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Co-occurrence of pseudopregnancy, pyometra, and bacterial parasitic anaplasmosis in a persian cat

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Abstract

An intact female tabby Persian cat, 26-month-old, was brought to IPB University Veterinary Teaching Hospital on the 21st of February, 2023, due to vaginal discharge, inappetence, and lethargy. Physical examination revealed a distended abdomen, mammary gland enlargement, and fever (40.1 °C). The heart and respiratory rates were 128 beats per minute and 32 times per minute, respectively. The patient body weight was 3.87 kg. The veterinarian palpated masses inside the abdominal cavity and observed obvious purulent and haemorrhagic vaginal discharge. Haematology showed leukopenia and thrombocytopenia, and the eosinophil percentage of white blood cells was increased (eosinophilia). Blood chemistry analysis showed hyperglycaemia. Ultrasonography showed two hyperechoic masses inside two hypoechoic pouches in the queen's uterus, suspected as two dead foetuses (no heartbeat). Roentgen revealed abnormalities in the *vena cava caudalis*, bronchial pattern in the lung, radioopaque masses in the ventral abdomen, radio-opacity changes in the small and large intestines, and abnormalities in the spleen. The patient's diagnosis was pyometra, and ovariohysterectomy surgery was performed on the patient. The patient was treated with additional medicines (antibiotics and others) according to the symptoms. The patient's symptoms disappeared, except for the fluctuating febrile and pale mucous membrane. A blood smear performed on the 1st of March 2023 revealed *Anaplasma phagocytophilum* (*A. phagocytophilum*) in the white blood cells, and immediately doxycycline treatment (10 mg/kg body weight, twice daily) was given. The patient was discharged from the hospital on the 14th of March, 2023, with no febrile or other symptoms reported. This case is the first report of open cervix-type pyometra and anaplasmosis co-occurrence in a Persian cat from IPB University Veterinary Teaching Hospital in Indonesia.

Keywords: Anaplasma phagocytophilum, open cervix-type feline pyometra, intra-leukocytes, parasitic bacteria, ovariohysterectomy, pseudo-pregnant cats

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Introduction

Pseudopregnancy in cats is a syndrome in which a female cat exhibits pregnancy-like behaviours such as nesting, mammary gland growth, and lactation despite not being pregnant. These symptoms are brought on by hormonal changes in the cat's body. Cat pseudopregnancy is a normal physiological response that resolves independently after a few weeks (England and White, 2016). Cats are classified as induced ovulators since they are subjected to numerous cycles of estrogenic stimulation. If a queen ovulates

but does not conceive, she may undergo pseudocyesis (the synonym of pseudopregnancy). However, in some circumstances, pseudopregnancy can cause complications like mastitis, endometritis, and even pyometra (Al-Zubaidi et al., 2023; England and White, 2016; Verstegen et al., 2008).

Pyometra in cats is a bacterial infection in the uterus. Pyometra can be acute or chronic, characterized by pus-filled uterine inflammation resulting from progesterone stimulus (Misk and El-Sherry, 2020). The possible reasons for pyometra are the changes in hormones, anatomy, and physiology of the ovaries and uterus that create a suitable environment for the bacteria to grow. There are two types of pyometra, namely open-cervix and closed-cervix pyometra (Talukdar et al., 2022). In the open cervix pyometra, the discharge from the vagina might be noticed with non-specific and mild symptoms. In contrast, the patient might have peritonitis, sepsis, and even death in closed cervix pyometra. The primary option for cats having pyometra is an ovariohysterectomy, a surgery to remove uterine and ovarian organs (Farghali et al., 2020; Hasan et al., 2021; Martini et al., 2023; Misk and El-Sherry, 2020; Palupi et al., 2022). Additional medical therapies for cats having ovariohysterectomy procedures, such as antibiotics, fluid replacement, and local antiseptic covering at the incision site, will be needed (Farghali et al., 2020; Palupi et al., 2022). However, in rare cases where ovariohysterectomy is not a valid resolutive option, such as owners who do not agree with surgery and request medical treatment, uterine drainage, and flushing have been performed (Martini et al., 2023) (Hasan et al., 2021; Martini et al., 2023, 2023; Misk and El-Sherry, 2020; Palupi et al., 2022). Pyometra is a sporadic sickness in cats, but it has become more common in veterinary clinics in Indonesia because cat owners normally do not take elective spaying, and also reported in domestic and stray cats (Adnyana et al., 2022; Mulyana and Sari, 2022; Nurrurozi and Indarjulianto, 2019; Palupi et al., 2022; Prayoga et al., 2021; Rahayu et al., 2021; Rahman et al., 2021; Sendana et al., 2019; Soesatyoratih and Esfandiari, 2022).

Anaplasmosis is a disease transmitted through ticks caused by the bacterium *Anaplasma phagocytophilum*. Anaplasmosis is more common in dogs; cats can also be contracted with the bacterium. The disease's clinical symptoms are non-specific: stupor, inappetence, pyrexia, conjunctivitis, hepatosplenomegaly, ataxia, and elevation of *membrana nictitans*. Cats inhabiting endemic areas of *Ixodes* species are at high risk of tick infections, thus increasing their risk of anaplasmosis. The diagnosis of feline anaplasmosis is by detecting *A. phagocytophilum* DNA or bacterial parasite inside the white blood cells from blood samples or by serologic tests. Feline anaplasmosis treatment can be done by administering antibiotics, such as doxycycline. The administration of ectoparasiticides can carry out the prevention of anaplasmosis in cats to prevent and treat cats from infestation by ticks, especially where the ticks are endemic (Savidge et al., 2016; Schäfer and Kohn, 2020). This study reported co-occurrence of pyometra and anaplasmosis in a Persian cat in IPB University Veterinary Teaching Hospital.

Case History

A 24-month-old female Persian cat with a black-brown tabby (Figure 1) was brought to the IPB University Veterinary Teaching Hospital on the 21st of February, 2023, with white-to-red coloured vaginal discharge, inappetence, and stupor. The owner reported that the cat was mated with a male several weeks before the hospital visit; thus, the owner suspected the cat was pregnant. The patient survived a positive feline panleukopenia virus infection when the patient was seven months old.

The veterinarian did a physical examination of the cat patient and found abdominal distention, enlargement of mammary glands, turgor < 3 seconds, capillary refill time < 2 seconds, fever (40.1 °C), and the oral mucous membrane was pink. The heart and respiratory rates were

128 beats per minute and 32 times per minute, respectively. The patient body weight was 3.87 kg. Obvious purulent- and bloody- vaginal discharge was observed. Abdominal palpation revealed masses inside the abdominal cavity. According to the physical examination, the cat was diagnosed with open cervix-type pyometra. Thus, the veterinarian suggested further laboratory (haematology and blood chemistry) and medical imaging (ultrasonography and roentgen) examinations.

Figure 1 (Left). The patient (black-brown tabby Persian cat) in the cage during hospitalization after ovariohysterectomy surgery; Figure 2 (Right) Ultra sonograph of the hyperechoic mass inside an anechoic pouch inside the patient's uterus.

Laboratory Results

Haematology examination (21st of February 2023) of the patient presented leukopenia 3.5 x 10^3/µL, reference interval (RI), 5.5–19.5 x 10^3/µL) and thrombocytopenia (82 x 10^3/µL, RI 100–514 x 10^3/µL). The haematology showed normal erythrocyte (red blood cells, RBC), 8.63 x 10^6/µL, RI, 4.6–10.0 x 10^6/µL); haemoglobin (Hb), 11.3 g/dL, RI, 9.3–15.3 g/dL; haematocrit (HCT), 35.1%, RI, 28.0–49.0%; mean corpuscular volume (MCV) 40.7 fL, RI, 39.0–52.0 fL; mean corpuscular haemoglobin (MCH), 13 pg, RI, 13.0–21.0 pg; mean corpuscular haemoglobin concentration (MCHC), 32.1 g/dL, RI, 30.0–38.0 g/dL; red cell distribution width (RDW), 15.1%, RI, 14.0–18.0%; mean platelet volume (MPV), 10.8 fL, RI, 5.0–11.8 fL; platelet distribution width (PDW), 15.3 %, RI, 0–50%; procalcitonin (PCT), 0.207%, RI, 0.1–0.5%; lymphocyte, 1.0, RI, 0.8–7.0 x 10^3/µL; monocyte, 7.6 x 10^3/µL, RI, 0.0–1.9 x 10^3/µL; and granulocyte 2.2 x 10^3/µL, RI, 2.1–15.0 x 10^3/µL. The percentage of granulocytes was the highest (63.8%, RI, 12.0–45.0%), with other white blood cell percentages within the normal range (monocytes 5.3%, RI 2.0–9.0%; granulocyte 44.0%, RI 35.0 – 85.0%;), except for eosinophils that showed eosinophilia (eosinophile 4.6%, RI 0.0–10.0%).

The blood chemistry analysis (21st of February 2023) presented hyperglycaemia (177 mg/dL, RI, 70–150 mg/dL). However, other parameters were within the normal range, namely, alkaline phosphatase (ALP), 52 IU/L, RI, 10–90 IU/L; serum glutamic oxaloacetic transaminase/aspartate transaminase (SGOT/AST), 26 IU/L, RI, 9–40 IU/L; alanine aminotransferase (ALT), or serum glutamic pyruvic transaminase (SGPT), 20 IU/L, RI, 20–100 IU/L; total protein (TP), 7.4 g/dL, RI, 5.4–8.2 g/dL; blood urea nitrogen (BUN), 15 mg/dL, RI, 10–30 mg/dL; and creatinine, 0.8 mg/dL, RI, 0.3–21 mg/dL. Besides, the blood smear examination was carried out on the 1st of March 2023. The blood smear was stained with Giemsa staining, and the result revealed the presence of bacterial parasites (*Anaplasma phagocytophilum*) in the white blood cells (Figure 3) and Howel Jolly Bodies in the red blood cells (Figure 4). Ultrasonography (21st of February 2023): Ultrasonographic findings of the cat were two hyperechoic masses inside two hypoechoic pouches in the queen's uterus, suspected as two dead foetuses (no heartbeat) (Figure 3). Thus, according to the ultrasonography of the uterus, the cats were

diagnosed with pseudopregnancy, pyometra, and death foetuses.

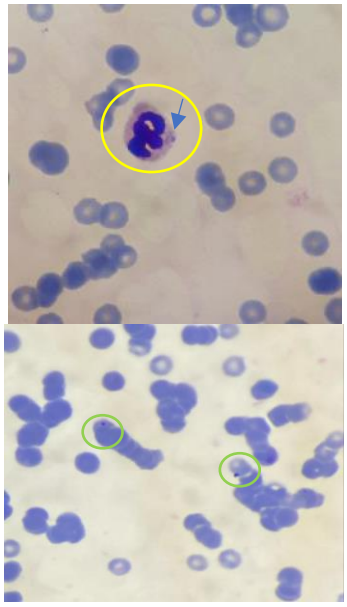


Figure 3 (Left). Blood smear of the cat from periphery blood vessels stained with Giemsa (Objective magnification: 100 x). The presence of *A. phagocytophilum* inclusion bodies (blue arrow) inside the cytoplasm of the white blood cells (yellow colour circle) of the cat patient; Figure 4 (Right). Blood smear of the cat from periphery blood vessels stained with Giemsa (Objective magnification: 100 x). The presence of Howel Jolly Bodies (Green colour circle). In 11 fields of view in the microscope, 8 Howel Jolly Bodies can be found.

Roentgen (22nd of February 2023): X-rays of the thoracic and abdominal cavity were obtained from the patient. Peripheral Structures: The result of roentgen of the thoracic region showed no abnormalities of the peripheral structures; there were no abnormalities in soft tissue, trachea, thoracic spine, diaphragm, stomach, liver, fat, sternum, skinfold, and ribs. Heart: The analysis of the clock analogy of the heart did not reveal any abnormalities in the left atrium, left ventricle, right ventricle, main pulmonary artery, right atrium, and aorta (Figure 5). The result of the vertebral heart size (VHS) score calculation in the lateral view was 7.2 V, which is within the normal range (6.8–8.1 V) (Figure 6). The calculation results show that there is no enlargement of the heart organ. Abnormalities found in the *vena cava caudalis* (Figure 7) might be due to atrioventricular enlargement, increased blood pressure, etc. There were also abnormalities found in all lobes of the lung field in the form of a bronchial pattern (blue arrows) (Figure 8) that can be due to bronchitis, bronchiectasis, pneumonia, etc. Pleural Space and Mediastinum: No abnormalities were found in the pleural space and mediastinum (trachea, thymus, lymph nodes, oesophagus, and aortic arch).

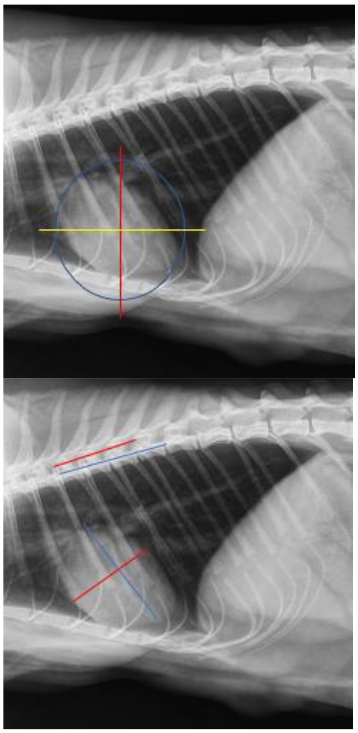
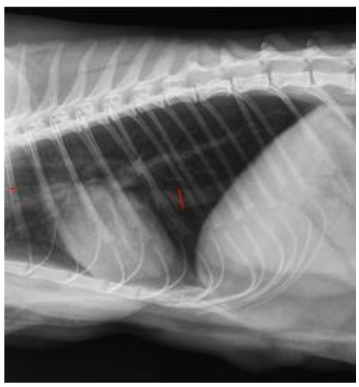


Figure 5 (left). The X-ray of the thoracic cavity. The clock analogy of the patient's heart revealed no abnormality in the position of each part of the heart; Figure 6 (right). The X-ray of the thoracic cavity. The vertebral heart size (VHS) score calculation in the lateral view was 7.2 V.



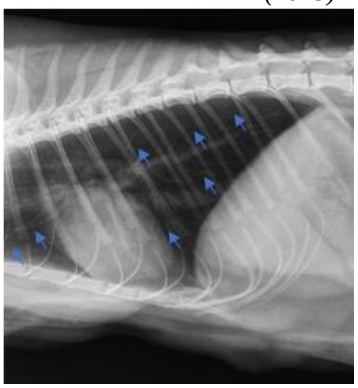
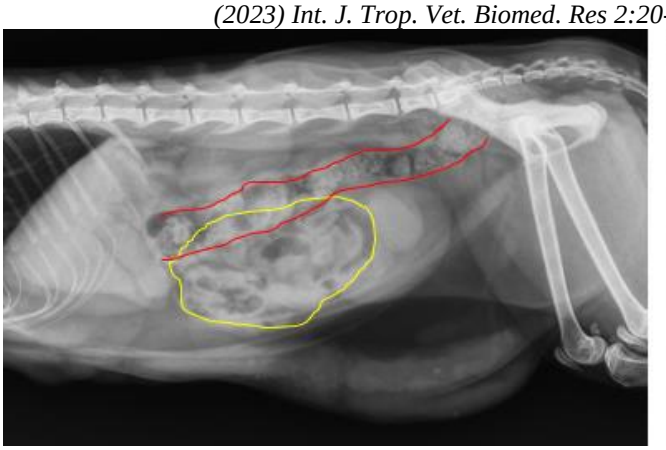


Figure 7. Lateral view of the X-ray of the thoracic cavity noted enlargement of *the vena cava caudalis* (red colour); Figure 8. Lateral view of the X-ray of the thoracic cavity showed a bronchial pattern within all lobes of the lungs (blue arrows).

Roentgen of Abdominal Region. Peripheral Structures: The result of the roentgen of the abdominal region showed no abnormalities found in peripheral structures in the abdominal area (hind legs, thoracic, lumbar spine, sacrum, coccygeal, pelvis, and diaphragm). However, abnormalities found in the soft tissue of the ventral abdomen were radioopaque masses (Figure 9) that can be caused by pseudopregnancy, breast-feed/pregnant period, mastitis, mammary tumours, abscesses, etc. The radio-opaque masses confirm the physical examination of the enlargement of mammary glands. Liver and stomach: No abnormalities were found in the liver and stomach. Intestines: The X-ray showed abnormalities in the small intestine (yellow line) and large intestine (red line). The lumen of the small intestine was seen to be mostly radiolucent (gas) opaque, and the rest were metabolic products with wall margins that appeared to have changed in opacity to become more radiopaque. The changes in radio-opacity in the small intestine can be caused by enteritis, gas accumulation, etc. The large intestine (colon) seems to have a normal lumen size with radiopaque opacities (faeces that appear solid) and slightly radiolucent (gas) (Figure 10 and Figure 11). Changes in the large intestine can be caused by enteritis, accumulation of body metabolic waste, etc. Kidneys: There was no abnormality found in both right and left kidneys. Spleen: Abnormalities of the spleen were observed in the form of changes in shape and size that can be caused by splenomegaly, congestion, etc. The spleen looks large and has a blunt tail (Figure 12 and Figure 13). Urinary Bladder: Abnormalities were found in the urinary bladder in the form of changes in size and opacity. The urinary bladder appears large, with a radiopaque lumen (Figure 14 and Figure 15). Peritoneum: No abnormality was found in the peritoneum. Reproductive Organs: Abnormalities were found in the uterus in shape, size, and opacity changes. A large uterus appears superimposed on the urinary bladder (Figure 16 and Figure 17).

Figure 9. Lateral view of the X-ray of the abdominal region. Radio-opaque masses in the ventral part of the abdomen (marked with yellow colour) (Figure 7)



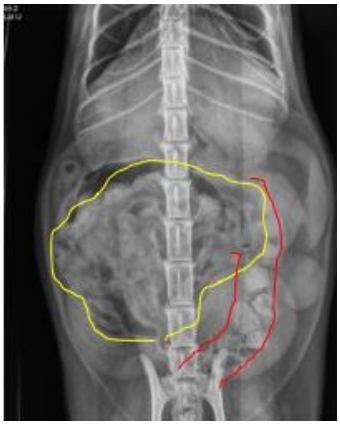


Figure 10 (Left; lateral view of the X-ray of the abdominal region and Figure 11 (Right; Ventrodorsal view of the X-ray of the abdominal area). The small intestine (yellow-coloured mark) lumen was seen to be mostly radiolucent (gas) opaque, and the rest were metabolic products with wall margins that appeared to have changed in opacity to become more radiopaque. The large intestine (colon) (red-coloured mark) seems to have a normal lumen size with radiopaque opacities (faeces that appear solid) and slightly radiolucent (gas).

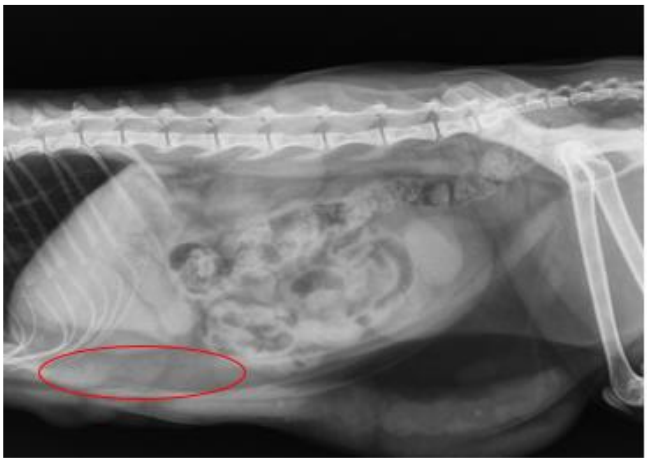


Figure 12 (Left; lateral view of the X-ray of the abdominal region and Figure 13 (Right; Ventrodorsal view of the X-ray of the abdominal area). The spleen (red-coloured mark) was seen as abnormal in shape and size. The spleen is enlarged and has a blunt tail.

Figure 14 (Left; lateral view of the X-ray of the abdominal region and Figure 15 (Right; Ventrodorsal view of the X-ray of the abdominal area). The urinary bladder (blue colour mark) showed changes in size and opacity. The urinary bladder appears large, with a radiopaque lumen.

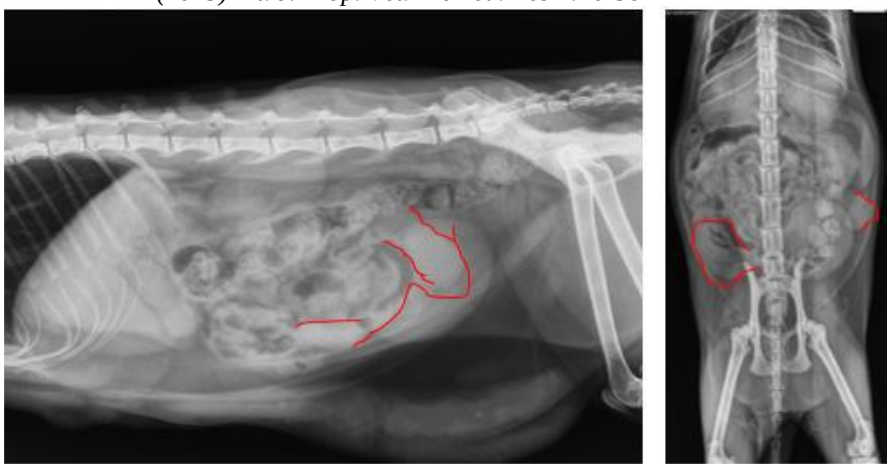


Figure 16 (Left; lateral view of the X-ray of the abdominal region and Figure 17 (Right; Ventrodorsal view of the X-ray of the abdominal area). There are abnormalities in reproductive organs (red colour marks) shown as shape, size, and opacity changes. A large uterus appears superimposed on the urinary bladder.

Diagnosis: The patient's diagnosis according to physical examination with additional information from haematology, blood chemistry analysis, blood smear, and medical imaging (ultrasonography and roentgen) were pseudopregnancy, open cervix-type pyometra, and bacterial parasitic anaplasmosis. Thus, the main treatment option is ovariohysterectomy surgery, with pre-and post-surgery medications according to the patient's symptoms or needs.

Prognosis: The prognosis of the patient was dubious.

Surgery: The ovariohysterectomy surgery was performed on the 2nd day of hospitalization (22nd of February 2023). (1) Surgery Preparation. The surgical procedure begins with anaesthesia using a combination of parenteral injections (ketamine HCl 10% and acepromazine). The dose of ketamine given was 10 mg/kg body weight, while the dose of acepromazine was 0.5 mg/kg. Both ingredients were mixed in a syringe and administered via an intramuscular route. Subsequent maintenance anaesthesia was given by inhalation of isoflurane. After the animals were anesthetized, the operation area was prepared by shaving and scrubbing with 4% chlorhexidine, water, 70% alcohol, and 10% povidone-iodine sequentially. The patient's surgery site was 10 cm each to the left, right, cranial, and caudal from the midline *medianus ventralis*. After the surgery site was clean, the patient was placed on the surgery table in the spinal recumbency position, and the four legs were restrained to the corner of the table. The surgical gauze and sterile surgical instruments were prepared on the tool table near the surgery table.

(2) Surgery Procedures. The incision was made 1 cm caudal to the umbilical cord. A skin incision was made 4 cm long. Then, the dissection was carried out until the *linea alba* was exposed, and this was continued by opening the abdominal muscles around 4 cm. Abdominal cavity exploration begins with the hypogastric direction to find the urinary bladder and bifurcation. Exploration was carried out manually through the surgeon's hands. After the bifurcation was found, the surgeon continued to search the ovarian pedicle. The ovariectomy technique used was the three-clamp method. Clamping was carried out using three haemostatic forceps in the cranial area of the ovary. Ligation was performed using 3/0 absorbable polyglycolic acid on the cranial haemostats. Ligation was performed in duplicate using two ligation techniques: figure-eight ligatures and full ligation. Ligation was confirmed to form an anaemic ring. Ovariectomy (cutting the ovary) was performed between the two haemostats. The same technique was performed on the

other ovary. The surgeon checked the bleeding on the remaining ovarian pedicles before placing them back into the abdominal cavity. When there is no bleeding, the remaining ovarian pedicle can be inserted into the abdominal cavity through the surgical incision.

The next stage was a hysterectomy (cutting the uterus) using the same method (three-clamp method). Clamping with haemostatic forceps was carried out as far as possible from the bifurcation to reach much of the uterine body. Ligation was carried out in duplicate using 3/0 absorbable polyglycolic acid. The first ligation position was the caudal haemostat in each blood vessel of the uterine corpus. The second ligation was caudal to the first ligation, with full ligation reaching the entire uterine body. Ligation was confirmed to form an anaemic ring. The hysterectomy procedure was performed between two haemostats. The surgeon checked the possible bleeding on the remaining uterine body before placing it back into the abdominal cavity. When there is no bleeding, the remaining uterine body can be inserted into the abdominal cavity through the surgical incision. The ovaries and the uterus were cut in this pyometra case, as seen in Figure 18. Then, the last procedure of surgery was closure. The closure of the abdominal cavity was performed in 3 layers, namely, (1) abdominal muscles and peritoneum using a simple interrupted suture technique; (2) abdominal fat using a simple continuous suture technique; and (3) subcuticular tissue with subcuticular suture technique (Figure 19). Suturing Layer 1 and Layer 2 was carried out using 3/0 absorbable polyglycolic acid, while suturing Layer 3 was performed using 4/0 absorbable polyglycolic acid.

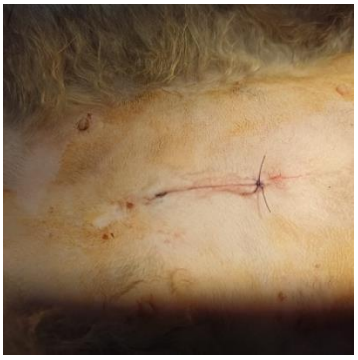


Figure 18 (Left). The uterus and ovaries after ovariohysterectomy surgery. Figure 19 (Right) The skin surface of the patient with subcutaneous sutures in the *linea alba* of the abdomen after the ovariohysterectomy surgery procedure.

Treatment: Immediately after physical examination (1st day of hospitalization), the patient was treated with intravenous infusion (NaCl and Lactated Ringer, interchangeably), Hematodin® (Imported by Romindo Primavetcom; consists of Taurine, Ammonium Ferric Citrate, DL-Methionine, Histidine HCI, DL-Tryptophan, Cobalt Acetate, Cyanocobalamin (B12), and Citric Acid Anhydrous) injection 0.5 mL, Biodin® (Imported by Romindo Primavetcom; consists of Adenosine Triphosphates (ATP), Magnesium Aspartate, Potassium Aspartate, Sodium Selenite, and Vitamin B12) injection 0.5 mL, Tolfedin® (tolfenamic acid) injection 0.38 mL, and Baytril® (enrofloxacin) injection 0.2 mL. After the surgery, the treatments were Baytril® (enrofloxacin) at 5.0 mg/kg twice daily for seven days. Starting from the 25th of February 2023, the patient was also given a nonsteroidal anti-inflammatory drug (meloxicam) 0.1 mg/kg body weight that was mixed with Pronicy® (Cyproheptadine, an antihistamine), vitamin A, and vitamin E; and Coatex® (Linoleic Acid (LA), Gamma Linoleic Acid (GLA), Eicosapentaenoic Acid, Docosahexaenoic Acid, Vitamin E, Vitamin A, Vitamin D_3) one capsule daily for seven days. One tablet of Drontal Cat® anthelmintics (80 mg pyrantel and 20 mg praziquantel) was given once on the 25th of February, 2023. Starting from the 1st of

March 2023, the patient was given oral doxycycline 10 mg/kg body weight twice daily for 21 days. Starting from the 7th of March 2023, the patient was given Nutriplus Gel® (Produced by Virbac, which consists of Vitamin A, Vitamin D, Vitamin E, Vitamin B1, Vitamin B2, Vitamin B6, Vitamin B12, Nicotinamide, Calcium Pantothenate, Folic Acid, Fe, Iodine, Manganese, and Magnesium) one tablespoon twice daily.

After three days of hospitalisation, the cats showed clinical signs of a pale mucous membrane, while on the fourth day of hospitalisation, the capillary refill time of cats was more than 2 seconds. During hospitalisation, the body temperature of the patient was fluctuating. The body temperature was usually normal; at other times, it decreased to subnormal, such as on the day and the next day after ovariohysterectomy (36.8 and 37.4 °C). However, at different times, the cat showed pyrexia, especially on the 6th day (39.7 °C), the 7th day (39.5–39.7 °C), and the 8th day (40.0 °C) of hospitalisation. Bloody and purulent vaginal discharge was reported on the day of the hospital visit or the 1st day of hospitalisation and disappeared after ovariohysterectomy surgery or on the 2nd day of hospitalisation. Force-feeding was carried out when the patient was inappetence. After first-time doxycycline administration, the patient showed symptoms of vomiting; however, this symptom occurred once. After the blood smear findings were obtained, the cat was prescribed doxycycline 10 mg/kg twice daily for 21 days. The cat's condition improved three days after treatment began, the clinical indications faded, and the patient regained his appetite; the patient was discharged from the hospital, continuing doxycycline therapy at home.

Discussion

No information exists about co-occurrence, pseudopregnancy, pyometra, and *A. phagocytophilum* infection in cats. However, this case report confirmed the cat's diagnosis with pseudopregnancy, pyometra, and anaplasmosis due to *A. phagocytophilum* infection. Clinical features during physical examination, namely, enlarged abdomen, distended mammary glands, and mass inside the belly. Ultrasonographic findings exhibited two hyperechoic masses inside two hypoechoic pouches in the queen's uterus, suspected as two dead foetuses, as no heartbeat was observed. The confirmation of an enlarged uterus during the ovariohysterectomy procedure supported the diagnosis.

This case reported a pseudopregnancy that led to pyometra. The clinical signs of pyometra in the cat patients of this study were in line with previous reports, anorexia, haemorrhagic and purulent vaginal discharge, stupor, distended abdomen, and fever. However, vaginal discharge may not be observed in queens that have thorough grooming behaviour or in closed pyometra cases (Hasan et al., 2021; Holst, 2022). This study showed the presence of inflamed and enlarged uterus with the presence of discharge inside through ovariohysterectomy confirmed the diagnosis of open cervix-type pyometra (Hagman, 2022, 2018; Hollinshead and Krekeler, 2016; Holst, 2022). Ovariohysterectomy is strongly recommended to treat pyometra in cats. There is, however, a medicinal approach to pyometra treatment with risk and variability in the success rate (Hollinshead and Krekeler, 2016). The symptom of valvular discharge of the patient disappeared starting the next day after the ovariohysterectomy. Besides, in this case, the patient did not display any issues related to peritonitis or other health concerns related to the surgery. Still, the fluid therapy prevented any complications following the procedure.

Pyometra in feline patients is considered a medical emergency in veterinary medicine because of various symptoms and laboratory results despite its diagnosis difficulty (Talukdar et al., 2022). The consequence is pyometra can progress for longer before the client brings their cat to the veterinary clinic, thus leading to more severe disease (Hagman, 2018; Palupi et al., 2022). A tentative diagnosis of pyometra

can be determined through anamnesis, signalment (older intact queens), clinical findings, haematology, blood chemistry (including acute phase proteins), and medical imaging (ultrasonography and/or radiography of the ovaries and uterus) (Holst, 2022; Johnson, 2022; Talukdar et al., 2022). This diagnosis approach was performed in this case report. The risk of pyometra increases with age; the disease is most common in middle-aged and older cats (Holst, 2022). The presence of foetuses is effectively protective against the development of pyometra; thus, the risk of uterine disease increases for each non-pregnant oestrous cycle (Hollinshead and Krekeler, 2016). In this case study, in addition to the ovariohysterectomy procedure, the patient obtained medical treatment for patients' post-surgery (such as analgetic and antipyretic) and medical treatment of pseudopregnancy and pyometra according to the patient's symptoms. The antibiotic administered was enrofloxacin (5 mg/kg twice daily) for eight days (De Faria and Norsworthy, 2008). Enrofloxacin targeted the uterus directly (Davidson and Black, 2015) and broad-spectrum (Plumb, 2008).

In addition to hormonal factors, pyometra also involves bacterial elements. *E. coli* is the main isolated species in cat pyometra and is associated with severe clinical cases (Holst, 2022; Lopes et al., 2021). In the current study, bacterial species in pyometra were neither cultured nor identified. However, bacterial culture and antibiotic susceptibility test patterns should be carried out whenever possible, and antibiotic therapy should be adjusted to the results (Johnson, 2022). Bacterial species primarily isolated in most pyometra cases are uro-pathogenic *E. coli*. Other bacterial species reported to be isolated from cat pyometra cases are *Staphylococcus* spp., *Proteus* spp., *Streptococcus* spp., *Nocardia* spp., *Enterobacter* spp., *Pasteurella* spp., and *Klebsiella* spp. Infection of the uterus is assumed to occur via vaginal ascension of faecal bacteria during oestrus during cervix dilatation (Hagman, 2018; Hollinshead and Krekeler, 2016; Lopes et al., 2021).

Infection by *A. phagocytophilum* between owned cats in Indonesia has rarely been described. However, nationwide prevalence studies have not been performed. Disease caused by *A. phagocytophilum* infection, called anaplasmosis, is essential in human medicine as the disease caused by it is zoonotic. This study, however, first reported the diagnosis of feline granulocytic anaplasmosis due to *A. phagocytophilum* infection in Indonesia. The current study's clinical signs of feline anaplasmosis are pyrexia, anaemia, and anorexia. Anaplasmosis diagnosis was approached with febrile on the first day of hospitalisation and fluctuating febrile, pale mucous membrane during patient hospitalisation, and inappetence. However, non-specific clinical signs of *A. phagocytophilum* infection in cats reported by previous studies are lethargy, anorexia, fever, and dehydration (Savidge et al., 2016). In addition, the clinical pathology abnormalities of cats infected with *A. phagocytophilum* are lymphopenia and hyperglycaemia with or without thrombocytopenia (Savidge et al., 2016). The blood sample taken for both haematology and blood chemistry analysis was performed on the day of the patient's hospital visit. However, haematologic features of the current case are thrombocytopenia, leukopenia, and eosinophilia. These haematologic and blood chemistry results, however, did not represent the case of pyometra, as cats with pyometra normally showed leukocytosis (Motawakkel et al., 2023; Uçmak and İslamoğlu, 2023). This case agreed with previous laboratory examination reports of *A. phagocytophilum* infection, namely, leukopenia due to lymphocytopenia, neutropenia, and thrombocytopenia (Woldehiwet, 2010). These haematologic alterations (including leukopenia) and platelet dysfunction (the synonym of thrombocytopenia) in *A. phagocytophilum* infection can cause further potential clinical manifestations such as bleeding diatheses, haemorrhage, toxic shock–like syndrome, and even death (Borjesson, 2005). In

addition, the patient considered hyperglycaemia in blood chemistry analysis. Hyperglycaemia has been documented in feline anaplasmosis due to *A. phagocytophilum* infection (Heikkilä et al., 2010; Schäfer et al., 2022; Schäfer and Kohn, 2020). Additional information from this study is the presence of *A. phagocytophilum* in cat granulocytes. *A. phagocytophilum* has been reported to infect granulocytes (Little, 2010; Morelli et al., 2021; Sainz et al., 2015), especially neutrophils and eosinophils (Heikkilä et al., 2010). However, *A. phagocytophilum* can also infect other cell types, such as megakaryocytes, myeloid cells, and endothelial cells, although the significance of these cell types in disease pathogenesis is unclear (Sykes and Foley, 2014). During the period of bacteraemia, the main targets of infection are eosinophils, neutrophils, and monocytes, with the latter being infected at the end of a primary bacteraemia.

Anaplasma phagocytophilum is a Gram-negative bacterium that lives as a parasites obligating inside host cells (Schäfer and Kohn, 2020). Previously, it was known as *Ehrlichia phagocytophilum* and *Ehrlichia equi* (Schäfer and Kohn, 2020). Another species of the genus *Anaplasma* is *Anaplasma platys,* but *A. platys* only infects dogs and does not infect cats (Sykes and Foley, 2014). Anaplasmosis, caused by *A. phagocytophilum* infection, is a tick-borne disease (Ahmed et al., 2020). The bacterium primarily infects dogs; however, cats can also suffer from the disease. *Anaplasma phagocytophilum* also infect other animals, such as sheep (Bauer et al., 2021), goats (Ghafar and Amer, 2019), horses (Gussmann et al., 2014), cattle (Dugat et al., 2017), deer (Santodomingo et al., 2023; Silaghi et al., 2020), and camels (Bahrami et al., 2018). The main reservoir hosts of *A. phagocytophilum* are wild ruminants and wild rodents (Schäfer and Kohn, 2020). Other wildlife animals, such as wild carnivores (Szewczyk et al., 2019), hares (Lesiczka et al., 2021), wild birds (Hoffman et al., 2020), reptiles (Nieto et al., 2009), and ticks (Hoffman et al., 2020), can also be the reservoirs for *A. phagocytophilum* for humans. To date, veterinary deaths from the pathogen have only been reported in sheep, cattle, horses, caribou, deer, elk, and dogs (Stuen et al., 2013).

In addition to animal infections, *A. phagocytophylum* can infect humans and cause granulocytic anaplasmosis; thus, the disease is zoonotic (Schäfer and Kohn, 2020), apart from *A. phagocytophilum*; *A. capra, A. ovis,* and *A. platys* of the genus *Anaplasma* have also been reported to infect humans (Hosseini-Vasoukolaei et al., 2014; Rikihisa, 2011; Sazmand et al., 2019). Human granulocytic anaplasmosis can lead to a possibly deadly emerging disease (Rikihisa, 2011). The transmission occurs by the vector ticks of the *Ixodes species* within the first 48 hours of attachment of the ticks to the hosts (Schäfer and Kohn, 2020). In addition, infection by opportunistic pathogens can also arise (Rikihisa, 2011). *A. phagocytophilum* cultivates only inside membrane-bound inclusions. This bacterium species multiply and reproduce, and the bacterium's inclusions occupy most of the cytoplasm of infected cells. Previous studies suggested three pathways of cytoplasmic vesicular transport for *A. phagocytophilum* inclusion formation, namely, (1) low-density lipoprotein (LDL) absorption, (2) autophagosomes, and (3) reprocessing endosome. *A. phagocytophilum* can acquire nutrients through these three pathways from the infected cells within the host while escaping NADPH oxidase and lysosomes (Rikihisa, 2011).

The identification of the causative agent, *A. phagocytophilum*, in this study was carried out through microscopy. The principle of microscopy of *A. phagocytophilum* is the detection of a single inclusion of a bacterium (0.54–1.3 µm) or colonies (synonym: morulae 1.5–5.0 µm) inside a granulocyte (neutrophile or eosinophile) cytoplasm (Heikkilä et al., 2010). The proliferation of *A. phagocytophilum* in host cells can be accumulated in vacuoles known as "morulae." These morulae (or

macrocolonies of this bacterium) can be observed using a light microscope after Romanowsky or Giemsa staining (Massung et al., 2006; Nguyen Trong et al., 2019). The detection of *A. phagocytophilum* was done by discovering the parasite recognized as dark blue-purple intracytoplasmic inclusions located in the cytoplasm of the white blood cells (peripheral blood monocytes or granulocytes). The variety of look of these morulae is due to highly basophilic loose or condensed aggregates of individual cells visible within the vacuole. However, there are some limitations of blood smear microscopy. It is a monotonous and time-consuming procedure, and detecting *A. phagocytophilum* presence with good staining is inadequate, especially when the animals are carriers. Microscopy is highly indicative, and despite its low sensitivity (Schäfer and Kohn, 2020), it is considered reliable diagnostics in the early stage of the disease (Matsumoto et al., 2006; Schäfer and Kohn, 2020; Woldehiwet, 2010; Zhang et al., 2022). However, other methods exist, such as polymerase chain reaction (PCR) and serology (Bauer et al., 2021). Commercially available test kits (For example, SNAP® 4Dx®Test and SNAP 4Dx® Plus Test) can detect the presence of antibodies to *A. phagocytophilum/A. platys* in the blood, along with up to 6 other diseases (heartworm infection, Lyme disease (*Borrelia burgdorferi*), and ehrlichiosis (antibodies to *Ehrlichia canis*, *E. chaffeensis*, or *E. ewingii*)) (Evason et al., 2019). The use of these test kits is widespread among Indonesian veterinarians.

In this case, feline anaplasmosis was treated by administration of doxycycline. As the drug of choice for feline anaplasmosis, the dose of doxycycline was 10 mg/kg once to twice daily for 14 to 28 days (Plumb, 2008; Schäfer et al., 2022). In this study, doxycycline was administered to the patient with a single dose of 10 mg/kg for 21 days. Doxycycline may trigger gastrointestinal side effects, such as vomiting (Plumb, 2008), that can be observed in this study. However, the cat only vomited once when given the first doxycycline administration. Doxycycline has also been used for the treatment of canine borreliosis (5 mg/kg body weight twice daily) (Gatellet et al., 2019) or ehrlichiosis (10 mg/kg body weight twice daily) (Lang et al., 2011). The application of acaricidal products, such as (1) spot-on combination of fluralaner and moxidectin (Rohdich et al., 2018), (2) spot-on lotilaner (Cavalleri et al., 2018; Vatta et al., 2019), or (3) spot-on formulation of selamectin plus sarolaner (Geurden et al., 2017; Vatta et al., 2019), or (4) spot-on formulation of emodepside, praziquantel, and tigolaner (Cvejić et al., 2022), is advisable to cats living in tick endemic areas for treatment of acarine infections and prevention of anaplasmosis.

Conclusions

In conclusion, the diagnosis of pyometra and pseudopregnancy in cats can be approached from the history, clinical signs, laboratory, and imaging results. In the case of open cervix-type pyometra due to pseudopregnancy, immediate ovariohysterectomy surgery is the first procedure option once the diagnosis is confirmed in cats. According to the symptoms, direct antibiotic treatment and intravenous infusion with other therapies were performed in this study to increase the survival rate. Blood smear examination of cats with clinicopathology to anaplasmosis can be carried out to confirm the diagnosis of *A. phagocytophilum* infection to detect morulae in the granulocytes. Therapy applied in this patient for anaplasmosis was with doxycycline (10 mg/kg) twice daily for 21 days. Thirteen days after the first administration of doxycycline, all clinical signs of the patient disappeared, and the patient was discharged with continuing antibiotic treatment at home.

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