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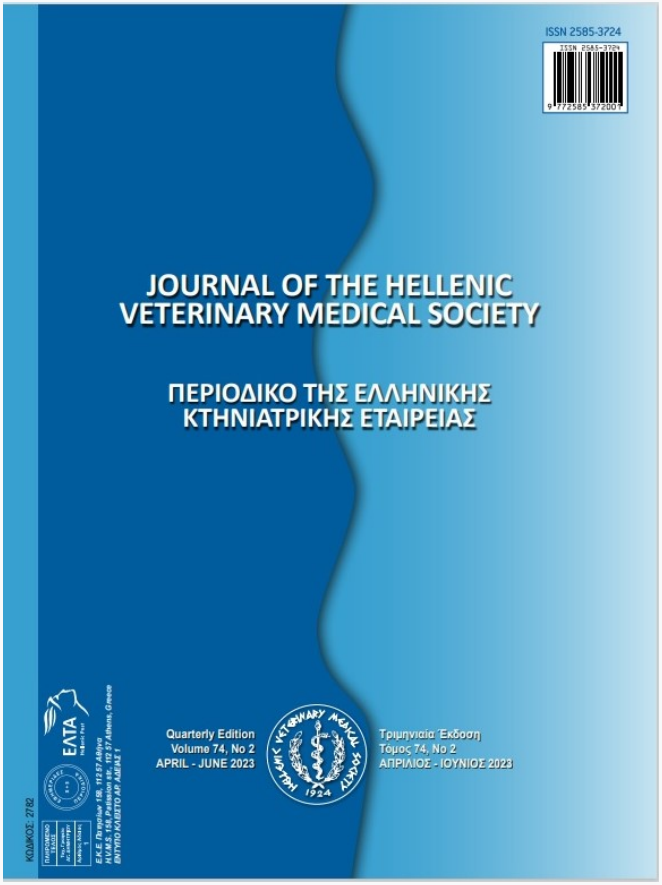


# Vaginal bacteria of healthy cats during different stages of their oestrous cycle

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Short communication
Σύντομη ανακοίνωση

# Vaginal bacteria of healthy cats during different stages of their oestrous cycle

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**ABSTRACT:** Little is known about vaginal and uterine bacteria in clinically healthy cats and their correlation with different stages of the oestrus cycle. The differences in vaginal bacterial flora between household and stray queens remains unknown. The aim of this study was to investigate the occurrence of vaginal and uterine bacteria in clinically healthy household and stray queens and to correlate culture findings with specific stages of the oestrous cycle. Vaginal and uterine samples from 40 clinically healthy queens were collected for isolation of bacteria and cytological examination. Bacteria were isolated from 31 vaginal swabs (77.5%) from stray (16/20; 80%) and household (15/20; 75%) cats. The isolates were more frequently detected in pure culture (18/31; 58%) than in mixed cultures (13/31; 41.9%). *Streptococcus* spp. was the most commonly identified bacteria (*n* = 16; 51.6%), followed by coagulase negative *Staphylococcus* spp. (n = 15; 48.4%) and *E. coli* (n = 12; 38.7%). A mixed bacterial culture of *E. coli* and *Streptococcus* spp. was commonly detected (50%), mainly in households (66.7%), whereas a mixed culture of *Staphylococcus* spp. and *Streptococcus* spp. (41.6%) was commonly isolated from stray cats (60%). The frequency of isolation of pure or mixed bacterial cultures and the isolates did not vary significantly during the different stages of the oestrus cycle. All uterine samples tested were negative for bacteria. This study identified the most common bacteria in the vagina of clinically healthy cats. The isolation of vaginal bacteria in pure or mixed cultures should be considered as normal finding. The stage of the oestrous cycle apparently does not affect vaginal bacterial flora. Vaginal bacteria may differ between stray and households cats. No bacteria can be isolated from the uterus of clinically healthy cats.

***Keywords:*** bacteria; household and stray queens; oestrous cycle; uterus; vagina

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## INTRODUCTION

A wide variety of bacteria have been detected in the vagina of healthy cats, among which staphylococci, streptococci and *Escherichia coli* are the most commonly isolated (Clemetson and Ward, 1990; Holst et al., 2003; Hariharan et al., 2011). These bacteria have also been associated with reproductive disorders of queens, such as pyometra (Graham and Taylor, 2012; Hagman, 2018). The next-generation sequencing of the reproductive tract (vulva and prepuce) of healthy cats revealed *Proteobacteria* as the most common phylum, followed by *Bacteroidetes*, *Firmicutes*, *Actinobacteria* and *Fusobacteria*. The most common family in the samples of vulva was *Enterobacteriaceae* (Older et al., 2017). The vaginal bacteria can be isolated in pure or mixed cultures (Clemetson and Ward, 1990; Holst et al., 2003; Hariharan et al., 2011). Some previous studies suggested that isolation in pure or 2-isolate mixed culture may contribute to more accurate diagnosis of canine reproductive diseases (Hirsh and Wiger, 1977; Bjurström, 1993). However, a more recent study (Maksimović et al., 2012) showed that isolation of pure bacterial culture does not necessarily indicate the presence of infection, and can be influenced by the stages of the oestrus cycle. Likewise, pure growth of bacteria in culture of vagina of clinically healthy cats has been indicated to be normal finding (Howard et al., 1993; Holst et al., 2003). The isolation of bacteria from uterus of healthy cats is uncommon and the presence of low numbers of organisms most likely represent normal flora. Bacteria isolated from the uterus of healthy queens include: *Acinetobacter* sp., *Bacillus* sp., *E. coli*, *Lactobacillus* sp. and *Streptococcus* sp. (Clemetson and Ward, 1990; Schultheiss et al., 1999). Although the differences among vaginal or uterine bacterial flora during different stages of the oestrus cycle of queens have been indicated, the results of a few conducted studies remain inconclusive (Clemetson and Ward, 1990; Schultheiss et al., 1999; Holst et al., 2003). Moreover, there has been no research reported with respect to the differences in vaginal bacterial flora between household and stray queens, to our knowledge. The aim of this study was to investigate the occurrence of vaginal and uterine bacteria in clinically healthy household and stray queens and to correlate culture findings with specific stages of the oestrus cycle.

## MATERIALS AND METHODS

The household cats' estimated age ranged from seven months to 3.5 years and the average age was 15 ± SD 9 months. The age of stray cats was unknown. All cats were mixed breed, except two household, which were Persian breed. Written informed consent was obtained from owners and caretakers of the cats described in this work. Vaginal and uterine swabs from household ($n$ = 20) and stray ($n$ = 20) clinically healthy cats were collected for isolation of bacteria and cytological examination. All samples were obtained under the general anaesthesia required for surgery (Holst et al., 2003). Sampling from the vagina and the determination of the stage of the oestrus cycle were conducted as described previously (Mills et al., 1979; Howard et al., 1993; Holst et al., 2003; Ververidis and Boscos, 2013). The air-dried and methanol fixed vaginal smears were stained by Diff-Quik staining for on-site cytological examination. To prevent cross-contamination fresh staining solution was used for each smear. Epithelial cells were classified as parabasal, intermediate and superficial (nucleate and anucleate), and the percentage of the observed epithelial cell populations (i.e., maturation index) was used for the determination of the stages of the oestrus cycle as described previously (Mills et al., 1979; Olson et al., 1984; Ververidis and Boscos, 2013). A swab of the mucosa from the uterine horns was aseptically taken for bacteriology immediately following ovariohysterectomy (Maksimović et al., 2012). Shortly after collection, the samples were inoculated onto blood agar, MacConkey's and bromocresol purple lactose agar, followed by aerobically incubation for 24–48 hours at 37°C. To improve detection efficiency, uterine swabs were also inoculated into nutrient and trypticase soy broths and incubated in the same manner. Identification of the isolates was performed by cultural, microscopic and biochemical examination (Markey et al., 2013). The biochemical tests included oxidase, catalase, urease, indole, citrate, coagulase, L-pyrrolidonyl-β-naphthylamide (PYR), esculin, nitrate, triple sugar iron agar (TSI), oxidative-fermentative (OF), bacitracin susceptibility (0.04 units), CAMP and the Analytical Profile Index System (api® 20 Strep, bioMérieux). Statistical evaluations were made using a chi-squared test and Fisher's exact test; $p \leq 0.05$ was considered statistically significant.

## RESULTS

The most of household cats were in oestrus ($n$ = 15/20; 75%), followed by interestrus (n = 4/20; 20%), and dioestrus ($n$ = 1/20; 5%), compared with stray cats ($n$ = 9/20, 45%; $n$ = 7/20, 35%; $n$ = 4/20, 20%, respectively) (Supplementary table 1, Table 2). Bacteria were isolated from 31 vaginal swabs (77.5%) from stray (16/20; 80%) and household (15/20; 75%) cats.

The isolates were frequently detected in pure culture (18/31; 58%). *Streptococcus* spp. was the most commonly identified bacteria ($n$ = 16; 51.6%) (Supplementary table 1, Table 1), followed by coagulase negative *Staphylococcus* spp. ($n$ = 15; 48.4%) and *E. coli* ($n$ = 12; 38.7%). Pure growth of *Staphylococcus* spp. was frequently detected (44.4%). A mixed bacterial culture of *E. coli* and *Streptococcus* spp. was commonly detected (50%), mainly in households (66.7%), whereas a mixed culture of *Staphylococcus* spp. and *Streptococcus* spp. (41.6%) was commonly isolated from stray cats (60%). A significant difference was found between household cats and stray cats in the numbers of vaginal samples from which *S. canis* was isolated (P=0.018) (Table 1). The isolates did not vary significantly during the different stages of the oestrus cycle. The frequency of isolation of pure or mixed bacterial cultures during specific stages of the oestrus cycle was not observed (Supplementary table 1, Table 2). All uterine samples tested ($n$ = 40) were negative for bacteria.

**Table 1:** Bacteria isolated from the vaginas of 31 household and stray clinically healthy queens

| Isolates | Household ($n$ = 20) | Stray ($n$ = 20) | % of total number of samples | % of positive samples | % of the isolates | P |
|---|---|---|---|---|---|---|
| *Staphylococcus* spp. (coagulase negative) | 6 | 9 | 37.5 | 48.4 | 34.1 | 0.535 |
| *Streptococcus canis* | 5 | 0 | 12.5 | 16.1 | 11.4 | 0.018 |
| *Streptococcus* spp. (β haemolytic) | 1 | 3 | 10 | 12.9 | 9.1 | 0.608 |
| *Streptococcus dysgalactiae* subsp. *equisimilis* | 0 | 3 | 7.5 | 9.67 | 6.8 | 0.234 |
| *Escherichia coli* (β haemolytic) | 5 | 5 | 25 | 32.2 | 22.7 | 1.000 |
| *Streptococcus* spp. (nonhemolytic) | 2 | 0 | 5 | 6.4 | 4.5 | 0.222 |
| *Streptococcus* spp. (α haemolytic) | 1 | 1 | 5 | 6.4 | 4.5 | 1.000 |
| *Escherichia coli* (nonhemolytic) | 1 | 1 | 5 | 6.4 | 4.5 | 1.000 |
| *Klebsiella* spp. | 0 | 1 | 2.5 | 3.2 | 2.3 | 1.000 |

[n] number of animals in specified group

**Table 2:** Bacteria isolated from the vaginas of clinically healthy queens during different stages of the oestrus cycle

| Species | Oestrus ($n$ = 24) | Interestrus ($n$ = 11) | Dioestrus ($n$ = 5) | Total | P |
|---|---|---|---|---|---|
| *Staphylococcus* spp. (coagulase negative) | 9 | 4 | 2 | 15 | 0.990 |
| *Streptococcus canis* | 2 | 2 | 1 | 5 | 0.617 |
| *Streptococcus* spp. (β haemolytic) | 2 | 2 | 0 | 4 | 0.484 |
| *Streptococcus dysgalactiae* subsp. *equisimilis* | 2 | 1 | 0 | 3 | 0.790 |
| *Escherichia coli* (β haemolytic) | 5 | 4 | 1 | 10 | 0.593 |
| *Escherichia coli* (nonhemolytic) | 2 | 0 | 0 | 2 | 0.496 |
| *Streptococcus* spp. (nonhemolytic) | 2 | 0 | 0 | 2 | 0.496 |
| *Streptococcus* spp. (α haemolytic) | 1 | 0 | 1 | 2 | 0.225 |
| *Klebsiella* spp. | 1 | 0 | 0 | 1 | 0.710 |
| Pure bacterial culture | 10 | 5 | 3 | 18 | 0.755 |
| Mixed bacterial culture | 8 | 4 | 1 | 13 | 0.803 |
| Total (%) | 18 (58) | 9 (29) | 4 (13) | 31 (77.5) | - |

[n] number of animals in the indicated stage of the oestrus cycle

**Supplementary table 1:** Vaginal bacteria and the stage of the oestrus cycle in individual queens

| Sample ID | Bacteria isolated | The stage of the oestrus cycle |
|---|---|---|
| H1 | *Staphylococcus* spp. (coagulase negative), *Streptococcus* spp. (nonhemolytic) | Oestrus |
| H2 | *Staphylococcus* spp. (coagulase negative), *Streptococcus* spp. (α haemolytic) | Oestrus |
| H3 | *Escherichia coli* (nonhemolytic), *Streptococcus* spp. (β haemolytic) | Oestrus |
| H4 | negative | Oestrus |
| H5 | negative | Oestrus |
| H6 | negative | Oestrus |
| H7 | negative | Oestrus |
| H8 | *Staphylococcus* spp. (coagulase negative) | Oestrus |
| H9 | *E. coli* (β haemolytic), *Streptococcus canis* | Oestrus |
| H10 | *E. coli* (β haemolytic), *S. canis* | Interestrus |
| H11 | *S. canis* | Interestrus |
| H12 | *S. canis* | Dioestrus |
| H13 | *Staphylococcus* spp. (coagulase negative) | Oestrus |
| H14 | negative | Interestrus |
| H15 | *S. canis* | Oestrus |
| H16 | *Staphylococcus* spp. (coagulase negative) | Oestrus |
| H17 | *Staphylococcus* spp. (coagulase negative) | Oestrus |
| H18 | *E. coli* (β haemolytic), *Streptococcus* spp. (nonhemolytic) | Oestrus |
| H19 | *E. coli* (β haemolytic) | Interestrus |
| H20 | *E. coli* (β haemolytic) | Oesturs |
| S1 | *Staphylococcus* spp. (coagulase negative), *Streptococcus* spp. (β haemolytic) | Interestrus |
| S2 | *Staphylococcus* spp. (coagulase negative) | Interestrus |
| S3 | *Staphylococcus* spp. (coagulase negative), *Streptococcus* spp. (α haemolytic) | Dioestrus |
| S4 | negative | Oestrus |
| S5 | *Staphylococcus* spp. (coagulase negative), *Streptococcus* spp. (β haemolytic) | Interestrus |
| S6 | *Staphylococcus* spp. (coagulase negative) | Oestrus |
| S7 | *Streptococcus dysgalactiae* subsp. *equisimilis* | Oestrus |
| S8 | negative | Interestrus |
| S9 | *Streptococcus* spp. (β haemolytic) | Oestrus |
| S10 | *Staphylococcus* spp. (coagulase negative) | Dioestrus |
| S11 | *Staphylococcus* spp. (coagulase negative) | Interestrus |
| S12 | *E. coli* (β haemolytic) | Dioestrus |
| S13 | *E. coli* (β haemolytic), *S. dysgalactiae* subsp. *equisimilis* | Oestrus |
| S14 | *E. coli* (nonhemolytic) | Oestrus |
| S15 | *E. coli* (β haemolytic), *Staphylococcus* spp. (coagulase negative) | Oestrus |
| S16 | *Klebsiella* spp., *Staphylococcus* spp. (coagulase negative) | Oestrus |
| S17 | *E. coli* (β haemolytic), *S. dysgalactiae* subsp. *equisimilis* | Interestrus |
| S18 | negative | Oestrus |
| S19 | negative | Dioestrus |
| S20 | *E. coli* (β haemolytic) | Interestrus |

[H]Household queens; [S]Stray queens

## DISCUSSION

The frequency of the isolation of bacteria from the vaginal samples was similar to those of previous studies (Clemetson and Ward, 1990; Howard et al., 1993; Holst et al., 2003; Hariharan et al., 2011). *Streptococcus* spp., *Staphylococcus* spp. and *E. coli* were the most commonly detected bacteria, which correlates with the findings of Clemetson and Ward (1990). In several other studies, *E. coli* was found to be the most prevalent isolate (Howard et al., 1993; Holst et al., 2003; Hariharan et al., 2011). This bacterium is also the predominant pathogen involved in pyometra of queens (Hollinshead and Krekeler, 2016; Hagman, 2018). In this study, most of *E. coli* isolates were hae-

molytic (83%). However, the finding of haemolytic or nonhaemolytic *E. coli* in the vagina of clinically healthy cats is not considered to be an indication of reproductive disorder (Howard et al., 1993; Holst et al., 2003). The bacterial isolates did not vary significantly during the various stages of the oestrus cycle. Opposite to our results, Holst et al. (2003) isolated a significantly higher proportion of members of the family *Pasteurellaceae* from cats in oestrus than from cats not in oestrus, and *S. canis* was detected more often in cats in oestrus. *Staphylococcus* spp. was only isolated from cats not in oestrus (Holst et al., 2003). In a study by Clemetson and Ward (1990), *Staphylococcus* spp. and *E. coli* were frequently recovered from cats in interestrous. In our study, the isolates were more frequently detected in pure culture than in mixed cultures. Similarly, Holst et al. (2003) detected pure culture in 27 of 66 (41%) of vaginal samples, while mixed cultures were less frequently isolated (32%). In a study by Hariharan et al. (2011), 75% (35/37) of the positive samples had pure growth of single bacteria. Clemetson and Ward (1990) reported a high isolation rate of pure culture from adult cats (12/13; 92%). In contrast, mixed bacterial cultures were commonly found in kittens (7/10; 70%). In the present study, no correlation between the frequency of isolation of pure or mixed cultures and the age of household cats was observed. Moreover, the frequency of isolation of pure or mixed cultures was not related to specific stages of the oestrus cycle. However, the differences in the isolation of vaginal bacteria in mixed cultures between household and stray queens were observed. A mixed bacterial culture of *E. coli* and *Streptococcus* spp. was frequently found in households, whereas a mixed bacterial culture of *Staphylococcus* spp. and *Streptococcus* spp. was commonly isolated from stray cats. *E. coli* was equally detected in both groups, and *S. canis* was isolated only from the vagina of households cats. Opposite to our findings, in a study by Hariharan et al. (2011), the most common species isolated from vagina of feral cats was *E. coli*, followed by streptococci.

During our study, no bacteria were isolated from the uterus of clinically healthy queens, which is in agreement with a study by Holst et al. (2003). The explanation may lie in the patency of cervix (Holst et al., 2003), which might prevent the entrance of bacteria in the uterus. In previous studies, the bacteria were isolated in low numbers from 2 of 29 (7%) (Clemetson and Ward, 1990) and from 4 of 37 (11%) uterine samples (Schultheiss et al., 1999). *Acinetobacter* sp., *E. coli*, *Lactobacillus* sp. and *Streptococcus* sp. were isolated from the queens in oestrus, while *Bacillus* sp. was recovered during anoestrus and pregnancy (Clemetson and Ward, 1990; Schultheiss et al., 1999). Pyometra commonly occurs during dioestrus (Hollinshead and Krekeler, 2016). Schultheiss et al. (1999) frequently recovered bacteria from the uterus of clinically healthy bitches in dioestrus. By contrast, the same study (Schultheiss et al., 1999), as well as other studies (Clemetson and Ward, 1990; Holst et al., 2003), did not report any bacteria in the uterus of clinically healthy queens in this stage of the oestrus cycle. Maksimović et al. (2012) recovered bacteria by standard bacteriological procedures from only one (2.5%) of 40 uteri of clinically healthy bitches. However, the frequency of isolation of bacteria was significantly enhanced (25/40; 62.5%) by the use of nutrient or trypticase soy broths. During our study, all uterine samples were negative for bacteria, regardless of the method used for the isolation. In contrast to the previous findings in bitches (Schultheiss et al., 1999; Maksimović et al., 2012), the results of the present study suggest that bacteria can not be isolated from the uterus of clinically healthy cats, regardless of stage of the oestrus cycle and the method used for the isolation.

## CONCLUSIONS

Despite the limitations imposed by relatively small numbers of samples, the results of the present study should contribute to a better understanding of vaginal and uterine bacteriological status of clinically healthy cats. The isolation of vaginal bacteria in pure or mixed cultures should be considered as normal finding, whereas the stage of the oestrous cycle apparently does not affect vaginal bacterial flora. Vaginal bacteria may differ between stray and households cats, which should be further investigated. In contrast to the previous findings in bitches, no bacteria can be isolated from the uterus of clinically healthy cats, regardless of stage of the oestrus cycle and the method used for the isolation.

## CONFLICT OF INTEREST

None declared by the authors.

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