

## CORRELATION BETWEEN DIFFERENT SEXUAL CYCLE STAGES AND VAGINAL BACTERIAL FLORA IN BITCHES OF DIFFERENT BREEDS

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**Summary.** This study determined the correlation between bacterial vaginal flora and the different sexual cycle (prooestrus, oestrus, metoestrus and anoestrus) of bitches of seven different breeds over 18 month period. Vaginal swab samples were taken from the vagina of 46 bitches of the following breeds: English bulldogs (10), Dobermans (6), Collie (9), Great Danes (6), German shepherds (5), Labrador retrievers (5) and Scottish terrier (5). The most common bacterial species isolated from 46 investigated bitches of different breeds were *Staphylococcus* spp. (57.6%) and *Staphylococcus aureus* was isolated from 32.6% of samples. In addition, *Escherichia coli* (in 15.1% of samples), *Streptococcus* spp. (9.1%), *Staphylococcus* spp. *Pasteurella* spp. (15.1%), *Pseudomonas aeruginosa* (3.1%) were identified. Our results were in concert with previous findings, that the lowest distribution of vaginal bacteria was found during metoestrus (2.0%) and significantly higher number of bacteria were identified during oestrus - 12.2% ( $P < 0.05$ ) and prooestrus - 28.6% ( $P < 0.001$ ). Our results showed that bacteria growth at different stages of the sexual cycles was variable and the vaginal flora had mixed character.

**Key words:** bitch, vaginal microflora, reproductive cycle.

## ĮVAIRIŲ VEISLIŲ KALIŲ MAKŠTIES MIKROFLOROS KORELIACIJA SKIRTINGOMIS LYTINIO CIKLO FAZĖMIS

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**Santrauka.** Įvairių veislių kalių makšties mikroflora buvo tirta skirtingų lytinio ciklo fazių metu. Tirtos anglų buldogų, dobermanų, dogų, labradoro retriverių, koli, škotų terjerų ir vokiečių aviganių veislių kalės. Mėginiai buvo imti skirtingų lytinio ciklo fazių metu – priešrujo, tikrosios rujos, porujo ir ramybės periodu. Dažniausiai išskyrėme *Escherichia coli* (15,1 proc.), *Streptococcus* spp. (9,1 proc.), *Staphylococcus* spp. (57,6 proc.), *Pasteurella* spp. (15,1 proc.), *Pseudomonas aeruginosa* (3,1 proc.). Šie mikroorganizmai laikomi oportunistiniais ir yra dažniausiai išskiriama aerobinė kalių makšties mikroflora. Visoms 46 tirtoms kalėms skirtingu lytinio ciklo fazių metu dažniausiai pasireiškė *Staphylococcus* spp. (32,6 proc.). *Staphylococcus aureus* yra dažniausiai išskiriama sveikų kalių bakterija. Nustatyta, kad mažiausiai mikroorganizmų išskirta porujo metu (2,04 proc.). Mūsų tyrimai patvirtino literatūros duomenis, kad daugiausia mikrobų išskiriama priešrujo ir tikrosios rujos metu (atitinkamai 28,6 proc. ir 12,2 proc.). Lygindami išskirtus mikrobus priešrujo ir tikrosios rujos metu, gavome patikimus rezultatus ( $p < 0,05$ ), kaip ir lygindami priešrujo ir porujo fazes ( $p < 0,001$ ). Patikimus rezultatus gavome lygindami išskirtų mikrobų rūšis tikrosios rujos ir porujo ( $p < 0,05$ ) bei porujo ir ramybės periodus ( $p < 0,01$ ).

**Raktažodžiai:** kalė, makšties mikroflora, lytinis ciklas.

**Introduction.** The vaginal tract of the bitch houses a multitude of various organisms, and any of them can become opportunistic pathogens under certain conditions. Some of these bacterial organisms that have been associated with spontaneous abortions or post-delivery complications most commonly include *Escherichia coli* and *Streptococcus* spp., or less commonly *Salmonella* spp. or *Campylobacter* spp. (particularly if diarrhea has been observed in the bitch or any humans in contact with her). Infections can occur in any bitch, however, endometrial hyperplasia that occurs with aging places older bitches at increased risk for these infections. Approximately 60 % of normal bitches harbor aerobic bacteria in the cranial vagina and 90 % – in the caudal vagina (Olson et al., 1986). Therefore, merely isolating bacteria does not constitute a basis for incriminating the isolate(s) with the reproductive disease. Frequently, bacteria may be very abundant during prooestrus and oestrus (Allen and Dag-

nall, 1982; Baba et al., 1983). High proportions of infectious cases were found in cases of limitation of fertility (67.8%) in vaginal discharge in the oestrus (60.8%) (Wendt and Stellmacher, 1996). The vagina is not sterile and a larger number of contaminants or normal flora are routinely cultured from the caudal vagina (2.2 isolates per bitch in anoestrus and 2.3 isolates per bitch in prooestrus) than from the cranial vagina (0, 7 isolates per bitch in anoestrus and 1.0 isolates per bitch in prooestrus) (Olson, 1976).

Microorganisms most commonly isolated from the vagina of normal bitches are *Escherichia coli*, *Streptococcus canis*, *Pasteurella multocida*, *Staphylococcus aureus*, *Staphylococcus intermedius*,  $\beta$ -haemolytic streptococcus,  $\alpha$ -haemolytic streptococcus, *Proteus mirabilis* (Osbaldiston, 1972; Platt et al., 1974; Hirsh, 1977; Ling 1978; Olson, 1978; Schaefer et al., 1978; Van Duijkeren, 1992; Laznicka, 1995). Aerobic and anaerobic microflora were

identified and quantitated in most vaginal and uterine samples obtained from mature bitches during different stages of the oestrus cycle (Baba et al., 1983). In all stages of the cycle *Escherichia coli* and *Streptococcus* spp. were isolated, except during the pregnancy period. It was determined that beta hemolytic streptococci grew during the oestrus and early metoestrus stages while  $\alpha$ -hemolytic streptococci grew during other stages of the sexual cycle (Findik et al., 2003). It was confirmed that different bacteria could grow as well as the same bacteria at different stages of oestrus cycle and vaginal flora of mixed character was isolated (Günay et al., 2004). A larger number of microorganisms are cultured during oestrus than during metoestrus or anoestrus (Noguchi et al., 2003). It was confirmed that more microorganisms are retrieved from bitches with reproductive tract disease than from normal bitches (Hirsh et al., 1977).

The qualitative and quantitative analysis of vaginal bacterial microflora of healthy bitches has received relatively little attention and limited information is available. Therefore the goal of our study was to investigate the vaginal bacterial flora in bitches of various breeds during the different sexual cycle (prooestrus, oestrus, metoestrus and anoestrus).

**Materials and methods.** From 2006 to 2007 year vaginal swab samples were taken from 46 bitches of the following breeds: English bulldogs (10), Dobermans (6), Collie (9), Great Danes (6), German shepherds (5), Labrador retrievers (5) and Scottish terriers (5). All animals were chosen randomly from 7 different kennels. Bitches were sampled at the different stages of sexual cycle: in prooestrus, oestrus, metoestrus and anoestrus. Stages of the sexual cycle were determined based on history, physical examination, vaginal cytology, vaginoscopy and serum progesterone levels. Animals were adequately restrained in a standing position. The perivulvar area was scrubbed with a potent antiseptic Manorapid (Antiseptica, Germany). Sterile swab (Liofilchem, Italy) sticks were passed into the vagina. The stick was initially directed cranio-dorsally (avoiding the clitoral fossa and the urethral orifices) and then longitudinally. The swab then was rolled around the vaginal wall gently and withdrawn care-

fully into sterile container for transporting to the laboratory. Microorganisms were identified using standard procedures. Swabs were transferred to the microbiological laboratory (Department of Infectious Diseases, LVA.). Samples were inoculated in Meat Peptone Broth (MPB) and parallelly on solid media: blood agar (BA) and Mac – Conkey agar (Oxoid, England). After inoculation Petri plates were incubated at 37°C temperature, for 24–48 hours. The bacteria colonies were analyzed. The smears from microorganisms cultures were stained by Gram (Diagnostica Merck, German) and microscopy to determine microorganism morphology was performed. *Staphylococcus* spp. was identified by Coagulase activity (Liofilchem, Italy), "Staphytest Plus" (Oxoid, England). *Staphylococcus aureus* strains producing beta lactamase were determined by Beta Lactamase Test (Liofilchem, Italy). *Streptococcus* spp. were identified by Streptococcal Grouping Test (Oxoid, England). For enterobacteria identification selective medium Manitol agar, Hectoer enteric agar, Brilliant Green, XLD (Oxoid, England), Oxidase test Bactident® Oxidase (Merck, Germany) and biochemical test – BD BBL™ Enterotube™ II (Becton. Dickinson, GmbH. Diagnostic systems, Germany), Hygicult E/β/ - Gur (Orion diagnostica, Finland) were used.

*Pseudomonas aeruginosa* was identified by selective medium for pigment production (Pseudomonas agar P, Pseudomonas agar F), (Difco, USA) and *Pseudomonas* (cetrimide) agar (Liofilchem, Italy). *Pasteurella* spp. were identified by Drigalsky lactose agar, Mac Conkey agar (Liofilchem, Italy).

**Statistical analysis.** In all analyses data from different bitches are assumed to be independent. The arithmetic average values (X), standard deviation (SD), and coefficient of variation (CV) were calculated for all data. The differences between groups were analysed by an analysis of variance (ANOVA) assuming that the transformed counts  $\log_{10}(x+10)$  for each bitch are multivariate normal with an unconstrained covariance common to all bitches. The significance of differences between groups was calculated using Student's test, where  $p < 0.05$  was considered statistically reliable.

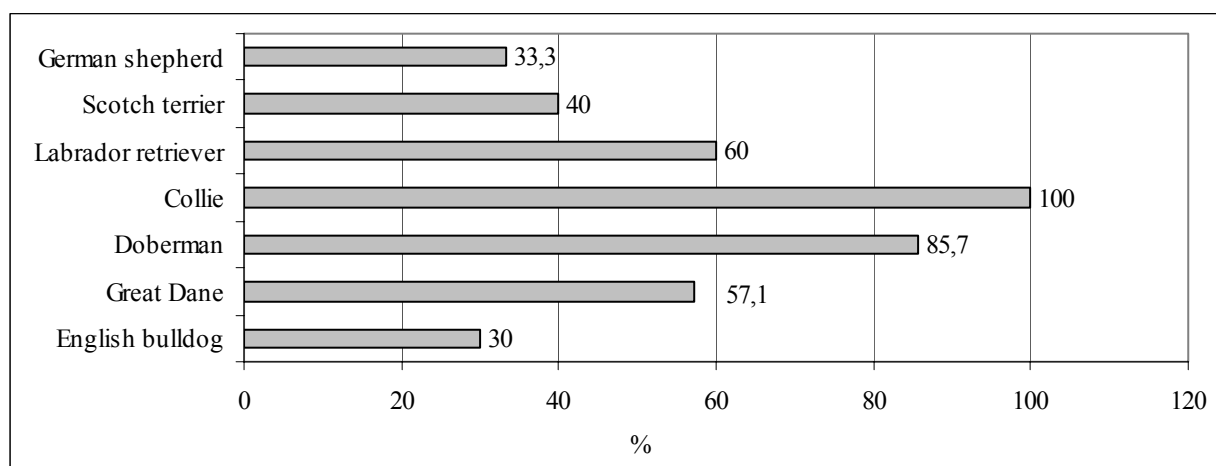


Figure 1. Vaginal microflora of different breeds bitches

**Results.** Details on the microbiological investigations in 46 bitches at the different stages of sexual cycle are shown in Figure 1 and Table 1. The most common bacterial species isolated were *Staphylococcus* spp. (57.6%) and *Staphylococcus aureus* was isolated from 32.6% of

samples. In addition, *Escherichia coli* (in 15.1% of samples), *Streptococcus* spp. (9.1%), *Staphylococcus* spp., *Pasteurella* spp. (15.1%), *Pseudomonas aeruginosa* (3.1%) were identified.

Table 1. Microorganisms isolation from various breed bitches in different stages of sexual cycle

Breed	Microorganism % in different stages of cycle			
	prooestrus	oestrus	metoestrus	anoestrus
English bulldog	10.0	30.0	0	0
Great Dane	28.6	14.3	14.3	14.3
Doberman	42.9	28.6	0	28.6
Collie	33.3	0	0	66.7
Labrador retriever	60.0	0	0	0
Scotch terrier	20.0	0	0	20.0
	16.7	0	0	33.3

Different bacteria were isolated from 12.2% of bitches on oestrus. The most common species in this group were *Staphylococcus aureus* (66.6%) and *Pasteurella multocida* (33.4%). Among the bitches in anoestrus bacteria were isolated in 24.5% samples. *Staphylococcus aureus* (50.0%), beta hemolytic streptococci (16.7%), *Escherichia coli* (8.3%), *Pasteurella multocida* (16.7%), *Pseudomonas aeruginosa* (8.3%) were identified. In metoestrus *Staphylococcus aureus* was found (2.0%) and it was a solid isolate in this stage. (Microorganisms were isolated in 28.6% of the bitches pro oestrus.) The most common bacteria isolated were: *Staphylococcus aureus* (57.1%), *Escherichia coli* (28.6%), beta hemolytic streptococcus (7.1%) and *Pasteurella multocida* (7.2%). The lowest distribution of vaginal bacteria was found during metoestrus (2.0%) and significantly higher number of bacteria were identified during oestrus - 12.2% ( $P < 0.05$ ) and prooestrus - 28.6% ( $P < 0.001$ ). The content of microorganisms was significantly higher in bitches on prooestrus compared to animals on oestrus ( $P < 0.05$ ) and on metoestrus ( $P < 0.001$ ). Furthermore, the content of microorganisms in oestrus was significantly higher than in ones on metoestrus ( $P < 0.05$ ) and anoestrus ( $P < 0.01$ ), respectively. The highest rate of bacteria per bitch was recorded in older bitches.

**Discussion.** This study demonstrates that the stages of sexual cycle in bitches has a significant effect on bacteria growth and the vaginal flora has a mixed character. The sparse bacterial growth from bitches can be ascribed to their individual characteristics and the stage of the sexual cycle. It is well documented that bitches generally have a lower total count of vaginal bacteria during metoestrus and anoestrus than during prooestrus and oestrus (Allen and Dagnall, 1982; Baba et al., 1983). In our studies the lowest total count of vaginal bacteria was found in metoestrus (2.0%). The bacteria isolated from bitches usually include *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., *Proteus* spp. (Osbaldiston, 1972; Platt et al., 1974; Hirsh, 1977; Olson, 1978; Schaefer et al., 1978; Ling, 1978; Van Duijkeren, 1992; Laznicka, 1995). In our study the most common bacterial species isolated from 46 investigated bitches of different breeds were *Staphylococ-*

*cus* spp. (57.6%) and *Staphylococcus aureus* was isolated from 32.6% of samples. In addition, *Escherichia coli* (in 15.1% of samples), *Streptococcus* spp. (9.1%), *Staphylococcus* spp., *Pasteurella* spp. (15.1%), *Pseudomonas aeruginosa* (3.1%) were identified. These microorganisms could therefore be considered as a part of the opportunistic vaginal bacterial flora in investigated bitches. According to the reports of Allen and Dagnall (1982) and Baba et al., (1983) an increased number of microorganisms appear to be present during prooestrus and oestrus. This finding is in agreement with our data (in prooestrus 28.6%).

The different frequency of isolation of the most common bacteria strains in various breeds could be attributed to breed-related differences in ambient environmental conditions. A more likely explanation is that there are genetically determined differences between breeds in vaginal characteristics such as oxygen tension, pH, moisture content, and amount of debris (Larsen, 1985). Our study demonstrates the value of vaginal microflora in the different breed bitches. When positive cultures are obtained during prooestrus and oestrus they must be considered normal, but therefore it could have an influence on the future fertility.

#### Conclusion

1. On the basis of our findings, the most common bacteria isolated from the 46 vaginal cultures were *Staphylococcus* spp. (57.6%), *Escherichia coli* (15.1%), *Pasteurella* spp. (15.1%), *Streptococcus* spp. (9.1%) and *Pseudomonas aeruginosa* (3.1%).

2. An increased number of microorganisms appear to be presenting during prooestrus – 28.6%, oestrus 12.2% and anoestrus 24.5%. The lowest total count of vaginal bacteria was during metoestrus – 2.0%.

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