

The Normal Microflora of the Female Rabbit's Genital Tract

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ABSTRACT

Microorganisms associated with the vagina, cervix and uterus of rabbits were isolated and identified. The predominant microorganisms isolated from the vaginas and cervixes were coagulase-negative staphylococci, micrococci, and nonfermentative bacilli. Coagulase-negative staphylococci were isolated frequently, but in small numbers, from the uteri. The pH of the rabbit vagina was found to be near neutrality. Our data indicate that the genital flora of female rabbits is relatively simple, regarding the number and type of microorganisms.

Key words: Microflora, genital tract, rabbit.

RÉSUMÉ

Cette expérience portait sur l'isolement et l'identification de la microflore des voies génitales de lapines. Les bactéries isolées le plus souvent du vagin et du col utérin comprenaient des staphylocoques coagulase-négatifs, des microcoques et des bacilles non fermentatifs. L'utérus recelait une flore fréquente, mais plutôt restreinte, de staphylocoques coagulase-négatifs. Le pH de vagin se rapprochait de la neutralité. Les résultats de l'expérience révèlent la simplicité relative de la microflore des voies génitales des lapines, quant au nombre et à la variété de ses composantes.

Mots clés: microflore, voies génitales, lapines.

INTRODUCTION

We chose the rabbit as a possible experimental model to study microbial colonization of intrauterine contraceptive devices (IUCD) because other aspects of IUCD implantation have been studied with this animal (1,2,3,4). Use of the rabbit for such a study requires a thorough understanding of the normal microflora of these animals. Thus, as a primary step in investigating IUCD microbial colonization, this microflora was studied. There have been a number of reports in the literature concerning genital microflora of different animal species such as the mouse (5), rat (6), dog (7), baboon (8), horse (9) and cow (10), but to our knowledge none have considered the rabbit. This report provides information on the various microorganisms found in vaginal, cervical and uterine tissues of healthy rabbits, and the frequency of isolation of each microorganism.

MATERIALS AND METHODS

ANIMALS

Thirty-five mature female New-Zealand rabbits, 2.5 to 3 kg (four to five months old), were purchased from Stoney Creek, Chase, British Columbia and Kleefeld Rabbitry, Niverville, Manitoba, Canada. Prior to their use all rabbits were housed individually from two to three weeks in stainless steel cages at a room temperature of $20 \pm 1^\circ\text{C}$ with 12 hours of light per day. Animals were fed commercial rabbit diet (United Feed, Calgary, Alberta) and water *ad libitum*. Animal care was conducted according to the

guidelines outlined by the Canadian Council on Animal Care. Vaginal, cervical, and uterine tissues were obtained for culture immediately following CO₂ euthanasia.

CULTURES

Portions of tissues were placed in 10 mL of phosphate buffered saline (PBS, pH 7.2), either homogenized with a Waring blender (Waring Products Division, New Hartford, Connecticut) or sonicated for ten minutes (Bransonic 220, Branson, Shelton, Connecticut); swabbings failed to recover a significant number of microorganisms. Aliquots (0.1 mL) of the suspensions obtained from homogenization or sonication were spread on different solid media purchased from Difco Laboratories, Detroit, Michigan. Blood agar (tryptose blood agar base containing 5% sheep blood), Mannitol salt agar, MacConkey agar, Rogossa SL agar and Sabouraud dextrose agar were incubated aerobically at 37°C for 48 hours whereas blood agar, blood agar containing 100 µg of kanamycin per mL and Rogosa SL agar were incubated at 37°C in an anaerobic chamber (Anaerobic system, model 1024, Forma Scientific, Marietta, Ohio) for up to seven days. Isolates were placed in a modified Ames transport medium (Culturette, Marion Scientific, Kansas City, Missouri) and sent to the Western College of Veterinary Medicine (University of Saskatchewan, Saskatoon, Saskatchewan, Canada) for bacteriological identification.

pH

Measurement of vaginal pH was

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accomplished by inserting a small diameter combined glass electrode (pH electrode and reference electrode) into the vagina immediately after euthanasia. Readings were taken using a standard pH meter.

RESULTS

Aerobic and anaerobic cultures were taken from genital tract tissues of 35 rabbits; microorganisms recovered from these samples are shown in Table I. Because no differences were observed between vaginal and cervical isolates, in terms of species and numbers, these two sites were considered together. Coagulase-negative staphylococci, micrococci, and non-fermentative bacilli (mainly pseudomonads) were the most frequent microorganisms isolated from the vaginas and cervixes. The predominant microorganisms recovered from the uteri were coagulase-negative staphylococci; they were always present in smaller numbers here than in vaginal and cervical samples. More than a third of the rabbits had at least three species composing the microflora. It was apparent that this group of rabbits was relatively homogenous with respect to the species forming the genital microflora, as evidenced by the small number of species reported in Table I.

Because of the apparent importance of vaginal acidity as a factor predisposing the human vagina to colonization by certain bacterial species (11), the vaginal pH of the rabbits was determined. The pH values ranged from 6.6 to 8.1 with a mean value \pm SD of 7.2 ± 0.4 .

DISCUSSION

Unlike other animal species which are known to support an important genital tract mucosal microflora, few bacteria were isolated from rabbit genital tract mucosal surfaces. We have not detected any differences between cervical and vaginal microflora; this may be due in part to the fact that the cervix is difficult to delimit and incorporation of a portion of the vagina with it might have occurred. The predominant constituents of the rabbit vaginal and cervical

TABLE I. Bacteria Isolated from the Vagina, Cervix and Uterus of 35 Rabbits

Bacteria	Number of Rabbits from which Microorganisms were Isolated (%)	
	Vagina/Cervix	Uterus
<i>Acinetobacter calcoaceticus</i>	1 (3)	
<i>Alcaligenes</i> sp.		1 (3)
<i>Bacillus</i> sp.	4 (11)	
<i>Corynebacterium kutscheri</i>	3 (9)	1 (3)
<i>Escherichia coli</i>	1 (3)	
<i>Lactobacillus</i> sp.	2 (6)	
<i>Micrococcus</i> sp.	10 (29)	5 (14)
Nonfermentative bacilli	5 (14)	
<i>Propionibacterium granulosum</i>	1 (3)	
<i>Pseudomonas maltophilia</i>	1 (3)	
<i>Pseudomonas</i> sp.	7 (20)	
<i>Staphylococcus</i>	21 (60)	12 (34)
<i>S. aureus</i>	3 (9)	3 (9)
<i>S. capitis</i>	3 (9)	
<i>S. epidermidis</i>	10 (29)	3 (9)
<i>S. haemolyticus</i>	7 (20)	2 (6)
<i>S. hominis</i>		1 (3)
<i>S. saprophyticus</i>	3 (9)	1 (3)
<i>S. sciuri</i>	1 (3)	1 (3)
<i>S. warneri</i>	2 (6)	1 (3)
<i>S. xylosus</i>	3 (9)	
<i>S. sp.</i>	1 (3)	1 (3)
<i>Streptococcus</i> alpha-hemolytic	2 (6)	
<i>Streptococcus faecalis</i>		1 (3)
<i>Streptococcus faecium</i>	2 (6)	
Strict anaerobes (unidentified)	5 (14)	2 (6)

microflora were coagulase-negative staphylococci, micrococci, and non-fermentative bacilli (mainly pseudomonads). High numbers of coagulase-negative staphylococci have been observed in the vaginal flora of dogs (7) and also in humans (12,13); micrococci have been isolated in dog and in rat vaginal microflora, but with a lower incidence (6,7). Streptococci, frequently encountered in our rabbits, have been observed in many vaginal microfloras (6,7,8,9,12,13).

Some strict anaerobes were recovered but they did not appear to form as major a component of the vaginal flora as observed in humans and in dogs (7,12,13). Another notable difference between rabbit and human vaginal flora included the almost complete absence of lactobacilli in rabbits while a high frequency was reported in humans (12,13). Because of the association of lactobacilli with acidic pH (11), the neutral pH values observed in the rabbits studied may be related to the observed low frequency of lactobacilli as well as account for some of the differences between human and rabbit genital microflora. Vaginal pH values near neutrality

were also noted with other animal species such as the rat (6) and the mouse (14).

We have isolated microorganisms (mainly coagulase-negative staphylococci) from 45% of the uterine samples; in many of them the composition of the microflora was quite similar to the corresponding vaginal microflora, the only major difference being the absence of nonfermentative bacilli which were observed in vaginal and cervical floras. There is continuing controversy over whether or not the normal human uterus is sterile (15), but in different animal species such as the cow (10), the horse (9), the dog (7), and the mouse (5), bacteria have been isolated from normal uteri. The predominance of staphylococci, mainly coagulase-negative, we noted in uterine microflora has also been observed in cows by Messier *et al* (10) and in dogs by Baba *et al* (7).

Our results indicate that the rabbit seems to possess a relatively simple genital tract microflora, regarding the number and type of microorganisms, and therefore might be advantageously used for IUCD microbial colonization studies.

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