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Canine pyometra associated with *Bacillus* species: a case report

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ABSTRACT: An 11-year-old, female Maltese was presented for evaluation of a mammary gland tumour. An abdominal sonographic examination showed an echogenic, fluid-filled, dilated uterus; pyometra was also diagnosed. Ovariohysterectomy and unilateral mastectomy was performed and the dog recovered uneventfully. However, 11 h later, the dog's condition suddenly deteriorated and it died on the day after the surgery. *Bacillus circulans* and *Bacillus megaterium* were isolated from its uterine content; these species had not been reported previously in canine pyometra. The two species were resistant to various antibiotics, including cefazolin used during the treatment. We describe for the first time the presentation, diagnosis, bacterial isolation and fatal outcome of *B. circulans* and *B. megaterium* infection in a Maltese dog with pyometra.

Keywords: dog; immunosuppression; opportunistic infection

Pyometra is the most common disease in female dogs but its aetiology has not been elucidated (Frances 2006). It is presumed that pyometra is caused by the response to progesterone stimulation, which leads to an increased predisposition to bacterial infection (Chotimanukul and Sirivaidyapong 2010; Shiju et al. 2011). The bacteria isolated from the uteri of dogs with pyometra belong to diverse species; *Escherichia coli* is most frequently found (Hagman and Greko 2005; Chotimanukul and Sirivaidyapong 2010). Other bacteria commonly isolated from uteri in cases of canine pyometra include *Klebsiella* spp., *Streptococcus* spp., *Enterobacter* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp. and *Citrobacter* spp. (Chotimanukul and Sirivaidyapong 2010). Although the bacteria found in the canine uterus are well-studied, there is a limited amount of information available on those that are considered non-pathogenic, such as the *Bacillus* spp.

Bacteria of the genus *Bacillus* are ubiquitous in the environment (Sliman et al. 1987). They are commonly found on inanimate objects and on the mucous membranes of healthy humans (Sliman et al. 1987). As *Bacillus* spp. are common laboratory contaminants and have been associated with pseudoepidemics in human and veterinary clinics, initially isolated *Bacillus* spp. may be ignored in treatment strategies (Alebouyeh et al. 2011). However, *Bacillus* species are considered as opportunistic pathogens, and predominate under conditions of suppressed or compromised immunity, metabolic disorders and neoplastic diseases (Logan 1988). Moreover, numerous reports on *Bacillus* infections have suggested that the species may also be primarily pathogenic (Banerjee et al. 1988). In human medicine, *Bacillus* spp. have been reported to be associated with abortion, meningitis, pneumonia, endocarditis and wound and ocular infections (Tuazon et al. 1979; Banerjee et al. 1988), but such reports are rare in veterinary medicine. *B. circulans* and *B. megaterium* were isolated from the uterine content in this case; to the authors' knowledge, this report is the first description of their

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involvement in canine pyometra. In the present report, we describe for the first time the presentation, diagnosis, bacterial isolation and outcome of *B. circulans* and *B. megaterium* infection in a Maltese dog with pyometra.

Case description

An 11-year-old, intact female Maltese was admitted to the Veterinary Teaching Hospital at Konkuk University (VTH-KU), for evaluation of a mammary gland tumour (MGT). On arrival at the hospital, the dog was alert and the following parameters were measured: body temperature, 38.3 °C; heart rate, 168/min; respiratory rate, 30/min. A firm and movable MGT was found between the 1st to 5th left and right mammary glands. A cardiac murmur (grade IV of VI) was detected on physical examination. Blood pressure was normotensive (systolic blood pressure, 120 mm Hg). Electrocardiography revealed P mitrale (0.055 s; reference range (RR): < 0.04 s) and sinus arrhythmia. Thoracic radiography revealed normal cardiac size (vertebral heart score: 10.5; RR: 8.5–10.5) and mild broncho-interstitial lung pattern. Two-dimensional echocardiography showed thickening and elongation of the anterior leaflet of the mitral valve. On spectral Doppler echocardiography, mitral regurgitant jet velocity (peak velocity: 6.0 m/s) was detected. Based on the diagnosis of myxomatous mitral valve disease (MMVD) with asymptomatic congestive heart failure ACVIM stage B1, the dog was treated with furosemide (Lasix; Handok Pharmaceuticals Co., Ltd., Seoul, Republic of Korea), 1 mg/kg twice daily per os, ramipril (Vasotop; Intervet Korea Co., Ltd, Seoul, Republic of Korea), 0.125 mg/kg once daily *per os*, pimobendan (Vet Medin; Boehringer Ingelheim, Seoul, Republic of Korea), 0.25 mg/kg twice daily *per os* and spironolactone (Spilacton; Daewon Pharm., Seoul, Republic of Korea), 1 mg/kg twice daily *per os*.

The history revealed that oestrus was seen four weeks before admission. For determining the stage of the oestrous cycle, a vaginal smear was performed. Vaginal samples were collected from the cranial vagina using a cotton swab and vaginal speculum. The vaginal swab was rolled over a slide, and left at room temperature to dry. This sample was then fixed in alcohol, stained with haematoxylin and eosin and evaluated under a microscope. Vaginal cytology revealed a number of intermediate and parabasal cells, a few superficial cells and high numbers of neutrophils. The vaginal smear and history confirmed that the dog was in diestrus.

On complete blood cell count (CBC), white blood cells (WBC, 10.26 × 10^9/l; RR: 6 to 17 × 10^9/l) were in the normal range with mild lymphopenia (0.71 × 10^9/l; RR: 0.72 to 5.1 × 10^9/l), and there was no evidence of anaemia (red blood cell [RBC] count 5.88 × 10^{12}/l, reference interval 5.5 to 8. 5 × 10^{12}/l; haemoglobin concentration 129 g/l, reference interval 120 to 180 g/l; haematocrit 42.52%, reference interval 37 to 55%). In the serum biochemical analysis, increased blood urea nitrogen (BUN, 14.28 mmol/l; RR: 2.5 to 9.64 mmol/l) and gamma-glutamyl transpeptidase (GGT, 0.22 µkat/l; RR: 0 to 0.2 µkat/l) were the only abnormalities noted; creatinine was in the normal range (61.88 µmol/l; RR: 44.2 to 159.12 µmol/l). D-dimer levels (200 µg/l; RR: less than 500 µg/l) were unremarkable. On abdominal sonographic examination, it was found that the uterus was dilated and filled with echogenic material; bilateral ovaries were normal in size with anechoic regions. Mastectomy and ovariohysterectomy were performed, respectively, for the treatment of MGT and pyometra. The dog was pre-medicated with glycopyrrolate (Glycopyrrolate Inj. SCD Pharm., Seoul, Republic of Korea), 0.01 mg/kg intramuscularly (*i.m.*) and butorphanol (Butophan Inj.; Myungmoon Pharm. Co., Ltd., Seoul, Republic of Korea), 0.1 mg/kg intravenously (*i.v.*) followed by anaesthetic induction with propofol (Provive Inj.; Myungmoon Pharm. Co., Ltd., Seoul, Republic of Korea), 6 mg/kg *i.v.* The dog was intubated and the anaesthesia was maintained with isoflurane (Isoflurane; Choongwae Co., Ltd., Seoul, Republic of Korea) and oxygen. Dobutamine (Toburex Inj.; Reyon Pharm. Co., Ltd., Seoul, Republic of Korea) was administered to maintain blood pressure at a rate of 5 µg/kg/min. The ovariohysterectomy was performed through a ventral midline celiotomy. The incision was initiated 2 cm caudal to the umbilicus and extended to the pubis. Both of the ovarian pedicles were double-ligated with 3-0 polydioxanone sutures and transected. The uterine body was ligated at the cranial tip of the cervix, the tissue was transected and the uterus was removed. Only left unilateral mastectomy was performed, because a two-stage procedure of mastectomy is better tolerated than a simultaneous bilateral mastectomy in most animals. An elliptical incision was made on the skin and subcutaneous tissues around

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the mammary glands to be excised from the cranial aspect of the first to the caudal aspect of the fifth mammary gland. The cranial and caudal superficial epigastric vessels were ligated with 2-0 polydioxanone sutures. The subcutaneous tissues were closed with interrupted and walking sutures using the 2-0 polydioxanone sutures. The skin was closed with interrupted sutures, using 3-0 polyamide sutures. The excised MGT was fixed by immersion in neutral buffered 10% formalin for microscopic evaluation. Additionally, the fluid in the uterus was aseptically collected to allow detection of the probable causative microorganism.

The uterine swab sample was streaked on blood agar and incubated in aerobic and anaerobic conditions at 37 °C for 18–24 h. A polymerase chain reaction (PCR) assay targeting the 16S rRNA gene was used to confirm the species of the isolated bacteria. PCR reactions were carried out with a universal PCR primer pair (Sim et al. 2012), and PCR conditions were the following: 94 °C for 10 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 90 s and 72 °C for 1 min. Nucleotide sequences of PCR products were determined using Sanger sequencing technology and the species of the isolated bacteria were confirmed using BLASTn analysis with Geneious software (Biomatters Ltd., Auckland, New Zealand). Two types of bacteria showing different colony characteristics were isolated from the uterine swab sample (Figure 1). One of the isolates produced a large white colony with an irregular margin, and the other produced a smaller, circular white colony with a grey margin. The isolates were identified as *Bacillus circulans* and *Bacillus megaterium* (Figure 2) with BLASTn analysis. With respect to the nucleotide sequences of 16S rRNA from the reference strains (*Bacillus circulans* strain ATCC 4513, GenBank accession No. NR_118446, *Bacillus megaterium* strain NBRC 15308, GenBank accession No. AB271751), that of the SM_BC strain *Bacillus circulans* showed 100% sequence identity with the *Bacillus circulans* reference strain ATCC 4513 (GenBank accession No. NR_118446), and *Bacillus megaterium* SM_BM strain showed 100% sequence identity with the *Bacillus megaterium* reference strain NBRC 15308 (GenBank accession No. AB271751). *Bacillus circulans* and *Bacillus megaterium* only showed 97.5% sequence identity with each other. In order words, the two strains were distinct species.

Antibiotic susceptibility testing was performed using the agar disc diffusion method. Isolated bacteria were suspended into nutrient broth (Becton Dickinson, New Jersey, USA), and the bacterial suspension was spread on Mueller-Hinton agar (Difco, Detroit, USA). For the analysis, 22 antibiotic discs containing zithromycin (15 µg, Oxoid), cefixime (5 µg, Oxoid), ciprofloxacin (5 µg, Oxoid), ceftazidime (30 µg, Oxoid), spiramycin (100 µg, Oxoid), amikacin (30 µg, Oxoid), clindamycin (2 µg, Oxoid), tetracycline (30 µg, Oxoid), sulfamethoxazole/trimethoprim (1.25/23.75 µg, Oxoid), gentamicin (10 µg, Oxoid), erythromycin (15 µg, Oxoid), cephazolin (30 µg, Oxoid), ampicillin (10 µg, Oxoid), cefovecin (30 µg, Oxoid), cefpodoxime (10 µg, Oxoid), enrofloxacin (5 µg, Oxoid), ofloxacin (5 µg, Oxoid), amoxycillin/clavulanic acid (20/10 µg, Oxoid), cefotaxime (30 µg, Oxoid), lincomycin (15 µg, Oxoid), Doxycycline (30 µg, Oxoid) and Cephalexin (30 µg, Oxoid) were used. Antibiotic inhibition zones were measured after incubation at 37 °C for 18~24 h. The SM_BC strain of *Bacillus circulans* was resistant to 11 antibiotics and susceptible to seven antibiotics, and the SM_BM strain of *Bacillus megaterium*

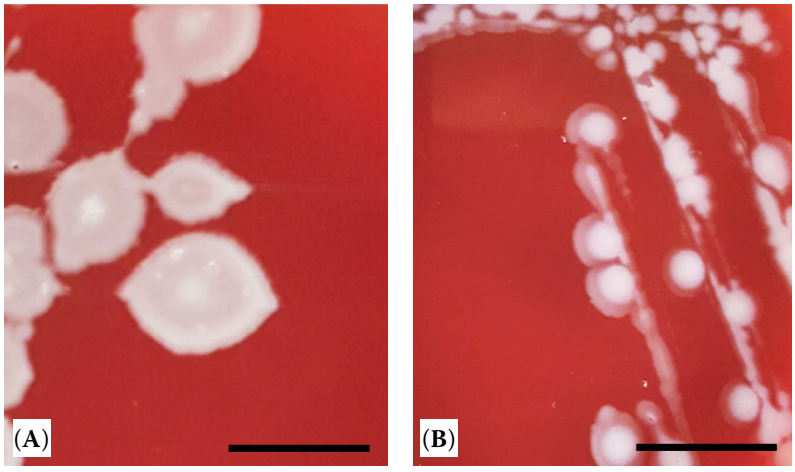

Figure 1. Colony characteristics indicating different phenotypes of the two bacteria, (**A**) *Bacillus circulans* SM_BC strain, (**B**) *Bacillus megaterium* SM_BM strain. The two bacteria were plated onto blood agar and incubated at 37 °C for 18~24 h (bar = 1 cm)

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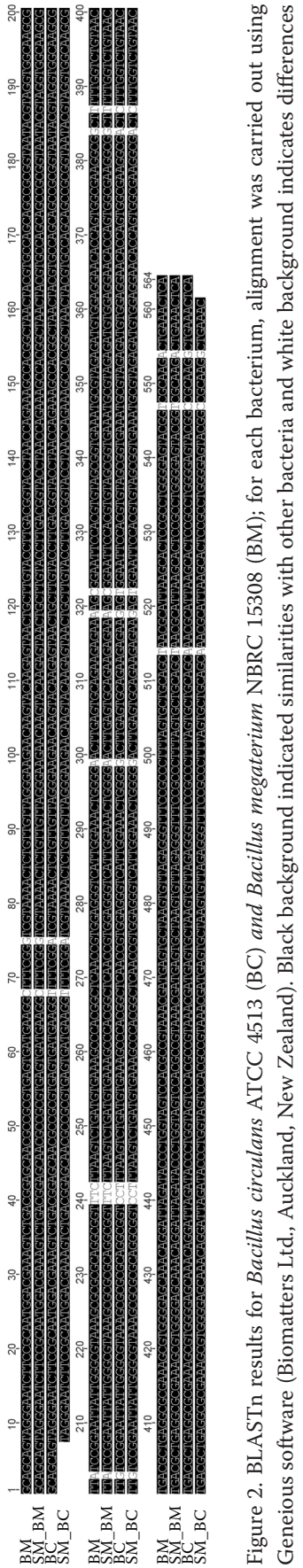

Figure 2. BLASTn results for *Bacillus circulans* ATCC 4513 (BC) *and Bacillus megaterium* NBRC 15308 (BM); for each bacterium, alignment was carried out using Geneious software (Biomatters Ltd., Auckland, New Zealand). Black background indicated similarities with other bacteria and white background indicates differences with other bacteria

showed resistance to six antibiotics and susceptibility to 12 antibiotics (Table 1). Both the isolates were resistant to cefixime, cefpodoxime, cefotaxime and lincomycin and susceptible to gentamicin, ofloxacin, amoxycillin/clavulanic acid and doxycycline.

Postoperative medications included cefazolin (Cefozol; Hankook korus Pharm., Seoul, Republic of Korea), 25 mg/kg *i.v*. and butorphanol, 0.1 mg/kg *i.v*. The dog recovered well from surgery; however, 11 h after surgery, there were signs of hypothermia, tachypnoea and increased abdominal pain. Based on the symptoms, sepsis was suspected. Although the patient was administered fluids and cefazolin as an antibiotic, it died on the day after surgery. Unfortunately, histopathological examination of the MGT and necropsy could not be performed as the owner refused to grant permission.

DISCUSSION AND CONCLUSIONS

The present study is the first report of a fatal case of mixed infection with *B. circulans* and *megaterium* in a dog with pyometra. *Bacillus* spp. were the only bacterial species to be identified in the microbiological tests in the present study. The bacteria were resistant to various antibiotics (11/22 [50%] and 6/22 [27%] for *B. circulans* and *B. megaterium,* respectively) including cefazolin which were administered during the treatment in this case. An infection was diagnosed in this dog and the empirically prescribed treatments were not effective.

Bacillus species include Gram-positive, rod-shaped, endospore-forming bacteria which are prevalent in the environment (Banerjee et al. 1988; Duncan and Smith 2011). *Bacillus* spp. are found in soil and spores and thus may be encountered during contact with soil. We suspect our patient acquired the opportunistic infection with *Bacillus* species while defecating or urinating in the soil, which is characteristic of its behaviour, most likely through the ascending route of infection.

Most *Bacillus* species are harmless, but some species are able to cause severe to self-limited disorders as primary or opportunistic pathogens (Alebouyeh et al. 2011). Although these bacteria have usually been assumed to be contaminants when recovered from clinical cultures (Tuazon et al. 1979; Banerjee et al. 1988), several recent reports have noted the importance of *Bacillus* spp. as putative pathogens

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Table 1. Antibiotic susceptibility of *Bacillus* spp. isolated from the uterine content of a dog with pyometra

Antibiotic	Class	SM_BC strain of *B. circulans*		SM_BM strain of *B. megaterium*	
		diameter of inhibition zones	susceptibility	diameter of inhibition zones	susceptibility
Azithromycin	aminoglycoside	13	R	18	S
Cefixime	cephalosporin	6	R	6	R
Ciprofloxacin	fluoroquinolone	19	I	21	S
Ceftazidime	cephalosporin	6	R	20	S
Spiramycin	aminoglycoside	16	R	18	I
Amikacin	aminoglycoside	17	S	16	I
Clindamycin	lincosamide	9	R	18	I
Tetracycline	tetracycline	25	S	22	R
Sulfamethoxazole/trimethoprim	antifolate	26	S	6	R
Gentamicin	aminoglycoside	15	S	17	S
Erythromycin	macrolide	16	I	19	I
Cephazolin	cephalosporin	13	R	22	S
Ampicillin	penicillin	18	I	20	S
Cefovecin	cephalosporin	6	R	25	S
Cefpodoxime	cephalosporin	6	R	6	R
Enrofloxacin	fluoroquinolone	18	I	21	S
Ofloxacin	fluoroquinolone	20	S	20	S
Amoxycillin/clavulanic acid	penicillin	22	S	27	S
Cefotaxime	cephalosporin	6	R	18	R
Lincomycin	lincosamide	6	R	10	R
Doxycycline	tetracycline	26	S	19	S
Cephalexin	cephalosporin	11	R	25	S

I = intermediate resistant; R = resistant; S = susceptible

(Alebouyeh et al. 2011). The increasing incidence of opportunistic *Bacillus* spp. infections is partly due to host predisposition, which in turn is attributed to compromised immunity and is also associated with advances in bacteriological techniques and interpretation (Logan 1988; Dib et al. 2003). Infections with these *Bacillus* species are usually observed in patients with diseases characterised by immunosuppression (Cotton 1987; Richard et al. 1988; Gatermann et al. 1991). *B. circulans* has been found in cases of meningitis, cerebrospinal fluid shunt infections, prosthetic heart valve replacements, endocarditis, endophthalmitis and wound and joint infection (Logan 1988; Gatermann et al. 1991; Alebouyeh et al. 2011). *B. megaterium* has not been reported to be the primary cause of an infection, but has been isolated from cases of eye infections after surgery; i.e., it may represent a secondary infection (Ramos-Esteban et al. 2005; Duncan and Smith 2011). These bacteria could be considered as potent pathogens for immunosuppressed patients (Alebouyeh et al. 2011).

Pyometra is an inflammatory condition which causes marked suppression of the immune system due to suppressed lymphocyte activity (Faldyna et al. 2001). The role of bacillus species in pyometra remains uncharacterised in veterinary medicine. However, *B. megaterium* has been reported as the cause of brain abscesses and other *Bacillus* spp. were described to cause primary cutaneous disease in human patients (Duncan and Smith 2011; Guo et al. 2015). In our patient, immunosuppression could have been caused by pyometra itself and may have contributed to the opportunistic clinical infection. A previous study revealed that altered immunological parameters in bitches with pyometra returned to normal seven days after hysterectomy (Bartoskova et al. 2007). However, in this case report, the dog deteriorated within one day after hysterectomy and could not ultimately recover

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from the operation. Furthermore, MGT, which is a neoplasia, also contributed to the immune suppression in this case as previously reported for a human patient with neoplasia where *B. circulans* was isolated from a wound infection as a primary causative agent (Logan et al. 1985; Logan 1988).

Pyometra is associated with many biochemical and haematological changes. The most commonly encountered blood parameters in dogs with pyometra are leukocytosis, neutrophilia, azotaemia and hyperproteinaemia (Frances 2006). Not all cases show these changes, and about 70% of dogs with pyometra exhibited leukocytosis, 2.5% showed elevated BUN while in 16% elevated creatinine levels were reported (Kitshoff et al. 2015). In the present case, an increased BUN and lymphopaenia without leukopaenia were the only abnormalities noted on the blood examinations. As shown in this case, increased BUN with normal creatinine could reflect the mild prerenal azotaemia.

In this case, the patient suddenly died on the day after the surgery. The dog had both heart disease and a bacterial infection, and the principal cause of death remains uncertain. Although the patient was diagnosed as MMVD ACVIM stage B1, the dog did not show any clinical signs related to congestive heart failure, and cardiac size and lung field were normal in the radiography after the surgery. Therefore, bacterial sepsis was the most likely cause of the sudden death in this case. In hospitalised patients with underlying diseases, sepsis is one of the leading causes of sudden death (Alebouyeh et al. 2011). Fatal sepsis and endocarditis caused by *B. circulans* was reported in an immunocompromised patient (Alebouyeh et al. 2011). In the absence of effective immune responses, *B. circulans* can multiply in the blood and host tissues (Alebouyeh et al. 2011). Unfortunately, we did not perform blood culture, but *B. circulans* was isolated from a culture of uterine content and was resistant to the prescribed antibiotic in this case. Thus, *B. circulans* could be considered as a potent pathogen responsible for the sudden death in the present study.

To the authors' knowledge, this is the first description of canine pyometra associated with isolation of *B. circulans* and *B. megaterium* from the uterine content. Furthermore, this case report should alert veterinary clinicians to the importance of microbiological tests in canine pyometra. Misdiagnosis or dismissal of *Bacillus* species as causative agents could result in the inappropriate choice of antibiotics, delayed recovery or mortality, as shown in this case. Finally, in immunocompromised patients such as dogs with neoplasia and pyometra, positive culture of *Bacillus* species from blood or body fluids should be evaluated carefully.

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