37 Saanen goats and removed after 6, 9 or 12 d (G6, G9 and G12). At sponge removal, all goats had clinical signs of vaginitis. Sampling was conducted just before sponge insertion and at 0, 24, 48, and 72 h after sponge removal. Vaginal secretions were subjected to standard bacteriological procedures, including isolation of bacteria, subculture, and determination of sensitivity to antimicrobials (gentamicin, cefalotin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, amoxicillin and clavulanic acid, penicillin G and cefoxitin). Ciprofloxacin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole were the most effective for coliforms (100% sensitivity), whereas ciprofloxacin, gentamicin and tetracycline were the most effective for cocci (100, 98.6 and 97.2% sensitivity, respectively). In contrast, the least effective antimicrobials were cefalotin for the coliforms, and penicillin for the cocci (37.5 and 64.4% sensitivity, respectively), regardless of duration of implant presence and interval from implant removal to sampling. In conclusion, insertion of intravaginal progestin-impregnated sponges induced clinical vaginitis in goats. Members of *Staphylococcus* genus were the most frequently recovered species of the vaginal samples cultured, and all isolates were resistant to several antimicrobials.

1. Introduction

Reproductive technologies in domestic ruminants have improved over the past decades and are currently widely used (Huang and Rosenwaks, 2012). Protocols involving exogenous hormones to synchronize estrus or ovulation are often employed in animals, including small ruminants (Ababneh and Degefa, 2006; Abecia et al., 2012; Zarazaga et al., 2012). In these animals, synchronization protocols usually include intravaginal sponges impregnated with progestogens (Manes et al., 2010; Abecia et al., 2012). However, the effect of these hormones, as well as the mechanical presence of the devices, may predispose to purulent vaginitis (Martins et al., 2009; Manes et al., 2010). This infection is often due to proliferation of the local microbiota and is typically characterized by erythema, a purulent vaginal discharge and abundant vaginal leucocytes (Martins et al., 2009). Coliforms, mainly *Escherichia coli*, as well as Gram-positive cocci, mainly *Staphylococcus* sp. and *Streptococcus* sp., are the most common bacterial species present

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in those infections (Sargison et al., 2007). Although this has been studied in ewes (Martins et al., 2009; Manes et al., 2010), there is an apparent lack of reports regarding the occurrence of vaginitis in goats due to the use of intravaginal devices. The objective was to determine the incidence of vaginitis and the antimicrobial sensitivity of vaginal bacteria in goats given intravaginal sponges.

2. Materials and methods

2.1. Animals

The study was conducted in October and November of 2012 (nonbreeding season), in a commercial caprine dairy farm in Rio de Janeiro, Brazil. A total of 37 Saanen goats were studied (13 multiparous and 24 nulliparous). The research protocol was reviewed and approved by the Committee of Ethics and Animal Care of Fluminense Federal University (UFF/0160-09).

2.2. Hormonal treatment

All goats were treated with intravaginal sponges containing 60 mg medroxyprogesterone acetate (MAP Progespon®, Schering Plough, São Paulo, SP, Brazil). Goats were randomly allocated in three groups, according to the day of the removal of the intravaginal device: G6 ($n = 13$; eight nulliparous and five lactating), G9 ($n = 11$; four nulliparous and seven lactating) and G12 ($n = 13$; four nulliparous and nine lactating), where sponges were removed after 6, 9 and 12 d, respectively. For all groups, 24 h before sponge removal, 37.5 μg D-cloprostenol (Veteglan®, Calier Laboratories Ltda, Minas Gerais, Brazil) and 200 IU eCG (Novormon 5000®, Sintex Industries Biochemistry, Buenos Aires, Argentina) were injected subcutaneously in the latero-vulvar area.

After sponge removal, estrus detection was done (using bucks) twice daily. Goats were considered to be in estrus when they allowed to be mounted. The bucks were not allowed to achieve intromission.

2.3. Bacteriology

Sterile swabs (Copan, Brescia, Italy) were used for collection of vaginal secretions on occassions: T1 (before sponge insertion), T2 (immediately after sponge removal), and T3, T4, and T5 (24, 48, and 72 h after sponge removal, respectively. Swabs were used to inoculate Agar Trypcase Soy (Difco®, São Paulo, SP, Brazil) plates, which were incubated at 37 °C for 24 h. Then, bacteria were subcultured according to current bacteriological methods and classified by biochemical reactions as previously described (Martins et al., 2009).

Since *Streptococci* have a stable, predictable pattern of antimicrobial sensitivity (reference[s]), antimicrobial sensitivity testing was not performed for those isolates. All remaining isolates were tested for antimicrobial sensitivity. A panel of eight antimicrobial agents was tested by the disk diffusion method in accordance with Clinical and Laboratory Standards Institute (2008). Isolates were tested for gentamicin – GEN (10 μg), cefalotin – CFL (30 μg), tetracycline – TET (30 μg), ciprofloxacin – CIP (5 μg), trimethoprim-sulfamethoxazole – SUT (25 μg), amoxicillin and clavulanic acid – AMC (30 μg), penicillin G – PEN (10 μg), and cefoxitin – CFO (30 μg).

3. Results

At sponge removal, all goats had signs of vaginitis. In that regard, 70.3% had purulent and/or bloody vaginal discharges, whereas 29.7% had production of gas and an obvious foul odor.

Gram-positive bacteria, mainly *Streptococcus* followed by coagulase-negative *Staphylococcus* (CoNS) and coagulase-positive *Staphylococcus* (CoPS), were predominant, regardless of the duration of sponge treatment or the interval from sponge removal to sampling (Table 1).

In regards to antimicrobial sensitivity, 64.5% of *Staphylococcus* isolates and 37.5% of coliforms were susceptible to all products tested. Ciprofloxacin, gentamicin, tetracycline and trimethoprim-sulfamethoxazole were the most effective for coliforms (100% sensitivity), whereas ciprofloxacin, gentamicin and tetracycline were the most effective for the cocci isolates (100, 98.6 and 97.2 sensitivity, respectively). Cefalotin (37.5%) and penicillin (64.4%) were the least effective compounds for coliforms and cocci, respectively. Sensitivity of all isolates is shown (Table 1).

4. Discussion

Knowledge regarding resident microbial populations is important in elucidating the pathophysiology of disease in humans and animals. Although the resident microbiota is usually harmless, in the presence of predisposing factors (e.g., trauma or concurrent infection) some of these organisms may become potential pathogens, multiplying and causing disease (Martins et al., 2009).

Although manufacturers of intravaginal hormonal sponges do not recommend their use in animals with preexisting vaginitis, it is well known that these sponges predispose to vaginitis caused by opportunistic microorganisms (Ababneh and Degefa, 2006; Sargison et al., 2007; Martins et al., 2009). In the present study, goats did not have vaginitis prior to devicc insertion; therefore, bacteriaat present at T1 were regarded as the normal pattern of vaginal microbiota. Comparing frequency of isolation of each microorganism in the present study, it is clear that *Streptococci* are major components of the vaginal microbiota of goats. In additional to causing mechanical irritation, intravaginal sponges are impregnated with progestins, which may also have a local immunosuppressive effect, reducing lymphocyte proliferation and PGF2α production, thereby impairing the capacity of the animal to prevent or resolve infections (Manes et al., 2010).

In the present study, insertion of intravaginal sponges containing medroxyprogesterone acetate and systemic administration of D-cloprostenol and eCG stimulated changes in the vaginal microbiota in goats. Goats do not have tendency to undergo microbiota changes (Ababneh and Degefa, 2006), whereas ewes are more susceptible to changes influenced by utilization of hormone treatment,

Table 1
Resistant isolates of bacterial colonies isolated from goats' vaginas to antibiotics at different moments of short-term protocol of estrus induction and synchronization.

Group	Moment	Isolate	%	%							
				PEN	CFO	AMC	CFL	GEN	SUT	TET	CIP
G6	T1	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	7(53.9)	14.3	0	0	0	0	NT	0	0
		Coliforms	1(7.7)	NT	0	0	0	0	0	0	0
		Streptococci	5(38.5)	NT	NT	NT	NT	NT	NT	NT	NT
	T2	CoPS	1(7.7)	0	0	0	0	0	NT	0	0
		CoNS	1(7.7)	100.0	0	100.0	100.0	0	NT	0	0
		Coliforms	0	NT	0	0	0	0	0	0	0
		Streptococci	11(84.6)	NT	NT	NT	NT	NT	NT	NT	NT
	T3	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	4(30.8)	25.0	0	0	0	0	NT	0	0
		Coliforms	1(7.7)	NT	0	0	0	0	0	0	0
		Streptococci	8(61.5)	NT	NT	NT	NT	NT	NT	NT	NT
	T4	CoPS	1(7.7)	0	0	0	0	0	NT	0	0
		CoNS	4(30.8)	25.0	0	0	25.0	0	NT	0	0
		Coliforms	1(7.7)	NT	0	0	0	0	0	0	0
		Streptococci	7(53.9)	NT	NT	NT	NT	NT	NT	NT	NT
	T5	CoPS	2(15.4)	50.0	0	0	0	0	NT	0	0
		CoNS	2(15.4)	0	0	0	0	0	NT	0	0
		Coliforms	1(7.7)	NT	0	0	0	0	0	0	0
		Streptococci	8(61.5)	NT	NT	NT	NT	NT	NT	NT	NT
G9	T1	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	5(45.5)	80.0	0	20.0	20.0	0	NT	20.0	0
		Coliforms	1(9.1)	NT	0	100.0	100.0	0	0	0	0
		Streptococci	5(45.5)	NT	NT	NT	NT	NT	NT	NT	NT
	T2	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	8(72.7)	37.5	0	0	0	0	NT	0	0
		Coliforms	3(27.3)	NT	33.3	0	100.0	0	0	0	0
		Streptococci	0	NT	NT	NT	NT	NT	NT	NT	NT
	T3	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	5(45.5)	60.0	40.0	40.0	40.0	0	NT	0	0
		Coliforms	1(9.1)	NT	0	0	100.0	0	0	0	0
		Streptococci	5(45.5)	NT	NT	NT	NT	NT	NT	NT	NT
	T4	CoPS	1(9.1)	100.0	100.0	0	0	100.0	NT	0	0
		CoNS	7(63.6)	14.3	0	0	0	0	NT	0	0
		Coliforms	1(9.1)	NT	0	0	0	0	0	0	0
		Streptococci	2(18.2)	NT	NT	NT	NT	NT	NT	NT	NT
	T5	CoPS	1(9.1)	100.0	0	100.0	0	0	NT	0	0
		CoNS	4(36.4)	0	0	0	0	0	NT	0	0
		Coliforms	2(18.2)	NT	50.0	0	100.0	0	0	0	0
		Streptococci	4(36.4)	NT	NT	NT	NT	NT	NT	NT	NT
G12	T1	CoPS	1(7.7)	0	0	0	0	0	NT	0	0
		CoNS	3(23.1)	0	0	0	0	0	NT	0	0
		Coliforms	0	NT	0	0	0	0	0	0	0
		Streptococci	9(69.2)	NT	NT	NT	NT	NT	NT	NT	NT
	T2	CoPS	3(23.1)	0	0	0	0	0	NT	0	0
		CoNS	0	0	0	0	0	0	NT	0	0
		Coliforms	2(15.4)	NT	0	0	100.0	0	0	0	0
		Streptococci	8(61.5)	NT	NT	NT	NT	NT	NT	NT	NT
	T3	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	2(15.4)	100.0	50.0	50.0	50.0	0	NT	0	0
		Coliforms	0	NT	0	0	0	0	0	0	0
		Streptococci	11(84.6)	NT	NT	NT	NT	NT	NT	NT	NT
	T4	CoPS	0	0	0	0	0	0	NT	0	0
		CoNS	4(30.8)	75.0	25.0	0	25.0	0	NT	0	0
		Coliforms	2(15.4)	NT	0	0	50.0	0	0	0	0
		Streptococci	7(53.9)	NT	NT	NT	NT	NT	NT	NT	NT
	T5	CoPS	1(7.7)	0	0	0	0	0	NT	0	0
		CoNS	4(30.8)	50.0	0	0	0	0	NT	0	0
		Coliforms	0	NT	0	0	0	0	0	0	0
		Streptococci	8(61.5)	NT	NT	NT	NT	NT	NT	NT	NT

CoPS, coagulase-positive staphylococci; CoNS, coagulase-negative staphylococci; PEN, penicillin; CFO, cefoxitin; AMC, amoxicillin + clavulanic acid; CFL, cephalotin; GEN, gentamycin; SUT, sulphamethoxazol + trimethoprim; TET, tetracyclin; CIP, ciprofloxacin.

and therefore are more likely to develop vaginitis (Suárez et al., 2006; Martins et al., 2009; Manes et al., 2010).

With regard to the antimicrobial sensitivity of the isolates obtained in this study, some unexpected and indeed alarming results were observed. Since only goats with no history of recent antimicrobial treatment were included, it was expected that the normal vaginal microbiota would be highly susceptible to the antibiotics tested. Indeed only one drug (ciprofloxacin) was effective against all isolates. Based on a detailed clinical history of the herd, there was no previous exposure to any of the tested antibiotics that would account for the observed resistance.

Most coliforms (62.5%) were resistant to at least one tested drug, and only two drugs, tetracycline and gentamicin, were effective against all isolates. In another study, these drugs were the most effective against bacteria isolated from vaginal discharge of small ruminants (Suárez et al., 2006). Although there was no history of the herd being treated with the tested antibiotics in the two months preceding sponge insertion, it is well known that antimicrobial drugs are frequently overused for other indications, including diarrhea and respiratory disease in juvenile animals, which could have contributed to selection of resistant strains in the microbiota.

5. Conclusions

In conclusion, insertion of intravaginal, progestin-impregnated sponges induced vaginitis in goats. Members of *Staphylococcus* genus were the most frequently recovered species of the vaginal samples cultured, and all isolates had a high rate of resistance to antimicrobials. Ciprofloxacin was the most effective drug to treat vaginitis, caused by either by Gram-positive or Gram-negative bacteria. Gentamicin was also an excellent choice for infections caused by Gram-negative and Gram-positive agents.

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