

# Bacteriological examination of the bitches with pyometra

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### Summary

Uterine contents of 40 bitches with pyometra were examined bacteriologically. Bacterial growth occurred in 37 (92.5 %) of the 40 uterine contents. *E. coli* (46.66%) was the most common bacteria isolated. The other microorganisms in descending order of frequency were *Enterobacter cloacae* (16%), *Klebsiella pneumoniae* (7%), *Streptococcus canis* (7%), coagulase-negative *Staphylococcus* sp. (7%), *Enterobacter aerogenes* (4.44%), *Streptococcus dysgalactia* subs. *equisimilis* (4.44%), *Proteus mirabilis* (2.22%), *Serratia marcescens* (2.22%), *Staphylococcus intermedius* (2.22%), and *Pseudomonas aeruginosa* (2.22%).

Susceptibility to antibiotics of the isolates was investigated. 73.33 % of the isolates were found to be sensitive to Cefpirom, while penicillin G was detected as the least effective antibiotic with a rate of 2.22%.

**Keywords:** pyometra, bitches

### Introduction

Pyometra is regarded as one of the most common illnesses in bitches. Middle aged or older, intact bitches that are in metoestrus are at high risk for the disease (5, 10). Common clinical signs are vaginal discharge, polydipsia, polyuria, lethargy, anorexia, vomiting, distended abdomen, depression; and these manifestations vary depending on the stage of the disease (5-7).

In the etiopathogenesis of the the disease, the importance of the presence of bacteria and their toxins in the uterus has been accepted by most of the researchers. On the other hand, it has been shown that inoculation of bacteria into normal uterus cannot alone cause the disease. A common etiological theory is that the primary cause is of a hormonal nature, an abnormal endometrial response to progesterone and that invasion of bacteria into uterus is a secondary event (5-7).

*Escherichia coli* is the most common bacteria isolated from the uterus of bitches with pyometra. The isolation frequency has varied in different studies between 59 and 85% (2, 4, 6, 10). This high isolation rate is associated with the ability of *E. coli* to bind specifically to uterine lining cells, changed by progesterone (6). Other bacteria isolated from pyometra infections in bitches are *Staphylococcus aureus*, *Proteus mirabilis*, *Actinomyces pyogenes*, *Streptococcus* sp., *Klebsiella* sp., *Pasteurella* sp., and *Pseudomonas* sp. However, in some cases no organisms at all could be isolated from diseased uteri (2, 7, 10).

Commonly, surgical removal of the uterus and ovaries is the preferred for the treatment of pyometra.

Antibiotics to fight the infection, are usually being use for supporting surgery. However in younger bitches which has a reel and important breeding career, antibiotic treatment can be selected by the owners instead of surgery (6).

This study was performed to determine the predominant bacterial species in the uterus of the bitches with pyometra and antimicrobial susceptibilities of the isolates.

### Materials and Methods

**Bitches:** 40 bitches with various manifestations and diagnosed as pyometra according to the history, ultrasound, clinical and laboratory findings were investigated.

**Uterine contents:** Uterine contents were collected immediately after the ovariohysterectomy, by using transport swabs. The swabs were brought to our laboratory within 15-30 minutes.

**Bacteriological examinations:** Swabs were inoculated into Nutrient Broths containing %2 horse sera and incubated aerobically and microaerobically at 37°C for 48 hours. The cultures were subcultured into 10% blood agar and MacConkey agar plates. All plates were incubated for 48 hours, both aerobically and microaerobically, again. The colonies that formed on the plates, were observed macroscopically and were Gram stained. Isolated bacteria were identified using routine biochemical tests (12).

**Antibiotic susceptibility test:** The antibiotic susceptibility of the isolates were determined by use of Kirby-Bauer single disk diffusion procedure (1). For this purpose antibiotic discs comprising ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefoperazone, cefpirom, ciprofloxacin, cloxacillin, enrofloxacin, gentamicin, lincomycin, penicillin G were used.

Tab. 1. Isolates from the uterine contents of the bitches with pyometra

Bacterial species	Number of isolates			Isolation rate (%)
	Pure culture	Mixed culture	Total	
<i>E. coli</i>	17	4	21	46.67
<i>Enterobacter cloacae</i>	5	2	7	15.56
<i>Klebsiella pneumoniae</i>	3	0	3	6.67
<i>Streptococcus canis</i>	0	3	3	6.67
Coagulase-negative <i>Staphylococcus sp.</i>	0	3	3	6.67
<i>Enterobacter aerogenes</i>	0	2	2	4.44
<i>Streptococcus dysgalactia subs. equisimilis</i>	0	2	2	4.44
<i>Proteus mirabilis</i>	1	0	1	2.22
<i>Serratia marcescens</i>	1	0	1	2.22
<i>Staphylococcus intermedius</i>	1	0	1	2.22
<i>Pseudomonas aeruginosa</i>	1	0	1	2.22
Total	29	16	45	100

**Results**

**Clinical examination:** The ages of the bitches ranged from 1.5 to 12 years and none had been treated with antibiotics. 95% of the bitches had a vaginal discharge, 80% had depression, 75% had anorexia, 45% had vomiting, 20% polydipsia, 20% had polyuria, and 5% had distended abdomen.

The uteri of the bitches scanned by ultrasonography were enlarged and filled with secret varying from anechogenic to hypoechogenic appearance, and often showing varying sized loops throughout the horn of the uteri.

As the results of clinical laboratory tests, all of the bitches (100%) had leukocytosis varying from middle to severe and 60% had anemia and hematocrit level under 40%. The blood urea levels were high in the 17.5% of the bitches (normal level is: 4-26 mg/dl).

**Bacteriological examinations:** 45 bacteria were cultured from 37 (92.5%) of the 40 uterine contents. 36 of the isolates were Gram negative, while 9 of were Gram positive. Of the 37 uterine contents, in the 29 (78.38%) were pure cultures and 8 (21.62%) were mixed cultures.

The most frequently isolated bacteria was *E. coli* with 46.67% isolation rate. *E. coli* was isolated from 21 of the 37 uterine contents; of these, 17 (80.95%) had pure cultures of *E. coli* and 4 (19.04%) had mixed infection with *Streptococcus species*.

The other bacteria in descending order of frequency isolated were *Enterobacter cloacae* (15,56%), *Klebsiella pneumoniae* (6,67%), *Streptococcus canis* (6,67%), coagulase-negative *Staphylococcus sp.* (6,67%), *Enterobacter aerogenes* (4.44%), *Streptococcus dysgalactia subs. equisimilis* (4.44%),

Tab. 2. Antibiotic susceptibility test results of the isolates: R: Resistant; I: Intermediate; S: Susceptible; AMP10: Ampicillin (10 µg); SAM20: Ampicillin/sulbactam (20 µg); AML25: Amoxicillin (25 µg); AMC30: Amoxicillin/clavulanic acid, (30 µg); CFM5: Cefixime (5 µg); CFP75: Cefoperazone (75 µg); CPO30: Cefpirom (30 µg); CIP5: Ciprofloxacin (5 µg); OB5: Cloxacillin (5 µg); ENR5: Enrofloxacin (5 µg); GM10: Gentamicin (10 µg); MY15: Lincomycin (15 µg); P10: Penicillin G (10 µg)

		<i>E. coli</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>S. canis</i>	Coagulase (-) <i>Staphylococcus sp.</i>	<i>E. aerogenes</i>	<i>S. dysp. ssubs. equisimilis</i>	<i>P. mirabilis</i>	<i>S. marcescens</i>	<i>S. intermedius</i>	<i>P. aeruginosa</i>
AMP10	R	13	7	3	2	2	2	1	1	1	1	1
	S	4	0	0	1	0	0	1	0	0	0	0
	I	4	0	0	0	1	0	0	0	0	0	0
SAM20	R	11	7	1	2	1	2	0	1	1	1	1
	S	9	0	1	1	2	0	2	0	0	0	0
	I	1	0	1	0	0	0	0	0	0	0	0
AML25	R	10	6	2	2	1	2	0	1	1	1	1
	S	10	0	1	1	2	0	2	0	0	0	0
	I	1	1	0	0	0	0	0	0	0	0	0
AMC 30	R	7	7	1	1	2	2	0	0	1	0	1
	S	11	0	2	2	1	0	2	1	0	1	0
	I	3	0	0	0	0	0	0	0	0	0	0
CFM5	R	3	5	0	2	3	1	0	0	0	1	1
	S	15	1	3	0	0	1	1	1	1	0	0
	I	3	1	0	1	0	0	1	0	0	0	0
CFP75	R	2	2	0	1	1	1	0	1	0	0	0
	S	14	5	3	2	2	1	2	0	1	0	1
	I	5	0	0	0	0	0	0	0	0	1	0
CPO30	R	2	3	0	1	1	1	0	0	0	0	1
	S	18	3	3	1	2	1	2	1	1	1	0
	I	1	1	0	1	0	0	0	0	0	0	0
CIP5	R	3	0	0	0	1	0	1	0	0	0	0
	S	16	4	3	0	2	2	0	1	1	1	1
	I	2	3	0	3	0	0	1	0	0	0	0
OB5	R	20	7	3	2	3	2	0	1	1	0	1
	S	1	0	0	0	0	0	2	0	0	1	0
	I	0	0	0	1	0	0	0	0	0	0	0
ENR5	R	2	1	0	2	1	0	1	1	0	0	1
	S	13	5	2	0	2	2	0	0	1	1	0
	I	6	1	1	1	0	0	1	0	0	0	0
GM10	R	3	2	0	3	3	1	2	0	0	0	0
	S	18	4	3	0	0	1	0	1	1	1	0
	I	0	1	0	0	0	0	0	0	0	0	1
MY15	R	17	5	3	3	1	1	2	0	1	1	1
	S	4	2	0	0	1	1	0	1	0	0	0
	I	0	0	0	0	1	0	0	0	0	0	0
P10	R	21	7	3	2	3	2	0	1	1	1	1
	S	0	0	0	1	0	0	1	0	0	0	0
	I	0	0	0	0	0	0	1	0	0	0	0

*Proteus mirabilis* (2.22%), *Serratia marcescens* (2.22%), *Staphylococcus intermedius* (2.22%) and *Pseudomonas aeruginosa* (2.22%).

The number of bacterial species isolated from uterine contents are presented in tab. 1.

**Antibiotic susceptibility test:** The results of the antibiotic susceptibility test of 45 isolates showed that Cefpirom was the most effective antibiotics (73.33%), followed by Cefoperazone (68.88%) and Ciprofloxacin (68.88%) while Penicilline G was the least effective (2.22%).

Ciprofloxacin (77.77%) and Gentamicin (77.77%) were the most effective antibacterial agents among the Gram negative bacteria, and Cefpirom (66.66%) and Cefoperazone (66.66%) were for Gram positive bacteria.

Antibiotic susceptibility test results of the isolates were shown in tab. 2.

## Discussion

Pyometra is a well-recognised disease of bitches and is perhaps the most common serious medical condition affecting entire bitches. Whenever levels of the progesterone rise, the uterine lining becomes susceptible to bacterial infections (5).

Many researches revealed that *E. coli* is the most common bacteria identified in pyometra (2, 10). Fransson et al (6) reported that *E. coli* have been isolated from 43 (90%) of the 48 uteri with pyometra. Similarly, Dhaliwal et al (4) isolated *E. coli* from 27 (79.4%) of the 34 pyometra cases, and the 25 of them (92.6%) were pure cultures. Also in a study of Pradhan et al (10) there were 14 *E. coli* isolates (29.16%) with 8 (16.66%) pure cultures. In this study, *E. coli* was the most frequent isolated organism with 46.67 per cent isolation rate. This finding confirms results of other studies (2, 4, 10). The affinity of *E. coli* for progesterone-sensitized endometrium could be the reason for this predominance.

Fransson et al (6), revealed that out of 48 cases of pyometra, bacteria were isolated in 47 (98%). Also they indicated that the number of pyometra cases with Gram positive uterine infection had been too small (6). In the present study, 20% of the isolates were Gram positive.

Pradhan et al (10), reported that 45.83% of the samples yielded single bacterial isolate whereas 54.16% had more than one bacteria, and they added that these results corroborated the findings of some researchers who stated that it was not uncommon to isolate a mixed bacteria from uterine samples of endometrial bitches. Bjurström (2), performed a retrospective survey to determine aerobic bacterial species found in the vagina of 203 bitches with genital disorders and the results of the study revealed that pure cultures were isolated more frequently from bitches with pyometra than from bitches with vaginitis and from those with fertility problems. In the present study, of the 37 uterine content 29 (78.38%) were pure cultures and 8 (21.62%) were mixed cultures.

Pyometra can be associated with the abnormal response of the bitches uterus to ovarian hormones either with or without a secondary bacterial infections. In some pyometra cases, the uterus could be sterile which suggests either that bacteria are not always involved in the pathogenesis of the disease, or that they have been eliminated by the time attempts to culture are made (4). Dhaliwal et al. (4) reported

that there had been no isolation from the uterine lumen of 6 (17.6%) of the 34 bitches. Bjurström (2) reported that of the 71 samples of bitches with pyometra 11.3% yielded no bacterial growth. In this study, there was no isolation in 3 (7.5%) of 40 uterine contents. The reason of this case may be the uterine defence mechanisms that killed the causative bacterial agent initially involved in the uterus, antimicrobial therapy that was used without the information of the authors.

As a result of different studies, in which antibiotic susceptibilities of the isolates from pyometra cases, different results have been obtained. Pradhan et al (10) reported that, the antibiogram of 48 uterine samples showed that the most sensitive antibiotics were ciprofloxacin (87.5%), chloramphenicol (54.16%) and gentamycin (50%) while oxytetracycline had the least (8.33%) inhibitory action. The researchers suggested that the highest sensitivity of ciprofloxacin *in vitro* might have been the result of its minimum use in the field of canine practice. Kılıçarslan et al (8), detected enrofloxacin, gentamicin sulphate, cephaperazone and amoxicillin as the effective antibiotics, respectively. Enrofloxacin and amoxicillin/clavulonic acid has been advised by Lemmer (9) as the result of antibiotic susceptibility test. Blendinger (3), revealed that the use of amoxicillin and cavulonic acid combinations have increased the success of the treatment in bitches with pyometra. Querol (11), suggested procain penicilline and neomycin combinations in the intrauterine treatment of pyometra. In the present study, we found Cefpirom was the most sensitive antibiotics (73.33%), followed by Cefoperazone (68.88%) and Ciprofloxacin (68.88%) while Penicilline G was least effective (2.22%) among both 45 isolates.

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