

DOI 10.24425/pjvs.2023.148293

*Original article*

# Vaginal aerobic bacteria of healthy bitches and those with fertility problems

D. Jagódka<sup>1</sup>, E. Kaczorek-Łukowska<sup>2</sup>, R. Graczyk<sup>3</sup>, P. Socha<sup>4</sup>

<sup>1</sup> AURA Veterinary Clinic, Dębowa 31, 86-065 Lochowo, Poland

<sup>2</sup> Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 13, 10-719, Olsztyn, Poland

<sup>3</sup> Department of Biology and Animal Environment, Bydgoszcz University of Science and Technology, Mazowiecka 28, 85-084 Bydgoszcz, Poland

<sup>4</sup> Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 14, 10-719 Olsztyn, Poland

## Abstract

The most common problems in veterinary practice in bitches are bacterial infections of the reproductive tract associated with fertility problems. Research to determine the correlation between the health status of female dogs and bacterial flora of the genital tract has been ongoing for years, but the results obtained by different authors are often contradictory, and do not always concern breeding bitches. Our study identified the most common aerobic bacteria in the genital tract of numerous breeding bitches population. A total of 275 breeding dogs in anestrus phase of the estrous cycle were included in this study. 198 were qualified to the first group with no genital tract infections and no reproductive disorders. 68 bitches were qualified to the second group with complications such as: infertility, abortion, foetus resorptions and newborn mortality. The type of bacterial isolates was almost the same in the healthy bitches and the group with fertility problems. The most common bacteria obtained from the vaginal tract of the tested dogs were *Streptococcus spp.*, *Staphylococcus spp.*, *Mycoplasma canis* and *Escherichia coli*. There were no significant differences in bacterial prevalence in the group with reproductive problems versus healthy dogs; however, we found a statistically significant difference between both groups when the numbers of bacterial strains were compared. The number of one-strain bitches was statistically higher in the problematic group than in the non-problematic one. Bacterial culturing of vaginal swab specimens from breeding bitches without clinical signs of genital disease is of little value. Furthermore, it should always be preceded by an examination (clinical, cytological or vaginoscopy etc.). The request or requirement to perform vaginal cultures that is made by some breeders, while common, is not diagnostic for any pathologic condition and the results of these cultures should never be used to determine if antibiotic therapy is indicated.

**Keywords:** aerobic bacteria, bitch, fertility problems, vagina

## Introduction

In veterinary practice of reproductive bitches, genitourinary tract infections and complications such as infertility, abortion, foetus resorptions and newborn mortality are common problems that might have a bacterial base (Smith 2006, Root Kustritz 2008, Pretzer 2008)

The canine vaginal lumen has natural contact with the external environment and is colonized by physiological microflora (Bjurström and Linde-Forsberg 1992, Noguchi et al. 2003, Concannon 2011). It is often reported that in vaginal smears of clinically healthy dogs the presence of bacteria is about 50% to 100% of cases depending on different factors e.g. age or the stage of oestrus cycle (Van Duijkeren 1992, Watts et al. 1996, Root Kustritz 2006, Zduńczyk et al. 2006, Janowski et al. 2008, Groppetti et al. 2012, Ruzauskas et al. 2016). This vaginal bacterial population is usually mixed, consisting of aerobic and anaerobic microorganisms, often opportunistic pathogens (Van Duijkeren 1992, Watts et al. 1996, Root Kustritz 2006, Groppetti et al. 2012).

The main composition of the physiological microflora consists of bacteria of the type  $\beta$ -hemolytic *Streptococcus spp.* (*S. canis*), *Staphylococcus spp.* (*S. intermedius*, *S. aureus*) and *E. coli*, *Enterococcus faecalis*, *Pasteurella multocida*, as well as *Proteus spp.*, *Bacillus spp.* and *Corynebacterium spp.* (Bjurström and Linde-Forsberg 1992, Zduńczyk et al. 2006, Janowski et al. 2008, Ruzauskas et al. 2016). The presence of *Klebsiella pneumoniae* and *Actinomyces spp.*, and *Neisseria spp.* is also observed (Delucchi et al. 2008, Janowski et al. 2008, Hutchins et al. 2014, Golińska et al. 2021). Some authors suggest that the appearance of *Lactobacillus spp.*, *Mycoplasma spp.* and *Ureaplasma spp.* is controversial (Janowski et al. 2008, Maksimowicz et al. 2018, Lyman et al. 2019, Golińska et al. 2021).

There is a discrepancy in the literature regarding the percentage of individual bacteria in all microflora population, but for example for  $\beta$ -hemolytic *Streptococcus*, the results of many authors are similar and the percentage of this bacteria's occurrence is about 20% (Hutchins et al. 2014, Maksimowicz et al. 2018). In microbiological tests, bacterial populations included between 1 and 4 strains, with an average of 2 strains (Bowen 1998, Janowski et al. 2008, Concannon 2011, Hutchins et al. 2014) isolated from bitches in 95% of cases. The study's results on the relationship between the species composition of the vaginal microflora and the phase of the oestrous cycle are still not clear. Bjurström (1993) showed that *Pasteurella multocida* and  $\beta$ -hemolytic streptococci were significantly more

frequently isolated in the proestrus phase relative to the other phases of the oestrus cycle. In the study of Groppetti et al. (2012) *E. coli* was found in all pregnant bitches that developed clinical symptoms of uterine disorders, resulting in uterine infection, resorption or abortion, but without a statistical significance. Vaginal presence of *Streptococcus spp.* in proestrus was instead negatively associated with the development of uterine infections. Therefore, according to Groppetti et al., *Streptococcus spp.* could have a protective competitive role against more dangerous pathogens affecting fertility of the bitch.

It is believed that genitourinary disorders are often associated with changes in vaginal microflora (Bjurström 1993, Delucchi et al. 2008, Pretzer 2008, Root Kustritz 2008). On the other hand, it has been shown that bacterial species isolated from bitches with reproductive disorders do not differ significantly from those found in healthy bitches (Van Duijkeren 1992, Zduńczyk et al. 2006) and it has been suggested that normal vaginal microbiota appear to protect the genitourinary tract against potentially pathogenic bacteria by competing for nutrients or interfering with adhesion to epithelial cell receptors (Golińska et al. 2021).

In our opinion, there is still limited information about the qualitative composition of the natural vaginal microflora of fertile breeding bitches compared with those are infertile or with reproductive problems. Moreover, even the results obtained by other authors on a pilot study using metataxonomic analysis are sometimes contradictory and therefore not conclusive (Lyman et al. 2019, Rota et al. 2020, Hu et al. 2022).

The aim of this study was to determine bacterial populations in the vagina of fertile non-problematic breeding dogs compared with the dogs with reproductive problems and eventually to find a correlation between the canine vaginal microbial environment and fertility outcomes (development of pregnancy, uterine infection, resorption, abortion and neonatal mortality).

## Materials and Methods

### Sample collection

Client-owned dogs were carried to the Clinic of the Department of Animal Reproduction, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland for routine gynecological examination of breeding bitches. The owners were informed about the purpose of the study and gave their written consent.

Two vaginal swabs for microbiology tests were collected from each of 275 anoestrus bitches of different breeds, aged from 1 year to 8 years. The study included

Table 1. Primer sequences and reaction conditions in PCR identification of *Mycoplasma canis*.

Molecular identification	Primer sequence (5'–3')	Amplicon size (bp)	Annealing temperature (°C)	Reference
<i>Mycoplasma canis</i>	F: CACCGCCCGTCACACCA R: CTGTCGGGGTTATCTCGAC	247	51	Chalker et al. 2004

bitches in good health only, showing no systemic or organ diseases (excluding the symptoms of vaginitis). Bitches undergoing any previous treatment were excluded. Qualifications for 2 clinical groups (healthy and with fertility disorders) were made based on the clinical examination, medical history and information obtained from owners.

Samples for microbiological examination and PCR test were taken from the vagina, using a sterile swab and a sterile vaginal specula for bitches, Hannover model (Eickemeyer, Tuttingen, Germany) with a size adjusted to the bitch. The microbiological samples were immediately delivered to the laboratory.

#### Identification of vaginal bacterial populations

For pre-incubation, the vaginal swabs were incubated into non-selective tryptic soy broth (Oxoid, Basingstoke, Great Britain) at 37°C for 24 h under aerobic conditions. Afterwards, samples were transferred with a calibrated inoculation loop onto the Columbia agar medium supplemented with 5% defibrinated sheep blood, MacConkey, Edwards, and Chapman medium. All agars were from Oxoid, Basingstoke, Great Britain. The bacteria were cultured at 37°C under aerobic conditions for 48 hours. The grown isolates were subjected to microbiological analysis, which included the evaluation of the morphology of the bacterial colonies, gram staining, selected biochemical tests (tests for catalase, coagulase and oxidase; API 20E and API 20NE tests (bioMérieux, Lyon, France)), CAMP reaction and selected latex tests (PathoDextra Strep grouping kit, Staphylect Plus (Oxoid, Basingstoke, Great Britain).

#### Identification of *Mycoplasma canis* by PCR reaction

All PCR reactions detecting *M. canis*. were performed with the use of the HotStarTaq Plus Master Mix Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations in a nexus gradient thermocycler (Eppendorf, Hamburg, Germany). The primer sequences, product size and annealing temperature are summarized in Table 1. All primers were synthesized by Genomed S.A. (Warsaw, Poland). All reactions were performed with positive and negative controls.

Once the reaction was completed, the PCR products were visualized as follows: 10 µl of PCR products were subjected to electrophoresis on a 2% agarose gel in the presence of Midori Green Advance (Nippon Genetics, Germany) at 120 V for 60 min. Results were read using the Quantum ST5 Gel Documentation System (Vilber, Collégien, France).

#### Statistical analyses

Statistical analyses were performed to demonstrate significant differences in the presence of each microbial species within the tested group. The frequency of microbial species was analyzed using Fisher's exact test.

The analysis of the results was performed using statistical inference methods (Stanisz 2006, McDonald 2009). In order to statistically characterize the studied community structure, statistical clustering was carried out using statistical series, determining the abundances of the absolute and relative percentages for the analyzed characteristics of the studied groups. The normality of the distribution was tested with the W Shapiro-Wilka test, while equality of variance in different samples was tested with a Levene test. Fixed factors were the type of bacterial strains. The assumption of normality or equality of variance was not met, so the non-parametric ANOVA rank Kruskal-Wallis test was used; and then in case of significant differences between averages multiple comparisons the test between average ranks was used. The level of significance for all statistical tests was accepted as  $\alpha = 0.05$ . The statistical calculations mentioned above were carried out with MS Excel 2019 software (Microsoft, Redmond, WA, USA, 2019) and Statistica 13.3 (Dell, Round Rock, TX, USA, 2022) software.

#### Results

A total of 275 breeding dogs were included in this study. The most common bacterial isolates obtained from the vaginal tract of all dogs were: *Streptococcus spp.* (107/275), *Staphylococcus spp.* (106/275), *Mycoplasma canis* (96/275) and *Escherichia coli* (88/275) (Table 2). The other, less important strains were: *Bacillus spp.*, *Proteus spp.*, *Pseudomonas spp.*, *Klebsiella spp.* and *Citrobacter spp.* (Fig. 1).

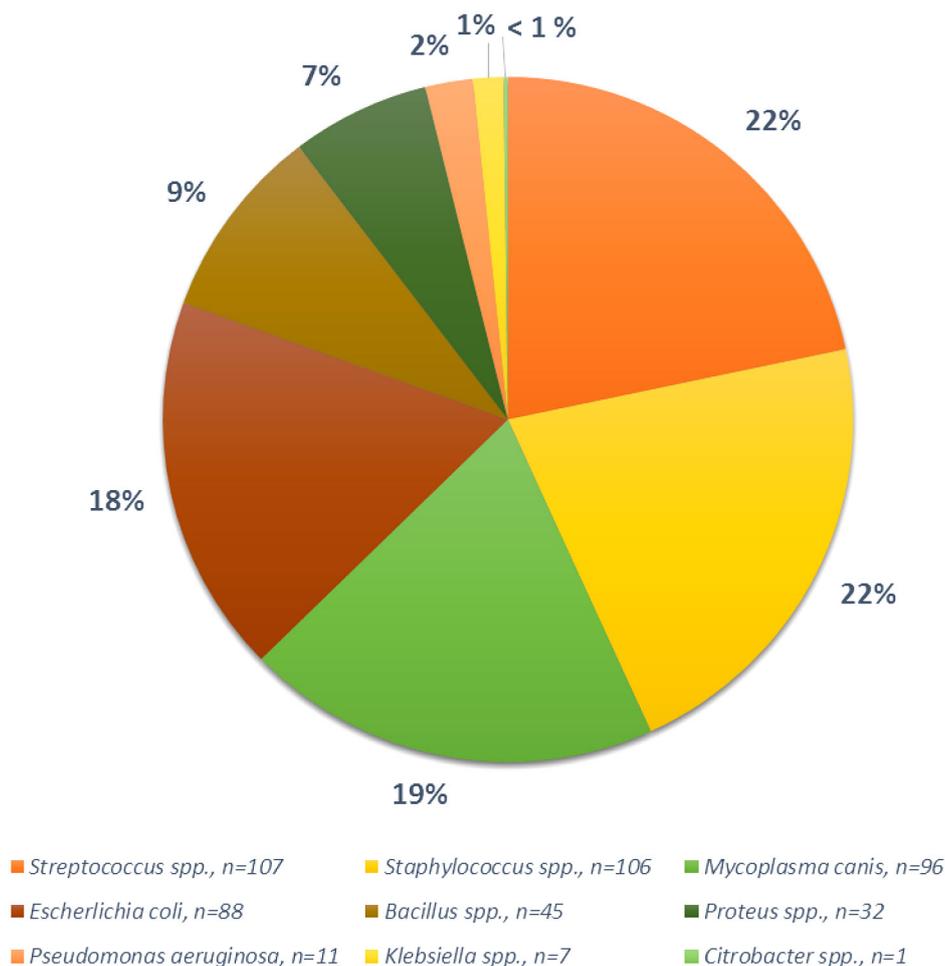


Fig. 1. Vaginal aerobic bacterial flora of breeding bitches population (n=275).

Table 2. Comparison of the number of bacterial strains (1, 2,  $\geq 3$ ) in the healthy and those with fertility disorders breeding bitches.

Number of strains	healthy bitches n=198		fertility disorders bitches n=68		ANOVA rank Kruskal-Wallis
	n	%	n	%	
1	79 <sup>b</sup>	16.49 <sup>b</sup>	24 <sup>c</sup>	5.01 <sup>c</sup>	H <sub>K-W</sub> = 3.01 p=0.008
2	92 <sup>a</sup>	38.41 <sup>a</sup>	25 <sup>d</sup>	10.44 <sup>d</sup>	
$\geq 3$	27 <sup>c</sup>	17.12 <sup>c</sup>	19 <sup>f</sup>	12.53 <sup>f</sup>	
ANOVA rank Kruskal-Wallis	H <sub>K-W</sub> = 265.01 p<0.001		H <sub>K-W</sub> = 197.02 p<0.001		

<sup>a-f</sup> – different letters indicate significant differences between groups at p $\leq$ 0.05

In the next step 198 bitches were qualified to the group without reproductive problems and another 68 qualified to the group with general problems, which were: infertility, abortion, foetus resorptions and newborn mortality, reported by owners. (Fig. 2).

*Streptococcus spp.* was isolated from 76 out of 198 (38.38%) dogs from the first group and from 28 of 68 (41.18%) from the second group, respectively. *Staphylococcus spp.* was isolated from 36.36% dogs in the first group and of 42.65% dogs from second group, respectively. *M. canis* was identified in 66 of 198 (33.33%) bitches from non-problematic bitches

and in 28 of 68 (41.18%) from the problematic group. *E. coli* was isolated from the vaginal tract of 30.81% dogs from the first group and from 35.29% dogs from the second group.

Generally, we found no difference in the prevalence of any bacterial strains (p>0.05) in the group of bitches with reproductive problems (68) versus healthy dogs (198) (Fig. 2).

The number of bacterial species isolated per single sampling from two different groups of bitches is presented in Tables 2 and 3. We found that 21.5 % of bitches had only one bacterial strain, other smears

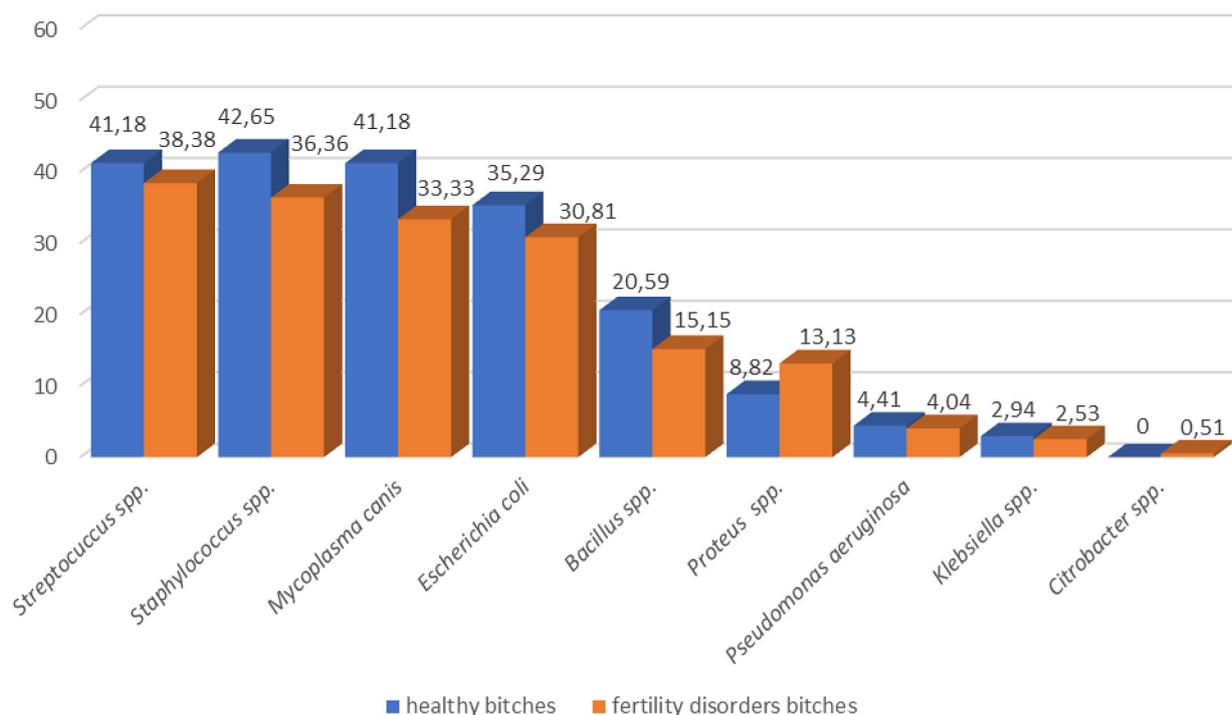


Fig. 2. Vaginal bacterial flora of the healthy and with fertility disorders breeding bitches.

Table 3. Difference matrix of the percentage of tested bitches with a different number of types of bacterial strain.

	Healthy bitches / Number of strains			
	1	2	3<	
Fertility disorders bitches / Number of strains	1	11.48% <sup>a</sup>	33.40% <sup>a</sup>	12.11% <sup>a</sup>
	2	6.05% <sup>b</sup>	27.97% <sup>b</sup>	6.68% <sup>b</sup>
	≥3	3.97% <sup>c</sup>	25.89% <sup>c</sup>	4.59% <sup>c</sup>
ANOVA rank Kruskal-Wallis	H <sub>K-W</sub> = 3.70 p<0.001			

<sup>a, b, c</sup> – different letters indicate significant differences between groups at p≤0.05

were characterized by two or more. A statistically significant difference was confirmed only when non-problematic and problematic groups of bitches with 1 strain were compared. The number of one-strain bitches was statistically higher in the problematic group than in the non-problematic one (Table 2 and 3). The most common bacterial species isolates in the one-strain group were: *Staphylococcus spp.* (16.18%), *E. coli* (11.76%), *Streptococcus spp.* (4.41%).

## Discussion

There are numerous bacteria that normally reside in the vaginal canal in the bitch. These bacteria are normal inhabitants, and their presence moderates a healthy mucosal environment. It is assumed that there is only one real venereal transmitted bacterial disease in the dog: brucellosis. All of the other bacteria already live in the normal reproductive tract of all dogs and normal

immune defence mechanisms will quickly clear any contamination that occurs during breeding for the bitch (Pretzer 2008). In fact, it is suggested by some authors that a negative culture would be a reason to suspect inadequate sampling (Golińska et al. 2021) and not actually a true negative culture except during anoestrus when vaginal bacterial numbers are usually very low (Zduńczyk et al. 2006, Rota et al. 2020). We confirm this opinion based on our own study that at least 1 bacterial strain was diagnosed in every vaginal swab from 275 bitches.

This study was aimed at determining bacterial populations in the vagina of breeding healthy dogs compared with those characterized by some selected reproduction problems, important from the clinicians' and breeders' point of view. Our results suggest that there is no variety in quantity and type of bacteria between healthy and breeding bitches with fertility disorders. The prevalence of common pathogens, such as *Streptococcus spp.*, *Staphylococcus spp.*, *M. canis* and *E. coli*,

was similar between the compared groups of dogs. Finally, a real novelty was the confirmation, with the methods of molecular biology, that *M. canis* is a quite common pathogen in the genital tract of breeding bitches (around 40%). Moreover, there was no relationship between the presence of *M. canis* and any specific symptoms of infections or other reproductive disorders. Our results are somewhat consistent with most of the conclusions of earlier research on *Mycoplasma spp.* (Janowski et al. 2008, Maksimowicz et al. 2018, Lyman et al. 2019), but the pathogenicity of this bacterium remains controversial.

Some results obtained in this study are in accordance with results presented by Hutchins et al. (2014) and Golińska et al. (2021). They explained that bacteria are normal inhabitants of the lower reproductive tract, and it is very difficult to determine, simply based on their presence, if they are potentially pathogenic or not. However, it is possible that a significant difference in the prevalence of some specific vaginal flora in correlation with other bacterial strains or in condition of immune system disorders would be identified as a source of serious reproductive complication such as infertility. It is indisputable that when specific bacteria invade the higher reproductive tract i.e. the uterus and the animal's immune system does not function properly and remove them in a timely manner, they can proliferate and cause disease i.e. endometritis or pyometra, abortion, foetus resorptions and newborn mortality (Smith 2006, Pretzer 2008). If the body's innate defence systems work properly, these bacteria are quickly cleared and do not cause diseases. In different authors results, bitches with vaginitis or other disorders of the reproductive tracts and those which were fertile hosted the same bacterial strains (Bjurström 1993, Zduńczyk et al. 2006, Root Kustritz 2008). However Golińska et al. (2021) suggested that *Streptococcus canis* is much more common in proestrus phase in healthy dogs, in contrast to *Enterococcus spp.* whose presence was more often noticed in dogs with some genital tract infections. Therefore, they suspected that *Staphylococcus spp.* and *Streptococcus spp.* can act as a protection against more dangerous pathogens, by competing for nutrients or interfering with adhesion to epithelial cell receptors. Hu J et al. (2022) also confirm that vaginal flora participate in vaginal self-cleaning functions, maintain vaginal microecological balance, and resist pathogens by releasing metabolites and competing with the natural microbiome. We strongly agree that this unknown aspect of the relationship between specific species of bacteria should be analysed in further research.

In our opinion, in line with Bjurström (1993) and Janowski et al. (2008), it is possible that bacterial

species isolated from a bitch having vaginitis or other reproductive disorders should be present in pure culture or maximum 2-species mixed culture to be regarded as of significantly pathogenic. In a few papers it was previously noticed that samples from bitches with vaginitis tended to have fewer species but a higher number of bacteria than samples from healthy ones (Zduńczyk et al. 2006, Root Kustritz 2008). We think that for reproductive bitches, where samples resulted in more than 2 bacterial species, other reasons than bacterial infection causing the infertility should be considered, such as *Canine Herpes Virus* infections, anatomic abnormalities, neoplasia and genetic disorders (Golińska et al. 2021) and there is a necessity for specific control of such bitches.

According to the results of Bjurström and Lindforsberg (1992), Delucchi et al. (2008), Gropetti et al. (2012) and Lyman et al. (2019) and our present investigation, bacterial sampling from the vagina of infertile bitches without clinical signs of genital infection is of low diagnostic value. The appropriate interpretation of microbiota growth of the vaginal canal is very difficult in terms of determining the need for possible treatment. We also suspect that antibiotic therapy in an animal without inflammatory disease will reduce or kill the population of bacteria which are protective of the mucosal environment of the lower tract. In consequence, it may predispose growth of real pathologic bacteria higher up in the tract and thereby increase the risk of developing significant reproductive disease in the future and other consequences in mating period (Dzięcioł et al., 2013).

On the other hand, it is suggested that in dogs where bacteriological reproductive tract infections and selected infertility problems are caused by an overgrowth of normal local microbial flora, it may be important for the outcomes of bacteriologic tests to be quantitative (Lyman et al. 2019, Rota et al. 2019, Golińska et al. 2021, Hu et al. 2022).

In conclusion, cultures of the canine vagina will almost always result in growth of bacteria and interpretation of this growth is very difficult in terms of determining the need for treatment. In fact, bacterial culturing of vaginal swab specimens from bitches without signs of genital disease is of little value. It should always be preceded by clinical examination of the reproductive tract and cytological examination of the vaginal epithelium. Our own results pay particular attention to the important aspect of collecting knowledge in the field of vaginal bacteria cultures as the starting point to effectively prevent serious reproductive disorders in specific group of breeding dogs.

## References

- Bjurström L (1993) Aerobic bacteria occurring in the vagina of bitches with reproductive disorders. *Acta Vet Scand* 34: 29-34.
- Bjurström L, Linde-Forsberg C (1992) Long-term study of aerobic bacteria of the genital tract in breeding bitches. *Am J Vet Res* 53: 665-669.
- Chalker VJ, Owen WM, Paterson C, Barker E, Brooks H, Rycroft AN, Brownlie J (2004) Mycoplasmas associated with canine infectious respiratory disease. *Microbiology* 150: 3491-3497.
- Concannon PW (2011) Reproductive cycles of the domestic bitch. *Anim Reprod Sci* 124: 200-210.
- Delucchi L, Fraga M, Perelmuter K, Cidade E, Zunino P (2008) Vaginal lactic acid bacteria in healthy and ill bitches and evaluation of in vitro probiotic activity of selected isolates. *Can Vet J* 49: 991-994.
- Dzięcioł M, Niżański W, Stańczyk E, Kozdrowski R, Najder-Kozdrowska L, Twardoń J (2013) The influence of antibiotic treatment of bitches in oestrus on their attractiveness to males during mating. *Pol J Vet Sci* 16: 509-516.
- Golińska E, Sowińska N, Tomusiak-Plebanek A, Szydło M, Witka N, Lenarczyk J, Strus M (2021) The vaginal microflora changes in various stages of the estrous cycle of healthy female dogs and the ones with genital tract infections. *BMC Vet Res* 17: 8
- Groppetti D, Pecile A, Barbero C, Martino PA (2012) Vaginal bacterial flora and cytology in proestrous bitches: role on fertility. *Theriogenology* 77: 1549-1556.
- Hu J, Cui L, Wang X, Gao X, Qiu S, Qi H, Jiang S, Li F, Yin Y (2022) Dynamics of vaginal microbiome in female beagles at different ages. *Res Vet Sci* 149: 128-135.
- Hutchins RG, Vaden SL, Jacob ME, Harris TL, Bowles KD, Wood MW, Bailey CS (2014) Vaginal microbiota of spayed dogs with or without recurrent urinary tract infections. *J Vet Intern Med* 28: 300-304.
- Janowski T, Zduńczyk S, Borkowska I, Jurczak A, Podhalec-Dzięgielewska M (2008) Vaginal and uterine bacterial flora at different stages of the estrus cycle in bitches. *Med Weter* 64: 444-446
- Janowski T, Zduńczyk S, Jurczak A, Socha P (2008) Incidence of mycoplasma canis in the vagina in three groups of bitches. *Bull Vet Inst Pulawy* 52: 533-535.
- Lyman CC, Holyoak GR, Meinkoth K, Wieneke X, Chillemi KA, DeSilva U (2019) Canine endometrial and vaginal microbiomes reveal distinct and complex ecosystems. *PLoS One* 14: e0210157
- Maksimovic A, Maksimovic Z, Filipovic S, Besirovic H, Rifatbegovic M (2012) Vaginal and uterine bacteria of healthy bitches during different stages of their reproductive cycle. *Vet Rec* 171: 375.
- Maksimović Z, Maksimović A, Halilbašić A, Rifatbegović M (2018) Genital mycoplasmas of healthy bitches. *J Vet Diagn Invest* 30: 651-653.
- McDonald JH (2009) Handbook of biological statistics, 2<sup>nd</sup> ed., Sparky House Publishing: Baltimore, MD, USA, pp 1-319.
- Noguchi K, Tsukumi K, Urano T (2003) Qualitative and quantitative differences in normal vaginal flora of conventionally reared mice, rats, hamsters, rabbits and dogs. *Comp Med* 53: 404-412.
- Pretzer SD (2008) Bacterial and protozoal causes of pregnancy loss in the bitch and queen. *Theriogenology* 70: 320-326.
- Root Kustritz MV (2006) Collection of tissue and culture samples from the canine reproductive tract. *Theriogenology* 66: 567-574.
- Root Kustritz MV (2008) Vaginitis in dogs: a simple approach to a complex condition. *Vet Med* 103: 562-567.
- Rota A, Corrà M, Patuzzi I, Milani C, Masia S, Mastroilli E, Petrin S, Longo A, Del Caro A, Losasso C (2020) Effect of sterilization on the canine vaginal microbiota: a pilot study. *BMC Vet Res* 16: 455
- Ruzauskas M, Couto N, Pavilionis A, Klimiene I, Siugzdiniene R, Virgailis M, Vaskeviciute L, Anskiene L, Pomba C (2016) Characterization of Staphylococcus pseudintermedius isolated from diseased dogs in Lithuania. *Pol J Vet Sci* 19: 7-14.
- Smith FO (2006) Canine pyometra. *Theriogenology* 66: 610-612.
- Stanisz A (2006) Easy course of statistic using Statistica PL and medicine examples. 1. Basic Statistic. StatSoft Polska, Kraków, Poland, p 532.
- Van Duijkeren E (1992) Significance of the vaginal bacterial flora in the bitch: a review. *Vet Rec* 131: 367-369.
- Watts JR, Wright PJ, Whithear KC (1996) Uterine, cervical and vaginal microflora of the normal bitch throughout the reproductive cycle. *J Small Anim Pract* 37: 54-60.
- Zduńczyk S, Janowski T, Borkowska I (2006) Vaginal and uterine bacterial flora in bitches with physiological and inflammatory. *Med Weter* 62: 1116-1119.