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Identification and antimicrobial susceptibility of bacteria from the uterus of bitches with pyometra

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Abstract : This study was performed to isolate and identify bacteria from uterus with pyometra and examine their susceptibility to antimicrobial agents. Uterus of 16 bitches with pyometra were surgically removed by ovariohysteroctomy and then bacteria were isolated and identified. Also, susceptibility test to 15 antimicrobial agents was performed. Out of 16 bitches, 11 strains of *Escherichia coli*, 2 strains of *Serratia marcescens*, and 1 strain of *Staphylococcus aureus* and *Salmonella spp.* were identified. In antimicrobial susceptibility test, the majority of isolates were susceptible to enrofloxacin, norfloxacin, chloramphenicol, nalidixic acid, gentamicin, trimethprim-sulfamethazole, tetracycline, and moderately susceptible to carbenicillin, amikacin, ampicillin, neomycin, but resistant to vancomycin, streptomycin, bacitracin and colistin. In conclusion, *E coli* was the most common bacteria isolated from bitches with pyometra and those susceptible antimicrobial agents could be recommended to medical therapy of pyometra.

Key words : bitch, pyometra, bacteria, antibiotics, susceptibility.

Introduction

Pyometra in bitches was the result of hormonal induced changes in the uterus that allow secondary infection to occur. It is related with the high plasma concentration of progesterone, estrogen during estrus and diestrus, and cystic endometrial hyperplasia (CEH)[1]. Pyometra was common disorder in aged bitches (> 6 years of age)[2] in diestrus and most of dogs with pyometra had clinical signs including vaginal discharge, depression, polyuria, polydipsia, anorexia, panting and abdominal distension[1]. Pyometra was diagnosed on the basis of the occurrence of clinical signs, hematology and the identification of a fluid-filled uterus on abdominal radiographs or sonograms.

There were two ways to treat pyometra ; medical therapy and surgical method. The decision to treat pyometra surgically or medically depends on the condition of the animal at the time of presentation, its age, and the importance to the owner of preserving the animal's reproductive capacity. In closed pyometra, development of toxicity is extremely rapid so immediate ovariohysterectomy has been recommended as a complete method. And, in open pyometra, surgical method was the treatment of choice if owner agreed[1,3].

However, in stable animals whose breeding potential is of utmost importance without sign of systemic illness, medical treatment could be attempted. The basis of medical treatment is reducing the plasma progesterone concentration, relaxing the cervix, promoting myometrial contraction, and preventing bacteremia. Generally, in medical treatment, a combined treatment of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and antibiotics had been used. $PGF_{2\alpha}$ acts by causing cervical dilation, myometrial contraction, and, at higher dosages, luteolysis[4-6]. And, concomitant systemic antibiotics were used to prevent bacteremia

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during uterine evacuation. In antibiotic therapy, broad-spectrum, bactericidal antibiotics were effective, generally. However, choice of antibiotics must be based on results of bacterial culture and antimicrobial susceptibility test[3].

The aims of present study were to isolate and identify bacteria from the uterus of bitches with pyometra admitted to the Veterinary Medical Teaching Hospital, Seoul National University and then operated with ovariohysterectomy during the period 1998-1999. And then, determining the appropriate antibiotics in medical therapy of canine pyometra.

Materials and Methods

Patients identification

Sixteen cases of pyometra that admitted to the Veterinary Medical Teaching Hospital, Seoul National University from 1998 to 1999 were used. The diagnosis was performed by base on clinical signs, hematology, radiography and ultrasonography.

Isolation and identification of bacteria

Ovary and uterus were removed by ovariohysterectomy[7]. After operation, aspirated specimens from the removed uterus (Fig 1) with a 18 gauge needle fixed to a 5 m*l* disposable syringe and stored in plain bottle at -20°C until use. Uterus biopsies were inoculated on blood agar plates containing 5% sheep blood and cultured aerobically and anaerobically for 24 hours at 37°C. A single colony was picked up and simple biochemical tests were carried out. Final identification of bacteria was performed using an automatic bacterial identification system, Vitek® (Vitek, USA).

Fig 1. Photograph of uterus removed by ovariohysterectomy.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed by disc diffusion test. The antimicrobial agents used in the test were amikacin, neomycin, colistin, vancomycin, streptomycin, gentamicin, ampicillin, bacitracin, enrofloxacin, nalidixic acid, chloramphenicol, carbenicillin, norfloxacin, sulfamethazole/trimethoprim (Tri-sulfa), tetracycline.

Results

As shown in Table 1. *E coli* were isolated from 11 bitches (73.6%). *Serratia marcescens* from 2 bitches (12.5%). *Staphyococcus aureus* and *Salmonella* spp. from each 1 case (6.3%).

Antimicrobial susceptibility test was performed as described in materials and methods. As shown in Table 2, all bacterial strains isolated from bitches with pyometra were susceptible to enrofloxacin and norfloxacin.

Table 1. Bacterial species isolated from the uterus of sixteen bitches with pyometra

Bacteria isolated	No. of dogs (%)
Escherichia coli	11 (73.8)
Serratia marcescens	2 (12.5)
Staphylococcus aureus	1 (6.3)
Salmonella spp.	1 (6.3)
Unidentified	1 (6.3)

Table 2. Antimicrobial susceptibility test to bacteria isolated from uterus of bitches with pyometra

Antibiotics	Susceptible (%)
Enrofloxacin	16 (100)
Norfloxacin	16 (100)
Chloramphenicol	15 (94)
Nalidixic acid	14 (88)
Gentamicin	12 (75)
Tri-sulfa*	12 (75)
Tetracycline	12 (75)
Carbenicillin	10 (63)
Amikacin	9 (56)
Ampicillin	6 (38)
Neomycin	4 (25)
Vancomycin	1 (6)
Streptomycin	1 (6)
Bacitracin	1 (6)
Colistin	0 (0)

*Trimethprim/Sulfamethazole.

And 15 bacterial strains were susceptible to chloramphenicol. Also nalidixic acid, Tri-sulfa, tetracycline, gentamicin and carbenicillin were effective in the susceptibility test. However, almost bacterial strains were resistant to colistin and bacitracin.

Discussion

The most likely sources of the bacteria that result in pathologic uterine infection are the vaginal vault, concomitant urinary tract infections and transient bacteremia. However, common vaginal flora are the logical source for uterine contamination[1,3]. The bacteria usually associate with pyometra are *E coli.* However, *Staphylococci, Streptococci, Klebsiella, Pseudomonas, Proteus, Haemophilus, Pasturella, Serratia*, and other bacteria are isolated from the uterus of bitches with pyometra[1]. These bacteria commonly ascend through the relatively dilated cervix into the uterus during proestrus and estrus.

The bacteria usually associate with pyometra are *E coli* and Gram-negative bacteria. They adhere to receptors via K-antigen in the progesterone-stimulated endometrium and myometrium and depend on the environment, they can produce extracellular polysaccharides, more frequently than *E coli* infecting other organs[8,9]. Moreover B. Wadas *et al*[10] found that *E coli* associated with canine pyometra derived from the fecal flora and that the urinary tract was infected by the same *E coli* clone as the uterus of a bitch with pyometra.

Serratis marcescens is a Gram-negative rod, anaerobic, has flagella and capsules. It is positive to the ornithine decarboxylase test. *Staphylococci* spp, *Micrococcus* spp and facultative anaerobes, grow abundantly on meat extract and infusion media at 37°C. And it is associated with a wide range of infections in humans and animals. *Staphylococcus aureus* has many toxins and enzymes that are hemolysins, coagulase, staphylokinase and enterotoxins. It is an opportunistic organism which may produce a variety of wound infections, abscess, surgical infection or infections of hair follicles.

Members of genus *Salmonella*, typical Gram-negative morphology of the Enterobacteriaceae are usually motile, facultatively anaerobic, produce gas from glucose, produce H_2S and utilize citrate as a sole source of carbon.

For treating pyometra, broad-spectrum antibiotics are administered before surgery and were continued for 7-10 days afterwards[3,7]. Prior to receiving culture and sensitivity data, tri-sulfa or enrofloxacin would be appropriate choices for the purpose[2]. Whereas this research reveal that enrofloxacin and norfloxacin were the most effective to control of pathogenic bacteria of pyometra. Fluoroquinolones including norfloxacine, enrofloxacin and ciprofloxacin exert their antimicrobial effects by inhibiting DNA gyrase, an essential enzyme for DNA replication within the bacterial cell. But unlike norfloxacin, enrofloxacin tend to accumulate in the serum after multiple doses[11,12]. As a result, 2.5 mg/kg BID dose[3,11] is enough to treat those infections caused by *Staphylococcus intermedius, E coli, Preteus mirabilis*, and *Klebsiella pneumoniae.* The most common side effect of enrofloxacin is gastric disturbance (anorexia, vomiting, diarrhea). Also because of the arthropathy side effects in dogs, enrofloxacin is contraindicated in dogs between the ages of 2 and 8 months, large breeds until 12 months, and the giant breeds until 18 months[11].

Conclusion

This study found that major pathogenic bacteria related with pyometra was *E coli* which could be originated from normal flora of urinary tract. Treating of pyometra, enrofloxacin, norfloxacin, chloramphenicol, etc. were very effective. But It is not appropriate to use colistin, vancomycin, streptomycin bacitracin for treating pyometra. In medical therapy of pyometra, antibiotics were administered by the results of bacterial culture and it's susceptibility test.

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개 자궁축농증에서의 원인균 분리 및 항생제 감수성 검사

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국문초록 : 본 연구는 1998년부터 1999년까지 서울대학교 수의과대학 부속동물병원에 내원한 개중 자궁축농증으로 진단된 개의 자궁내용물로부터 균을 분리, 동정하고 항생제 감수성 검사를 실시하여 개 자궁축농증 치료를 위한 기초 자료로 사용하고자 하였다. 위 병원에 내원하여 자궁축농증으로 진단된 개는 16두였으며, 이들로부터 자궁적출술을 이용하여 자궁을 적출한 후 원인균을 분리하였고, 분리된 균에 대하여 15종의 항균제에 대하여 감수성 검사를 실시하였다. 16두의 개로부터 분리된 균은 *Escherichia coli* 11주, *Serratia marcescens* 2주, *Staphylococcus aureus* 및 *Salmonella spp.* 각 1주 씩이었다. 이들 균주들에 대하여 항균제 감수성 검사를 실시한 결과, 대부분의 균주들이 enrofloxacin, norfloxacin, chloramphenicol, nalidixic acid, gentamicin, Trimethprim-sulfamethazole, tetracycline에 대하여는 높은 감수성을 나타내었고, carbenicillin, amikacin, ampicillin, neomycin에 대하여는 중등도의 감수성을을 나타내었으나, vancomycin, streptomycin, bacitracin, colistin에 대하여는 저항성을 나타내었다.

Key words : bitch, pyometra, bacteria, antibiotics, susceptibility.