Small Ruminant Research 81 (2009) 182–184

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progestin-impregnated intravaginal sponges

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ARTICLE INFO

Article history:
Received 1 September 2008
Received in revised form
12 November 2008
Accepted 5 December 2008
Available online 21 January 2009

Keywords:
Bacteria
Ewes
Vaginitis
Antimicrobials

ABSTRACT

The objective was to characterize vaginal bacteria in ewes with vaginitis. Intravaginal sponges impregnated with medroxyprogesterone were used to synchronize estrus in 22 multiparous Santa Inês ewes. At sponge removal (6 days later), all ewes had clinical signs of vaginitis. Purulent vaginal secretions were subjected to standard bacteriological procedures, including determining whether isolates were susceptible to trimethoprim-sulfamethoxazole, gentamicin, cefalotin, tetracycline, ciprofloxacin, nitrofurantoin, ampicillin, penicillin G, and amoxicillin. The majority of the isolates were coliforms (72.7% *Escherichia coli* and 18.2% *Klebsiella pneumoniae*), whereas the remainder were *Staphylococcus aureus*. Antimicrobial resistance was common, with all isolates resistant to at least one compound. Ciprofloxacin and trimethoprim-sulfamethoxazole were the most effective (100% susceptibility), whereas penicillins (including broad-spectrum penicillins), were the least effective (80–100% resistance). In conclusion, pathogenic bacteria, mainly coliforms, were present in association with vaginitis in ewes given intravaginal sponges; all isolates were susceptibile to at least some antimicrobials.

1. Introduction

Vaginitis, a very common disease of the genital tract of domestic ruminants, is often caused by opportunistic secondary invaders (Root Kustritz, 2006). In that regard, coliforms, mainly *Escherichia coli* species, have been frequently isolated from the vagina of ewes (Sargison et al., 2007), as well as from goats (Ababneh and Degefa, 2006) and cows (Sheldon et al., 2008).

Progestin-impregnated intravaginal sponges are commonly used to synchronize estrus in ewes (Suárez et al., 2006). Nevertheless, they are a predisposing factor for vaginal infections (Padula and Macmillan, 2006), leading to vaginitis, typically characterized by erythema, a purulent vaginal discharge, and abundant vaginal leucocytes (Donders et al., 2002).

Coliforms (of fecal origin) are opportunistic pathogens in the reproductive tract. That they have a highly variable pattern of antimicrobial susceptibility limits the efficacy of empirical therapies. Since there is little scientific information regarding the use of antibiotics to control reproductive infections in ewes (Suárez et al., 2006), a better understanding of the bacterial species that occur in vaginitis and their susceptibility to antimicrobial agents may enhance management of vaginitis, and perhaps other infections of the reproductive tract in ewes. The aim of the present study was to evaluate the prevalence and antimicrobial susceptibility of vaginal bacteria in ewes following treatment with intravaginal sponges.

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doi:10.1016/j.smallrumres.2008.12.003

Table 1
Resistance pattern of strains of bacteria isolated from the vagina of ewes.

	AMP	AMO	TET	CFL	GEN	NIT	PEN	CIP	SUT
Coliforms	18/20	16/20	15/20	14/20	14/20	4/20	N//T	0/20	0/20
Staphylococci	0/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	N/T
Total	18/22	16/22	15/22	14/22	14/22	4/22	2/2	0/22	0/20

AMP, ampicillin; AMO, amoxicillin; TET, tetracycline; CFL, cefalotin; GEN, gentamicin; NIT, nitrofurantoin; PEN, penicillin G; CIP, ciprofloxacin; SUT, trimethoprim-sulfamethoxazole; N/T, not tested.

2. Materials and methods

2.1. Animals

At the start of the 2007 breeding season, 22 multiparous Santa Inês ewes from a single flock were selected for an estrus synchronization program, based on an intravaginal sponge impregnated with medroxiprogesterone (Progespon®). There were no apparent vaginal abnormalities at sponge insertion. However, when sponges were removed 6 days later, all ewes had clinical signs of vaginitis, including a purulent vaginal discharge and varying degrees of vaginal erythrema. There was no contemporary control group that were not given a sponge.

2.2. Bacterial culture

A sterile cotton swab was used to collect samples from the posterior vaginal region of each ewe (immediately after sponge removal). Samples were transferred to the laboratory in transport medium (Stuart's medium, Copan, Italy), inoculated in 5% sheep blood agar (Merck), and incubated at 37 °C (aerobic culture only). If bacterial growth was apparent after 24 or 48 h of incubation, smears were made, Gram-stained, and examined microscopically. Samples with morphology consistent with Gram-negative rods were transferred to EMB Teague Agar (Merck), whereas those suggestive of *Staphylococcus* sp. were transferred to Mannitol-salt-Agar (Merck).

2.3. Bacterial identification

Bacteria were identified on the basis of colony characteristics, Gram stain, pigment production and biochemical reactions, including agar Triple Sugar Iron (TSI), citrate, urease, indol, Methyl Red (MR), Voges Proskauer (VP), nitrate and motility tests, catalase activity test, tube coagulase test, and aerobic fermentation of several carbohydrates. Bacteria were classified as described in previous studies (Otero et al., 2000) and according to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.4. Antibiotic sensitivity test

Susceptibility to a panel of eight of the most frequently used antimicrobial agents was determined by the disk diffusion method in Mueller-Hinton agar (Merck), in accordance with National Committee for Clinical Laboratory Standards (2003). Briefly, three to five well-isolated colonies of the same morphological type were selected from the agar plate culture and transferred to a tube containing Brain Heart Infusion broth (Merck). Standardized inocula culture with a turbidity equivalent to a 0.5 McFarland standard were used (corresponds to a concentration of approximately $1–2 \times 10^6$ CFU/mL). All isolates were tested with disks, with the following drug concentrations and minimum inhibitory zones: gentamicin (10 μg, 15 mm), cefalotin (30 μg, 18 mm), tetracycline (30 μg, 15 mm), ciprofloxacin (5 μg, 21 mm), nitrofurantoin (300 μg, 17 mm), ampicillin (10 μg, 17 mm), and amoxicillin (10 μg, 17 mm). In addition, trimethoprim-sulfamethoxazole (25 μg, 17 mm) was tested for Gram-negative rods, and penicillin G (10 U, 29 mm) was tested for Gram-positive cocci. *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as quality control organisms.

3. Results

3.1. Bacterial prevalence

All samples yielded abundant bacterial growth in pure culture. If more than one bacterial colony type was detected, the most prevalent (based on number of colonies) was incriminated as the cause of vaginitis. From the 22 isolates, 20 were coliforms, including 16 (72.7%) *E. coli* and four were *Klebsiella pneumoniae* (18.2%). The remaining two isolates (9.1%) isolates were classified as *Staphylococcus aureus*.

3.2. Antibiotic susceptibility

Resistance of isolates to antibiotics was common; all isolates were resistant to at least one tested drug (Table 1). In the coliform group, resistance to the penicillins was common, primarily to ampicillin (95% resistance) and amoxicillin (80% resistant). Resistance to other drugs was also observed, mainly to tetracycline (85%), gentamicin (70%) and cefalotin (70%). The most active antimicrobial agents against coliforms were ciprofloxacin and trimethoprim-sulfamethoxazole (100% susceptible), and nitrofurantoin (only four isolates were resistant). *Staphylococcus* strains had limited resistance; both of the two isolates were resistant only to penicillin G.

4. Discussion

Although manufacturers of intravaginal hormonal sponges do not recommend their use in animals with pre-existing vaginitis, it is well known that these sponges predispose to vaginitis caused by opportunistic microorganisms (Sargison et al., 2007). Changes in the vagina may be attributed either to the physical action and/or to the constant absorption and retention of the vaginal secretions by the intravaginal sponge, which stimulates bacterial growth (Suárez et al., 2006). Besides its mechanical action, intravaginal sponges are impregnated with progestins, which may have a local immunosuppressive effect, reducing lymphocyte proliferation and $PGF_{2\alpha}$ production, thereby impairing the capacity of the organism to prevent or resolve infections (Lewis, 2003). Although some of the sponge-induced vaginitis may be self-cured after its removal, other cases may require antibiotic therapy (Suárez et al., 2006), and therefore the adequate identification and susceptibility testing of the agents becomes necessary.

This was not a controlled study and no attempt was made to compare findings in a contemporary group of ewes

that were not treated with an intravaginal sponge. Nevertheless, it was suggested that intravaginal sponges may substantially increase bacterial load (up to 100-fold), with a peak after 5 days (Suárez et al., 2006). In the present study, all ewes with sponges had clinical evidence of vaginitis and, since samples were collected 6 days after sponge insertion, it was expected the bacterial population would have peaked. In relation to the etiology of the infection, the present study confirmed the importance of coliforms as opportunistic agents of bacterial vaginitis, consistent with studies conducted not only in ewes (Donders et al., 2002), but also in cows (Padula and Macmillan, 2006).

Regarding antimicrobial susceptibility, all coliforms isolated (20 samples) were resistant to at least one tested drug and only two drugs (ciprofloxacin and trimethoprim-sulfamethoxazole) were effective against all isolates. Although there was no history of the flock being treated with the tested antibiotics in the 2 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 a previous selection of microbiota. There is a paucity of studies regarding antimicrobial susceptibility of bacteria isolated from vagina of ewes, and it may vary not only due to the primary incriminating factor, but also with the region where the study was conducted. Although Suárez et al. (2006) suggested cefalotin and gentamicin were the most effective compounds to prevent bacterial growth following the use of progestin-impregnated intravaginal sponges in ewes from Uruguay, they were far less effective in the present study (63.6% of isolates were resistant to each of these drugs). In a more recent study conducted in cows from Canada (Carson et al., 2008), there was a high susceptibility of coliforms to ciprofloxacin and trimethoprim-sulfamethoxazole, consistent with the present findings.

5. Conclusion

In conclusion, vaginitis was apparent in all 22 ewes 6 days after insertion of intravaginal, progestin-impregnated sponges. Coliforms were recovered from 90.9% of the vaginal samples cultured. There was a high rate of resistance to antimicrobials. Ciprofloxacin was the most effective drug to treat infections by Gram-positive or Gram-negative bacteria. Trimethoprim-sulfamethoxazole was also shown to be an excellent choice for infections caused by Gram-negative agents.

Acknowledgements

The authors are thankful to Prof. John Kastelic for the critical review and valuable suggestions. This study was funded by FLAP and FAPERJ. GM and WL are CNPq fellows.

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