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First Report on Ovariohysterectomy for the Treatment of Pyometra in a Golden Retriever Dog in Mongolia

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ABSTRACT

A 4-year-old female Golden Retriever dog was brought to the Animal Health Centre with complaints of anorexia, purulent and blood-tinged discharge, and white foamy vomit. The dog was clinically diagnosed with pyometra and was successfully treated with ovariohysterectomy. Bacteriological testing revealed a bacterial infection with *Proteus vulgaris* that was resistant to antibiotics including vancomycin, novobiocin, and penicillin. Histopathological examination showed multifocal cystic hyperplasia of the endometrial glands, severe abscessation of the glandular lumen, and severe infiltration of lymphocytes and plasma cells in the muscle layer. The dog recovered and showed normal hematology and biochemical parameters after ovariohysterectomy, antibiotics administrations, and supportive care. This case report including the diagnosis and surgical management of pyometra will be a very important resource for the development of small animal clinical skills and clinical training in Mongolia.

Key words: Dog, Ovariohysterectomy, Pyometra, Treatment.

INTRODUCTION

Pyometra is one of the most common diseases in healthy female dogs and is a potentially life-threatening disease that develops as a result of a combination of hormonal and pyogenic bacterial infections. Pyometra often occurs due to the influence of the hormone progesterone, which causes hyperplasia of the endometrial glands and increased secretory activity. It can also occur due to bacterial infection such as infection with *Escherichia coli* (*E. coli*), *Proteus vulgaris* (*P. vulgaris*), *Streptococcus* spp., *Staphylococcus* spp. *Pseudomonas* spp., and *Klebsiella* spp. (Smith 2006; Kassé and Fairbrother 2016; Wareth et al. 2017; Castillo et al. 2018; Rainey et al. 2018; Fiamengo et al. 2020; Zheng et al. 2023). Pyometra can be classified into two main types based on whether the cervix is open or closed: Open pyometra is when the cervix remains open, allowing pus to drain from the uterus through the vagina. This often causes a noticeable discharge from the vulva, which may contain pus, blood, or both. Closed pyometra is a condition in which the cervix closes and prevents the drainage of pus. With no outlet for the infected material, the uterus fills with pus, leading to a more serious situation. Furthermore, pyometra in dogs usually begins with subtle clinical signs such as lethargy, anorexia, polydipsia, polyuria, vomiting, and abnormal vaginal discharge. Without timely treatment, it can progress to peritonitis, sepsis, and multiple organ dysfunction (Jitpean et al. 2014a; Jitpean et al. 2017; Hagman 2022). Therefore, pyometra is considered a life- threatening infection (Agostinho et al. 2014; Fieni et al. 2014; Jitpean et al. 2014b).

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Some studies have reported that one in five dogs develop this disease before the age of 10 years (Jitpean et al. 2012). All dog breeds are generally susceptible to pyometra, but Golden Retrievers are at an increased risk (Egenvall et al. 2001). In Sweden, 37% of all Golden Retrievers younger than 10 years are diagnosed with pyometra (Jitpean et al. 2012). Arendt et al. (2021) explained that this elevated risk may be due to the adenosine 5-triphosphate (ATP) binding cassette transporter 4 (ABCC4) gene, which encodes a transmembrane transporter important for prostaglandin transport.

Pyometra is often diagnosed symptomatically based on the presence of purulent vaginal discharge and other typical methods. Both qualitative and quantitative tests can be used to diagnose pyometra. For example, ultrasound can detect small amounts of intrauterine fluid and abnormal changes in the ovaries or uterine tissue (Fleischer 2003; Bigliardi et al. 2004; Davidson and Baker 2009; Hagman 2018; Mantziaras and Luvoni 2020; Batista et al. 2022). Radiography (X-rays) can also be used identify an enlarged and fluid-filled uterus (Mattei et al. 2018). Laboratory findings including haematology and biochemical analysis are nonspecific but reflect the presence of inflammation and secondary metabolic disorders. For example, a complete blood count (CBC) and serum biochemical parameters including alkaline phosphatase (ALP) and alanine transaminase (ALT/GOT) activities as well as blood urea nitrogen (BUN), creatinine (CRE) and total protein (TP) concentrations may be altered (Hamm and Dennis 2012; Paudel et al. 2023).

In recent years, the number of domestic dogs and cats of various breeds has been steadily growing due to the increasing interest of Ulaanbaatar residents in keeping animals. Therefore, the incidence of various common diseases is increasing and there is a need to further improve the clinical skills of veterinary students and veterinarians.

CASE PRESENTATION

A 4-year-old female Golden Retriever dog weighing 28.7kg was presented to the Animal Health Centre, School of Veterinary Medicine, Mongolian University of Life Sciences, Mongolia, with a 10-day history of anorexia, purulent blood-tinged vaginal discharge, and vomiting white foam. The dog had no known mating history and no additional significant clinical history. Antibiotics in the form of amoxicillin, and paracetamol were administered for 3 days before the examination.

Clinical examinations included a physical examination, CBC (Celltac Alpha MEK-6550, Nihon Kohden, Japan), comprehensive biochemical analysis (Dri-Chem NX500i, Fujifilm, Japan), bacteriology of the uterine swabs, histopathology of the uterine tissue, simple ultrasonography (GE Healthcare Ultrasound Docking Cart, V1510101, China), and radiography (EcoRay, ECO-CM-N, Korea) of the caudal abdomen. On physical examination, the dog's rectal temperature was 38.9°C, the heart rate was 128beats/min, the respiratory rate was 56 breaths/min, and the mucous membranes were pink. She had a dry nose and yellow ocular discharge and had become apathetic. The vulva was enlarged and had a moderate amount of yellowish-grey discharge.

A CBC revealed a leukocytosis, thrombocytosis, and low mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Table 1). A serum biochemical analysis revealed increased total protein (TP), total cholesterol (T-CHO), calcium (Ca), SGOT, GGT, lipase (v-Lip), total bilirubin (T-BIL), and ALP (Table 1).

On abdominal diagnostic imaging, radiography showed an enlarged tubular structure filled with fluid, as shown in Fig. 1, and ultrasonography revealed thickened uterine walls and an enlarged uterine horn filled with hypoechoic fluid, as shown in Fig. 2.

Table 1: Hematological and biochemical abnormalities in Golden Retriever with pyometra

Variables	Pyometra	Reference intervals
WBC (10^3/μL)	51.8	6.0-17.0
Lymphocytes (10^3/μL)	10.8	1.0-4.8
Monocytes (10^3/μL)	2.8	0.2-1.4
Eosinophils (10^3/μL)	1.1	0.1-1.3
Granulocytes (10^3/μL)	37.1	3.0-11.8
Thrombocytes (10^3/μL)	149	200-500
MCH (pg)	18.6	19.5-24.5
MCHC (pg)	29.5	32.0-36.0
TP (g/dL)	7.4	5.0-7.2
T-CHO (mg/dL)	334	111-312
Ca (mg/dL)	13.9	9.3-12.1
SGOT (IU/L)	50	17-44
GGT (IU/L)	37	5-14
v-Lip (IU/L)	242	10-160
T-BIL (mg/dL)	8.1	0.1-0.5
ALP (IU/L)	3500	69-333

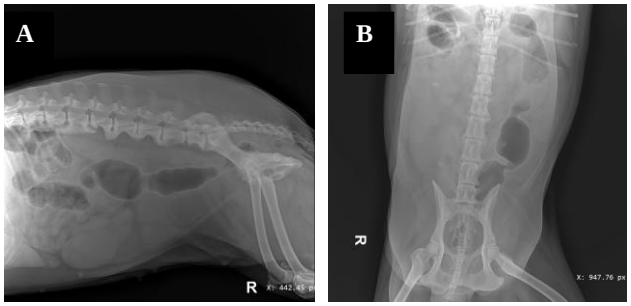

Fig. 1: Abdominal radiography. A) Right lateral view. The enlarged uterus is observed as a tubular structure in the ventral abdominal region; B) Ventrodorsally view. The enlarged uterine horns are observed along the lateral aspect of the descending colon (left) and the abdominal wall (right).

Fig. 2: Abdominal ultrasonography. The uterine is enlarged with thickened uterine wall and tubular horns filled with echogenic fluid.

RESULTS AND DISCUSSION

Lactated Ringer's solution (Cisen Pharmaceutical Co., Ltd. China) intravenous fluids were administered at 5mL/kg body weight (BW) per hour several times before surgery. The dog was intramuscularly premedicated with xylazine at 1mg/kg BW (Bimeda-MTC Animal Health Inc. Cambridge, Canada) and maintained on intravenous ketamine hydrochloride at 5mg/kg BW (Ciron Drugs & Pharmaceuticals Pvt. Ltd. India). General anaesthesia was induced with isoflurane (Piramal Enterprises Limited. India). The dog was maintained on isoflurane with oxygen at 2.0–2.5% at the beginning of surgery and reduced to 1.5–1.8% approximately 5–10min later (Ibis200 Anaesthesia Vaporizer).

The surgery site was clipped from the xiphoid to caudal end the of sternum, and the skin was scrubbed and disinfected for preparation of surgery. Laparotomy was performed through 10–12cm ventral caudal to mid-abdominal incision with a Thunderbeat electrosurgical pencil (Olympus ESG-400). The uterine horns and body were carefully exteriorized (Fig. 3). The ovaries, ligaments, and blood vessels were carefully identified and resected with the Thunderbeat open fine jaw instrument. Use of the Thunderbeat open fine jaw instrument is important to prevent the risk of bleeding during surgery (Devriendt et al. 2017). The laparotomy incision was closed following routine standard procedures. The size of the uterine horns and the blood supply around the uterine wall were significantly increased, and the uterine wall was thinner than normal.

Fig. 3: Uterus after exteriorization.

E. coli infections account for more than 80% of all urinary tract infections (Coggan et al. 2008). However, in this case, bacteriological isolation from the uterine swabs revealed *P. vulgaris* (Fig. 4a). *Proteus* spp. are opportunistic multidrug-resistant enterobacteria associated with diverse clinical diseases in domestic animals. The rise in infections caused by opportunistic enterobacteria, particularly those caused by *Proteus* spp. in domestic animals, is alarming. Such infections usually occur due to improper use of antimicrobials during treatments or after invasive diagnostic procedures (Zappa et al. 2017). This is a cause for concern and requires antimicrobial susceptibility testing. The antibiotic susceptibility of the pathogen was determined by the disk diffusion method according to the Kirby-Bauer technique (Jan Hudzicki 2009). The antibiotics tested were amoxicillin (10mcg), penicillin G (10mcg), tetracycline (10mcg), doxycycline (10mcg), vancomycin (10mcg), streptomycin (25mcg), novobiocin (10mcg), and colistin (10mcg) (Biolab, Hungary). After incubation, the diameters of the zones of incubation were measured to the nearest mm with a ruler. The zones were interpreted using EUCAST. The clinical results obtained were either evaluated as susceptible (S), intermediate (I), or resistant (R) for clinical application.

The results showed that the isolated bacteria were susceptible to the tetracycline group of antibiotics and were particularly more sensitive to doxycycline and tetracycline. Furthermore, *P. vulgaris* was found to be sensitive to streptomycin. *P. mirabilis* and *P. vulgaris* are naturally resistant to polymyxins (colistin), nitrofurans, tigecycline, and tetracycline (Stock 2003).

Bashir et al. (2021) recently published an antibiotic sensitivity profile of some bacteria isolated from patients diagnosed with urinary tract infections and showed similar results to what we found. Natural resistance is genetically supported by the bacterial chromosome; nevertheless, bacterial species may become susceptible via horizontal gene transfer. However, the isolates were resistant to vancomycin, novobiocin, and penicillin (Fig. 4b). In Gram-negative bacteria, an outer membrane surrounds the peptidoglycan layer. This membrane serves as a highly impermeable barrier, especially to antibiotics. Due to their large size, glycopeptides such as vancomycin (1448Da) and teicoplanin (1900Da) are unable to penetrate the external membrane of Gram-negative bacteria, thus rendering peptidoglycan inaccessible to these antibiotics. Enteric Gram-negative bacteria have an external lipopolysaccharide membrane that prevents penicillin G, rifampicin, novobiocin, fusidic acid, and macrolide antibiotics (such as erythromycin) from penetrating them (Todar 2008; Coculescu 2009). Administering amoxicillin to dogs before surgery does not affect the susceptibility or resistance of the pathogenic bacteria. The results showed that the isolated bacteria were more sensitive to the tetracycline group of than to other groups of antibiotics; this group was considered more convenient for treatment.

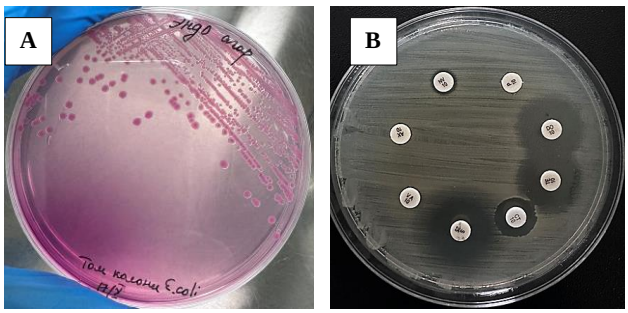

Fig. 4: A) *Proteus vulgaris* bacteria grow on Endo agar after 24 h, and B) its antibiotic susceptibility.

Histopathological examination of the affected uterine tissues was performed using common procedures. In detail, the uterine tissue samples were fixed with 10% neutral buffered formalin and then embedded (Tissue-Tek TEC5

Embedding Console System) in paraffin. The paraffin block was cut into slices (2–3µm) using a microtome (Tissue-Tek TrustomeTM [TTM-200]). Paraffin sections were stained with a conventional hematoxylin and eosin staining. Finally, histopathology of specimens was performed using a light microscope (Olympus CX33).

The results showed visible multifocal cystic hyperplasia of the endometrial glands with obvious flattening of the glandular epithelial cells, mild edema, and hemorrhages (Fig. 5a) into the lumen of the endometrial glands. Endometrial hyperplasia is the result of cystic deformation of the endometrial glands and interstitial proliferation of fibroblasts accompanied by an inflammatory response (De Bosschere et al. 2001). Severe diffuse degenerated epithelial cells with ulceration were also observed in the endometrium. In addition, severe abscesses (karyorrhectic neutrophils, macrophages, and bacterial mass) were observed in the lumen of the endometrial glands (Fig. 5b and 5d), and severe infiltration of lymphocytes and plasma cells was frequently seen in the muscular layer (Fig. 5c). The histological changes due to infection can vary depending on the bacterial cause and the time of infection. These findings were similar to those reported in previous studies (Varun et al. 2014; Binder et al. 2021; Woźna-Wysocka et al. 2021; Carlson et al. 2022).

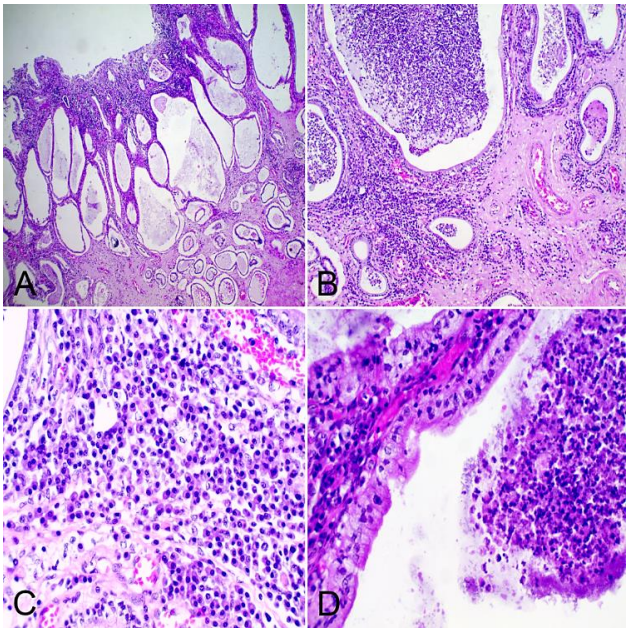

Fig. 5: Histopathological images of pyometra. A) Multifocal cyst formation, B, D) Micro-abscess in the lumen, and C) Infiltration of inflammatory cells

Lactated Ringer's solution intravenous fluids were administered postoperatively at 5mL/kg per hour for 4 days. Cefotaxime (PT. Sanbe Farma, Indonesia) was administered intravenously at a dose of 30mg/kg for 4 days. Metronidazole at 20mg/kg (PT. Bernofarm, Indonesia), doxycycline at 10mg/kg (Sintez OJSC., Kurgan, Russia), and omeprazole at 1mg/kg (Protech Biosystems Pvt., Ltd. India) were given orally twice daily for 7 days. In addition, daily dressing changes and wound checks were performed for 6 days. The conventional treatment for female dogs with pyometra is ovariohysterectomy and administration of broad-spectrum antibiotics. In particular, antibiotics can be administered immediately after pyometra is diagnosed to prevent life-threatening toxicosis or sepsis. Fluoroquinolones, such as enrofloxacin and amoxicillin/clavulanate, are the primary and secondary recommendations, respectively, for pyometra treatment. Other effective compounds include cephalothin, streptomycin, and gentamicin (Hagman and Greko 2005; Inoue et al. 2013; Agostinho et al. 2014; Lansubsakul et al. 2022). A recent retrospective review corroborated these findings in 10 of 16 animals by demonstrating that ampicillin or amoxicillin are effective antimicrobials for cases requiring antibiotic treatment, particularly in dogs exhibiting moderate to severe general depression (Turkki et al. 2023).

In this case, the patient's haematology and biochemicals parameters significantly changed 5 days after surgery. The CBC revealed a normal leukocyte count of $9.8x10^3$/µL (RI: 6.0–$17.0x10^3$/µL) and thrombocyte count of $368x10^3$/µL (RI: 200–$500x10^3$/µL). There were no other changes. The infectious process of pyometra can commonly cause leukocytosis. Several biochemical parameters that were relatively higher than normal were remeasured after treatment. In this case, SGOT and GGT were in the normal range at 30IU/L and 14IU/L, respectively. Bacterial endotoxemia and reduced liver perfusion are thought to contribute to increased liver enzyme activity during pyometra (Hamm and Dennis 2012). However, the dog's ALP and TP levels were 3050IU/L (RI: 69–333IU/L) and 2.4mg/dL (RI: 0.1–0.5mg/dL), respectively, which were still higher than the reference intervals. In general, serum ALP concentrations ($P<0.01$) are dramatically higher in dogs with pyometra than in healthy dogs (Sevelius et al. 1990; Ettinger and Feldman 1993; Dabhi et al. 2007; Hagman et al. 2009; Gupta et al. 2013; Ahn et al. 2021). These findings reflect damage to the vital organs and bone marrow due to toxemia in pyometra of bitches (Gupta et al. 2013). In generally, ALT and ALP are two liver enzymes used in the clinical assessment of liver injury (McGill 2016), however, ALP is not a specific marker of liver function (Gwaltney-Brant 2016). It can also be increased by intrahepatic cholestasis, damage to the heart, kidneys, and bone narrow, septicemia, endotoxemia, stress, and pain from infections (Lassend 2007). After surgery, it decreases to normal levels. This dog with pyometra recovered and showed normal hematology and biochemical parameters after ovariohysterectomy, administration of antibiotics, and supportive care.

Conclusion

This is the first case report on diagnostic methods, ovariohysterectomy, and treatment using modern equipment for open pyometra in a Golden Retriever dog in Mongolia. This case report will be a very important resource for the development of small animal clinical skills and clinical training in Mongolia.

Author Contributions

The hematology and biochemical parameters were analyzed by Kh.B., O.Ch., and Kh.Ch.. The surgery and treatment were performed by D.Y. K., B.G. and S.D. The microbiological diagnosis was performed by U.T. The histopathological evaluation was carried out by O.E.

Writing including-original draft preparation, review, and editing was performed by Kh.B. and G.J.C. All authors have read and agreed to the published version of the manuscript.

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