



Case report

Fatal vascular pythiosis in a dog from Thailand: Clinical presentation and pathological findings—first report: A case report

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Abstract

Vascular involvement in *Pythium insidiosum* infection is exceedingly rare in dogs and often results in delayed diagnosis due to nonspecific clinical signs. A one-year-old female German Shepherd presented with subcutaneous edema and mass-like lesions on the right hindlimb, caudal abdomen, and tail base. Computed tomography lymphangiography revealed lymphedema with impaired lymphatic drainage, and cytologic evaluation demonstrated pyogranulomatous inflammation, including multinucleated giant cells containing poorly stained, contorted fungal hyphae within the cytoplasm. Despite right hindlimb amputation, the disease progressed to ischemic necrosis, gangrene and eventual autoamputation of the left hindlimb and tail. Severe *P. insidiosum* infection was confirmed by histopathologic examination and immunochromatographic testing. Clinical deterioration continued despite aggressive antifungal and antimicrobial therapy, leading to euthanasia. Postmortem examination revealed extensive pyogranulomatous and necrotizing inflammation involving the aorta, caudal vena cava, kidneys, uterus and intestines. Broad, ribbon-like, sparsely septate hyphae were observed and confirmed as *P. insidiosum* by culture, and by Internal Transcribed Spacer Polymerase Chain Reaction. This case represents the first reported instance of vascular pythiosis in a dog, highlighting its rarity, severity and poor prognosis. Early recognition of vascular involvement is critical, as delayed diagnosis and limited therapeutic response are frequently fatal.

Keywords: Canine, Lymphangiography, Postmortem, Pythiosis, *Pythium insidiosum*, Vascular.

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INTRODUCTION

Pythium insidiosum is an aquatic oomycete that causes pythiosis, a potentially life-threatening infection affecting both humans and animals. Unlike true fungi, this organism lacks chitin in its cell wall and ergosterol in its cell membrane. *P. insidiosum* is endemic to tropical and subtropical regions, including Southeast Asia, northern Australia, South America and parts of the United States. It grows optimally at temperatures of 34–36 °C and can tolerate temperatures up to 45 °C (De Cock et al., 1987) which enables its persistence in warm surface waters. Infection typically occurs when motile zoospores gain entry through damaged skin or mucosal surfaces (Grooters, 2022). Pythiosis has been reported in multiple species, most commonly dogs, horses and humans (Gaastra et al., 2010).

Clinical classification of pythiosis is based on tissue tropism and histopathological features, and includes cutaneous/subcutaneous, gastrointestinal, ocular, vascular and disseminated forms (Krajaejun et al., 2006). In dogs, the disease predominantly affects young, large-breed individuals, most commonly presenting as cutaneous/subcutaneous or gastrointestinal forms (Yolanda and Krajaejun, 2022). Cutaneous pythiosis is characterized histologically by invasive granulomatous lesions with ulcerated nodules, draining tracts and pyogranulomatous inflammation, typically affecting the tail base, limbs, ventral neck or perineum (Grooters, 2022). Gastrointestinal involvement demonstrates transmural granulomatous inflammation, frequently associated with hemorrhage, necrosis and mucosal ulceration, which manifest clinically as vomiting, weight loss and hematochezia (Peano et al., 2023). Ocular pythiosis, although uncommon, may lead to severe granulomatous uveitis and corneal involvement (Yolanda and Krajaejun, 2022). Vascular pythiosis is exceedingly rare in animals and is defined by angioinvasion of medium-to-large arteries, leading to progressive granulomatous vasculitis, thrombosis, ischemic necrosis and gangrene of downstream tissues. Cases exhibiting vascular obstruction and contiguous progression from a cutaneous entry site are classified as vascular pythiosis, distinguishing them from the more common forms. In contrast, disseminated pythiosis is characterized by multifocal, noncontiguous involvement of various organs, including the brain, lungs, skin and abdominal viscera, resulting from hematogenous dissemination and generally associated with a poor prognosis.

Diagnosis of pythiosis relies on an integration of clinical, imaging and laboratory findings. Clinical signs such as limb swelling, non-healing ulcers or gangrene, combined with imaging evidence of vascular occlusion or aneurysmal changes, raise suspicion of vascular involvement. Histopathology demonstrating hyphal invasion of vessel walls, supported by cytological evaluation when available and molecular confirmation via Polymerase Chain Reaction (PCR) or serology are essential for definitive diagnosis (Krajaejun et al., 2006; Schloemer et al., 2013). Histopathological hallmarks of *P. insidiosum* include ribbon-like hyphae with pseudo-septation and right-angle branching. Although culture and identification of the organism remain the gold standard, molecular and serological methods provide critical diagnostic support, particularly in cases where fungal growth is limited (Norasetthada, 2025).

This report describes the first confirmed case of vascular pythiosis in a dog in Thailand. Comprehensive diagnostic evaluation, including clinical assessment, imaging, cytology, fungal culture, molecular testing, necropsy and histopathology, has documented progression from cutaneous to vascular involvement. The extensive vascular pathology observed parallels what has been reported in human cases, underscoring both the rarity and clinical significance of this presentation, and highlighting the importance of considering vascular involvement in dogs with compatible risk factors.

CASE DESCRIPTION

Signalment and history

A one-year-old intact female German Shepherd was presented with a three-month history of subcutaneous edema or mass formation involving the right hindlimb, right caudal abdomen and tail base (Figure 1A, 1B). There was no reported history of external trauma or accidents. The dog was housed in a backyard environment in the Salaya Subdistrict, Nakhon Pathom Province, Thailand.



Figure 1 Grossly visible lesions on affected body regions. A. A subcutaneous edema or a mass on the right hindlimb and right caudal abdomen. B. A subcutaneous edema or a mass at the base of the tail. C. Multiple progressive cutaneous ulcerative wound and necrotic lesions of the right hindlimb. D. Tail showing similar multifocal cutaneous ulcerative wound and necrotic lesions.

Physical examination

On clinical examination, the dog was in generally good condition, with a normal appetite and normal urination and defecation. However, partial weight-bearing and mild lameness of the right hindlimb were observed. Subcutaneous edema was evident in the right stifle region, and no external wounds were identified.

Diagnostic assessment and therapeutic management

At the initial visit, routine blood work, along with SNAP 4Dx® and multiplex PCR testing, was performed to assess general health status and to rule out common vector-borne diseases, including *Ehrlichia canis*, *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Dirofilaria immitis*, *Hepatozoon canis* and *Babesia* spp. The results revealed mild hypoalbuminemia, while all tests for blood-borne parasites were negative. Broad-spectrum antibiotic therapy (amoxicillin–clavulanic acid) and a nonsteroidal anti-inflammatory drug (NSAID)

were administered at the initial visit to address suspected infection and inflammation.

Approximately one week later, the dog was re-examined for clinical follow-up. At that time, there was no evidence of clinical improvement, and ulcerative skin lesions had developed. Therefore, fine-needle aspiration (FNA) was performed for further diagnostic investigation. Cytological examination of aspirates collected from the right hindlimb and tail base demonstrated numerous multinucleated giant cells mixed with neutrophils. Some giant cells contained poorly stained, contorted fungal hyphae within the cytoplasm, along with cellular debris and keratinous material, findings consistent with pyogranulomatous inflammation (Figure 2). Additionally, computed tomography (CT) imaging was scheduled to further delineate the size, extent and underlying characteristics of the subcutaneous mass.

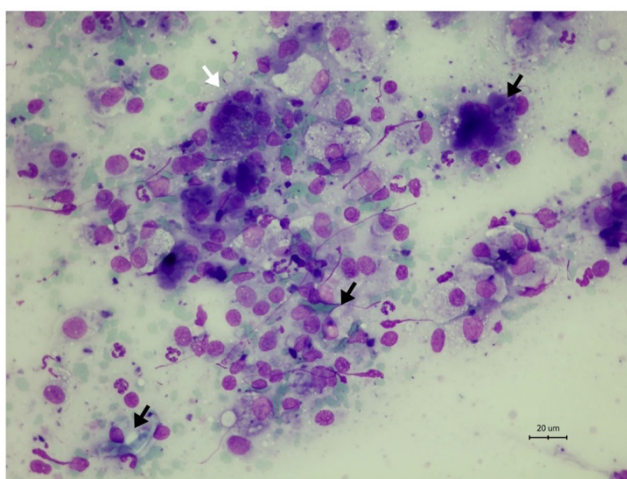


Figure 2 Cytological appearance (40x) of the subcutaneous lesion. Numerous multinucleated giant cells (white arrow), accompanied by neutrophils, are present within a background of cellular debris and keratinous material. Some giant cells contain poorly stained, contorted fungal hyphae within the cytoplasm (black arrow).

A Computed Tomography (CT) lymphangiography demonstrated lymphedema of the right hindlimb, a subcutaneous mass on the right caudal abdomen, sacral lymphadenopathy, and tail base swelling. This procedure, using an intra-metatarsal pad injection with iohexol (350 mg I/kg), identified abnormal lymphatic drainage. On the normal left hindlimb, contrast media migrated normally to the popliteal and medial iliac lymph nodes within 15 minutes. In contrast, on the right hindlimb, contrast media was retained in the edematous subcutaneous tissue for over 25 minutes, with only a few afferent lymphatic vessels enhanced. The right popliteal and medial iliac lymph nodes did not show any contrast enhancement, suggesting a compromised lymphatic system due to obstruction or damage of afferent lymphatic vessels (Figure 3). A post-contrast intravenous CT scan of the subcutaneous mass on the right caudal abdomen showed an ill-defined margin, shaped irregularly, and heterogeneous, contrast-enhancing parenchyma. The mass size was measured as 14 x 17 x 4.3 cm (width x length x thickness) with a surrounding hyperattenuating fat strand. By its location, some part invaded into the right pelvic cavity to the right iliac area via an inguinal ring and attached to the right external iliac artery and the right external iliac vein. The mass was likely able to obstruct vascular flow, causing a right external iliac artery aneurysm (Figure 4). The sacral lymph nodes, including both medial iliac, hypogastric and medial sacral lymph nodes, showed an elongated shape with a slightly enlarged size. The subcutaneous layer around the tail base was edema with mildly heterogeneous

contrast enhancement. Other organs, including urinary bladder, liver and both kidneys, were unremarkable.

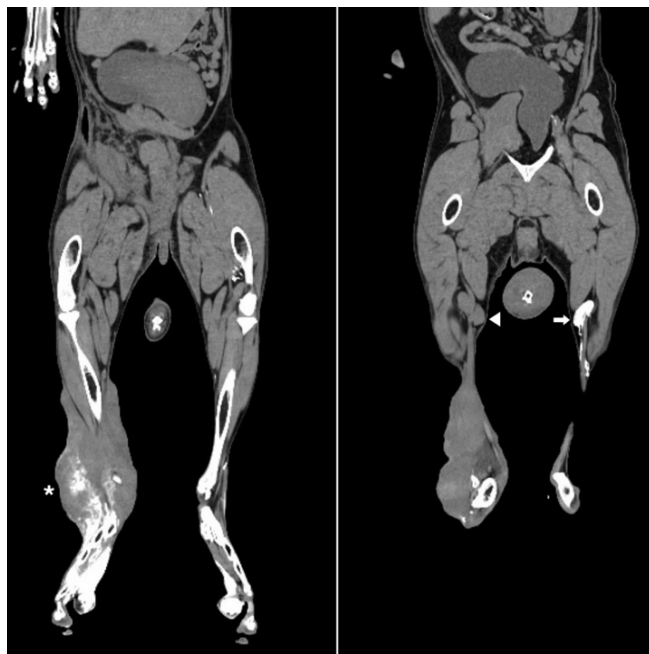


Figure 3 CT Lymphangiography. An obstruction or damage to afferent lymphatic vessels (star) resulting in the absence of contrast media in the right popliteal lymph node (arrowhead) compared to the left popliteal lymph node (arrow).



Figure 4 CT Angiography. The mass (black arrow), measuring 14 × 17 × 4.3 cm (width × length × thickness), extended into the right pelvic cavity and right iliac region through the inguinal ring and was adherent to the right external iliac artery and vein, resulting in vascular obstruction and aneurysmal dilation of the right external iliac artery (star), in comparison with the left external iliac artery (white arrow).

Three days following the CT scan, the right hindlimb and tail developed severe localized infections characterized by multiple progressive cutaneous ulcerative wounds and necrotic lesions (Figure 1C, 1D), necessitating amputation of the right hindlimb. Intraoperatively, a granulomatous mass with an associated fistulous tract was identified, extending from the caudal abdominal subcutaneous mass deep into the adjacent abdominal wall. Tissue samples were submitted for histopathological examination, bacterial culture and fungal culture to guide further therapeutic planning. Histopathological evaluation revealed an unencapsulated mass composed of multifocal to coalescing pyogranulomatous inflammation. Gomori's methenamine silver (GMS) staining identified poorly septate, ribbon-like fungal hyphae (6–12 µm wide) with irregular, nonparallel walls within granuloma centers and multinucleated giant cells. Peripheral infiltrates of lymphocytes and plasma cells, along with fibroblast proliferation, were also present. These histopathological features supported a diagnosis of multifocal to coalescing pyogranulomatous dermatitis with intralesional fungal organisms, most consistent with pythiosis. This diagnosis was further corroborated by a positive *Pythium insidiosum* antibody result, obtained using an immunochromatographic test (ICT). Bacterial culture yielded Group D *Streptococcus* and *Escherichia coli*, both of which were susceptible to amoxicillin–clavulanic acid, and showed intermediate susceptibility to marbofloxacin, whereas fungal culture showed no growth. Five days postoperatively, the amputation wound dehiscd, and the contralateral (left) hindlimb developed marked edema acutely. Further examination revealed weak to absent peripheral arterial pulses in the left hindlimb, followed by progressive tissue ischemia characterized by coldness, discoloration, and severe pain. These changes advanced to extensive tissue necrosis and gangrene of the left hindlimb and tail (Figure 5), ultimately resulting in auto-amputation.



Figure 5 Gross appearance of tail and hindlimb gangrene with auto-amputation. A. The tail showing extensive tissue necrosis and gangrene. B. Auto-amputation of the tail. C. Progressive tissue ischemia of the left hindlimb and tail, characterized by coldness, discoloration, severe pain, and gangrene. D. Early stage of auto-amputation of the left hindlimb.

The dog subsequently developed clinical signs consistent with sepsis. Blood culture was performed and yielded *Enterococcus faecium*, which was resistant to all tested antibiotics, and *Escherichia coli*, which was susceptible to amikacin, ceftriaxone, marbofloxacin and meropenem. Based on these results, meropenem therapy was initiated. Although fungal culture results were negative, antifungal therapy with itraconazole and terbinafine was administered due to a strong clinical suspicion of pythiosis, as described above. Wound management included twice-daily irrigation with sterile saline, surgical debridement of necrotic tissue, and topical application of terbinafine cream. Despite these interventions, necrosis progressed extensively, ultimately involving the left hindlimb and tail.

Treatment Response and Outcome

After several weeks of aggressive treatment, the dog developed severe gastrointestinal signs, including vomiting and hemorrhagic diarrhea. Abdominal ultrasonography indicated a grave prognosis, revealing marked thickening of the gastric and pyloric walls (0.6 cm and 0.9 cm, respectively) with hyperechoic mucosa. The right medial iliac lymph nodes were enlarged and exhibited heterogeneous parenchyma, and a heterogeneous mass was identified in the medial iliac region. Portions of this mass (measuring 0.5 × 2.0 cm and 0.8 × 2.0 cm) extended into the caudal vena cava, resulting in partial obstruction of blood flow. Both kidneys showed ultrasonographic features consistent with nephritis, including heterogeneous hyperechoic parenchyma, thin hyperechoic medullary rims, and mild right-sided pyelectasia (1.2 cm), with no evidence of obstructive uroliths. A small volume of peritoneal effusion was also present. At this stage, the disease had progressed extensively and was refractory to therapy. Given the poor prognosis and declining quality of life, euthanasia was ultimately elected as a humane course of action.

Postmortem Examination

Postmortem examination revealed the right hindlimb had been amputated at the coxofemoral joint via disarticulation, while the left hind limb and tail showed necrosis and autoamputation. At the right inguinal surgical site, extensive necrotic tissue and purulent exudate were observed near the abdominal aorta. An irregular, firm mass adhered to the external surface of the aorta. On longitudinal section, the vessel showed features of partial obstruction, including an intraluminal mass, poorly demarcated granulomatous tissue in the vessel wall, luminal narrowing, and an irregular luminal surface (Figure 6A). Adjacent to this, the abdominal caudal vena cava contained a large, firm, tan to reddish-brown thrombus that projected into the lumen, causing partial obstruction. The lesion appeared adherent to the luminal surface and was consistent with a mural thrombus (Figure 6B). Surrounding vascular tissue was congested, mildly thickened, and coated with purulent exudate. The right kidney exhibited severe disruption of its normal architecture. The cut surface revealed multifocal to coalescing white to yellowish caseous necrotic material extending from the cortex to the medulla and pelvis (Figure 6C). The corticomedullary junction was obscured by partially infiltrative lesions, and the renal pelvis was distorted. The left kidney showed mild involvement with similar lesions and mild congestion. The right uterine horn contained multifocal, firm, round, white to grey nodules that had likely originated in the myometrium. The uterine body was also enlarged and distended. A black firm thrombus was observed within the lumen of a small artery adjacent to the uterine body, causing complete obstruction. The gastrointestinal tract, especially the stomach and small intestine, showed mucosal thickening and dark red discoloration, while the large intestine had ulcerative lesions (Figure 6D).

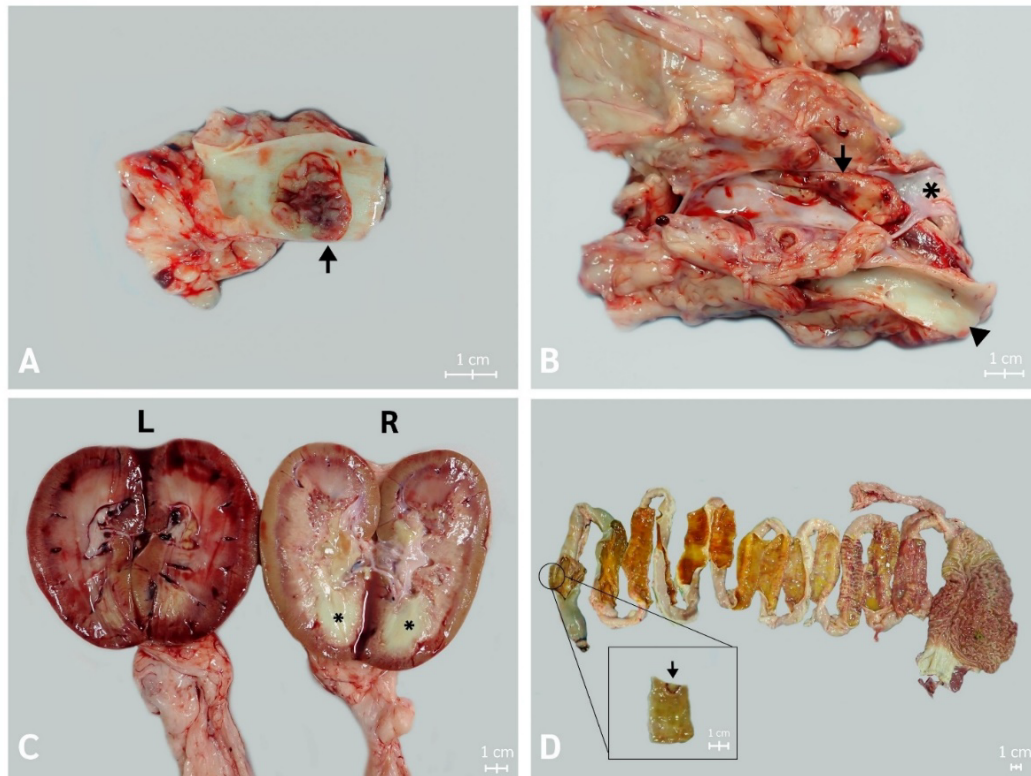


Figure 6 Macroscopic findings. A. Longitudinal-section of the aorta showing an intraluminal mass (arrow), granulomatous vascular wall, luminal narrowing, and irregular intimal surface. B. Abdominal segment of the caudal vena cava (star) adjacent to the aorta (arrowhead), containing a firm tan to reddish-brown thrombus (arrow) projecting into and partially occluding the vascular lumen. C. Cut surface of the right kidney showing multifocal to coalescing white to yellowish caseous necrotic areas extending from cortex to medulla and pelvis (star). D. Gastrointestinal tract showing diffuse mucosal thickening and dark red discoloration, most pronounced in the stomach and duodenum, with a large ulcerative lesion present in the intestine (arrow).

Histopathological Analysis

The tissues from the aorta, caudal vena cava, kidney, uterine horn, uterine body and large intestine, were collected and fixed in 10% neutral buffered formalin for 24 hours. Sections were cut to a 3 μm thickness and stained with hematoxylin and eosin (H&E), and Gomori's Methenamine Silver Stain (GMS). All samples showed extensive necrotizing and granulomatous inflammation characterized by scattered eosinophils intermixed with lymphocytes, plasma cells, neutrophils, and multinucleated giant cells. Broad, ribbon-like, sparsely septate hyphae (4–10 μm wide) were identified within the lesions. These hyphae exhibited irregular, non-dichotomous branching with non-parallel walls and stained poorly with H&E, but were GMS positive (Figure 7A, 7B). In both the aorta and the caudal vena cava, large granulomatous lesions were observed extending into and firmly adherent to the perivascular adipose tissue, accompanied by extensive areas of necrosis and dense inflammatory cell infiltration. The aortic lesion was more severe, showing transmural granulomatous inflammation predominantly involving the tunica media, with focal disruption and rupture of the tunica intima. The affected intimal region was replaced by necrotic debris, abundant inflammatory infiltrates, and invasive fungal elements (Figure 7C, 7D). In addition, fungal invasion was identified within small vessels in the tunica adventitia of the aorta, with extension into their tunica media (Figure 7E, 7F).

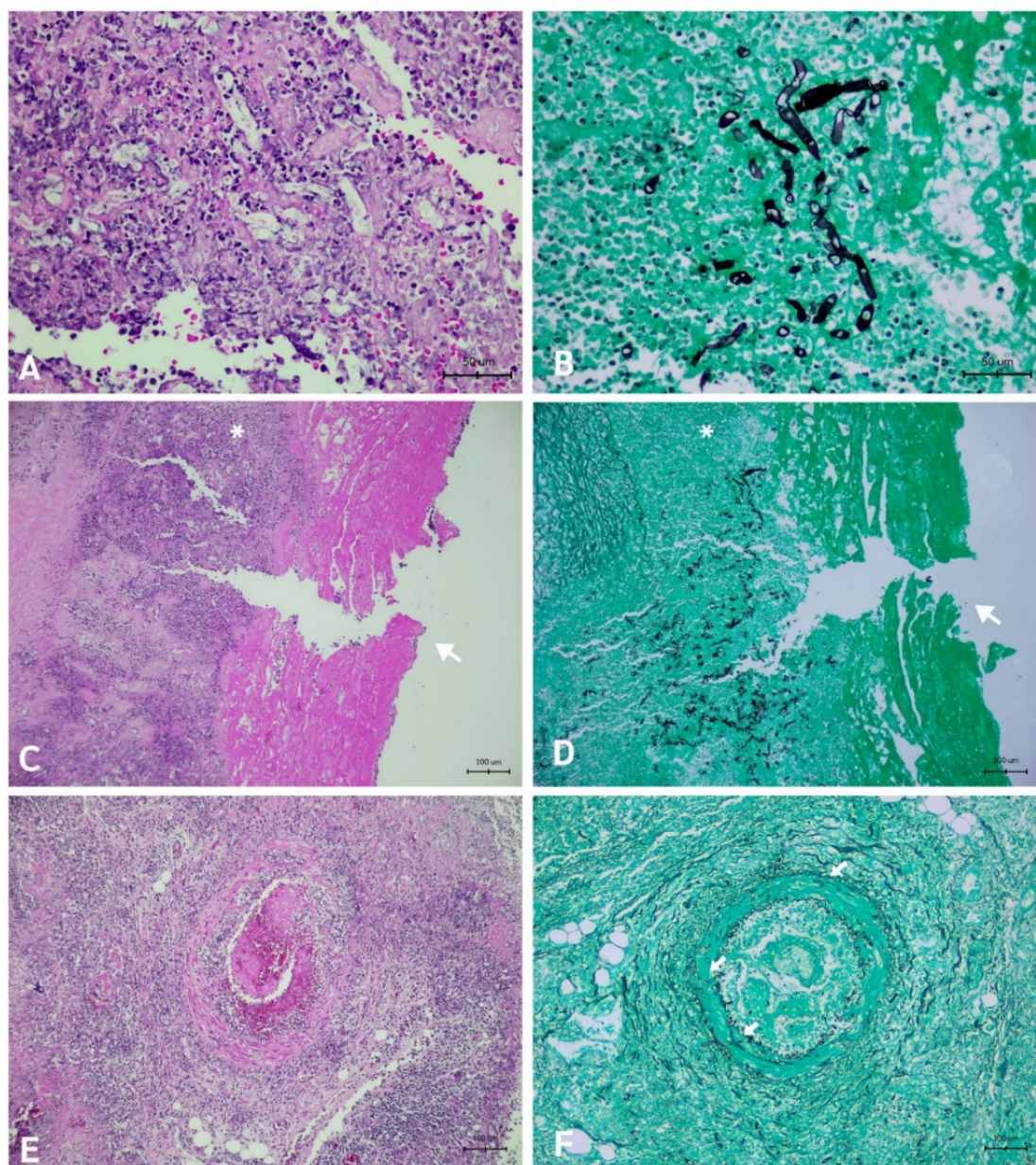


Figure 7 Microscopic findings. A–B. Broad, ribbon-like, sparsely septate hyphae (4–10 μm) with irregular, non-dichotomous branching, poorly stained with H&E, positive with GMS. C–D. Severe aortic lesion characterized by granulomatous inflammation involving the tunica media (star) and rupture of the tunica intima (arrow), which was replaced by necrotic debris, inflammatory infiltrates, and fungal invasion. E–F. Small vessels in the aortic tunica adventitia showing fungal invasion into the tunica media (arrows).

In the caudal vena cava, a mural thrombus was identified, firmly adherent to the vascular wall and projecting into the vascular lumen. Histologically, the thrombus was characterized by prominent inflammatory cell infiltration, extensive coagulative necrosis, and marked vascular congestion. Fungal hyphae were observed within the thrombus, with invasion extending into the tunica intima and tunica media. The right renal parenchyma exhibited multifocal to coalescing granulomatous and necrotizing interstitial nephritis, with granulomas primarily located in the cortex and extending into the medulla. These granulomas showed central necrosis surrounded by inflammatory infiltrates containing fungal hyphae

(Figure 8A, 8B). The right uterine horn showed multifocal granulomatous and necrotizing myometritis, corresponding to the nodules seen grossly. In addition, a blood vessel adjacent to the uterine body contained a granulomatous thrombus composed of necrotic debris, inflammatory cells, and numerous fungal hyphae, which completely occluded the vascular lumen (Figure 8C, 8D). The large intestine had severe ulcerative pyogranulomatous enterocolitis, with extensive mucosal erosion and replacement by necrotic debris, inflammatory exudate and granulation tissue. Hyphal elements were identified in the necrotic mucosa and submucosa (Figure 8E, 8F). Overall, broad, sparsely septate hyphal structures were consistently demonstrated within granulomatous and necrotic lesions of the vascular walls, renal parenchyma, uterine horn and large intestine, with uniform hyphal morphology observed across all examined tissue sections.

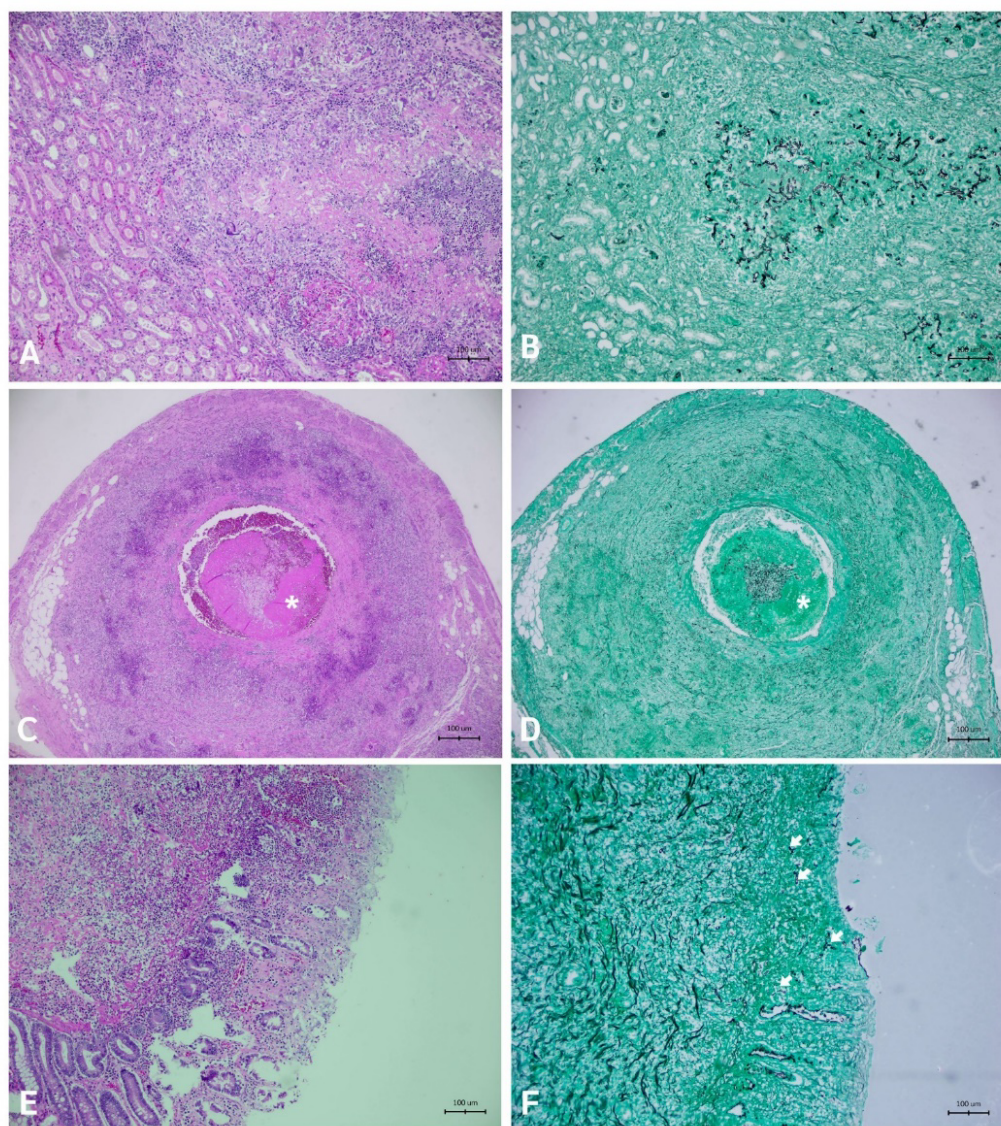


Figure 8 Microscopic findings. A–B. Right kidney showing multifocal to coalescing granulomatous and necrotizing interstitial nephritis with granulomas containing fungal hyphae. C–D. Granulomatous thrombus (star) in a vessel adjacent to the uterine body. E–F. Large intestine submucosa with marked inflammatory infiltration and hyphal elements (arrows) in necrotic mucosa and submucosa.

Microbiological and Molecular Analyses

Tissue samples from the lung, heart, liver, spleen, kidney and intestine were submitted for fungal culture at the Mycology Unit, Faculty of Veterinary Science, Mahidol University. Each specimen was inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol, and all procedures were conducted in a BSL-2 biosafety cabinet. The cultures were incubated at 30°C for up to 7 days. Fungal colonies were observed in cultures derived from kidney and intestinal tissues. These colonies were flat, cottony, and pale in color, with morphology consistent with *Pythium* spp. The microscopic examination revealed broad, sparsely septate, ribbon-like hyphae suggestive of oomycete infection. For species confirmation, genomic DNA extracted from positive cultures was amplified by PCR using universal primers targeting the internal transcribed spacer (ITS) region. The resulting ITS sequence was submitted to GenBank and assigned the accession number PX796458. Total genomic DNA was extracted with the MasterPure™ Yeast DNA Purification Kit (LGC Bioresearch Technologies, UK) according to the manufacturer's protocol. For PCR, the rDNA was amplified using ITS1 and ITS4 primers (White et al., 1990). The temperature program consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles at 94°C, 53°C and 72°C for 20 s, 20 s and 60 s at 72°C, respectively. The amplified PCR products were electrophoresed on a 1% agarose gel. Amplified DNA bands for the ITS region were cut for DNA extraction. The purified PCR products were submitted to Bioneer Corporation (Daejeon, South Korea) for Sanger sequencing using the ITS1 and ITS4 primers. The resulting sequences were compared with the NCBI GenBank database using BLASTn tool. Sequencing and a BLASTn search of the GenBank database identified the isolate as *P. insidiosum* isolate LPN17 (Accession No. EF016880.1) with a sequence similarity of 99%. Both isolates yielded positive PCR results, confirming the presence of *P. insidiosum*. No fungal growth was detected from the lung, heart, liver or spleen samples.

DISCUSSION

Large-breed dogs are considered to be at higher risk for developing pythiosis, as their outdoor lifestyle increases the likelihood of exposure to water contaminated with *P. insidiosum* zoospores (Rivierre et al., 2005). In the present case, this dog's breed and frequent outdoor activity likely contributed to increased exposure to a contaminated environment, consistent with previously reported risk factors.

Regarding disease presentation, the cutaneous and subcutaneous forms of pythiosis are the most commonly reported in dogs. However, the gastrointestinal form is associated with the highest mortality rate, reaching approximately 86% (Yolanda and Krajaejun, 2022). In contrast, vascular pythiosis is exceedingly rare in animals. A global review of cases reported between 1980 and 2021 demonstrated that approximately 22% of human pythiosis cases involved vascular structures, whereas no vascular cases had been documented in animals during that period (Yolanda and Krajaejun, 2022).

Pythiosis typically develops following penetration of the oomycete through the skin at the site of a preexisting wound. Histopathological studies of subcutaneous pythiosis have demonstrated a granulomatous inflammatory response characterized by cellular infiltration and edema of the vessel walls, which may progress to vasculitis and ultimately result in vascular pythiosis (Norasetthada, 2025). Although no history of cutaneous injury was reported in the present case, the possibility of a minor or occult wound that healed prior to the fungal incubation period cannot be excluded.

The pathogenesis observed in this dog closely mirrors that of human vascular pythiosis. Infection is presumed to initiate at a minor cutaneous wound (Pan et al., 2014), followed by subcutaneous or lymphatic spread to adjacent major

vessels (Krajaejun et al., 2006; Sudjaritruk and Sirisanthana, 2011). Once established within the vessel wall, the organism ascends along the arterial intima toward the iliac arteries and distal aorta, leading to progressive granulomatous vasculitis, thrombosis and arterial occlusion (Thongsuk et al., 2021). At this point, the disease can no longer be considered purely cutaneous, as involvement of the vascular system represents a distinct and more severe clinicopathological form.

This aggressive, yet regionally confined, vascular progression is likely driven by pathogen-intrinsic factors, including protease secretion, the mechanical force generated by hyphal elongation, and strain-specific virulence, in combination with the site of inoculation and a high local inoculum (Shipton, 1987; Imwidthaya, 1994; Ravishankar et al., 2001; MacDonald et al., 2002; Davis et al., 2006; Krajaejun et al., 2006). In the present case, disease progression extended from the right hindlimb to adjacent organs, including the right kidney, ipsilateral uterus and large intestine, with extensive involvement of major vessels and lymphatic pathways. The pattern of spread was anatomically contiguous and regionally confined, with a pronounced predilection for vascular structures. In contrast, disseminated pythiosis typically presents as multifocal disease involving noncontiguous organs, such as concurrent lesions in the brain, skin, gastrointestinal tract and nasal cavity, resulting from hematogenous dissemination (Chitasombat et al., 2018). Overall, the pathological and anatomical features observed in this case are more consistent with vascular pythiosis than with disseminated disease (Krajaejun et al., 2006). In humans, progression from cutaneous to vascular pythiosis has been associated with underlying conditions such as thalassemia and diabetes mellitus (Krajaejun et al., 2006; Norasetthada, 2025). Although no overt hematological abnormalities were identified in this dog, the presence of an underlying immunocompromised or subclinical condition cannot be entirely excluded.

The clinicopathological features in this case closely parallel those of human vascular pythiosis, supporting its classification as an angioinvasive, vascular-type infection. In humans, vascular pythiosis commonly presents with arterial insufficiency of the lower extremities, including limb swelling, chronic ulcers, absent arterial pulses, and localized masses associated with aneurysmal changes; severe cases may progress to gangrene and tissue necrosis (Krajaejun et al., 2006; Chitasombat et al., 2018). Notably, although the disease may initially arise from cutaneous or soft-tissue infection, progression to arterial invasion defines the vascular type (Krajaejun et al., 2006). Previous reports have described transient soft-tissue swelling or localized infection occurring weeks to months before the development of arterial insufficiency; for example, a 14-year-old boy in Thailand initially presented with cervical soft-tissue swelling for one week prior to vascular invasion of the external carotid artery (Thitithanyanont et al., 1998). These observations support cutaneous entry with subsequent extension to adjacent vascular structures and further reinforce the angioinvasive nature of this infection.

In this case, the pattern of disease progression closely resembled the transition from subcutaneous to vascular involvement reported in humans. The dog initially presented with cutaneous swelling, which progressed to reduced limb function, absent arterial pulses, tissue necrosis, limb and tail gangrene, and ultimately autoamputation over an average disease course of approximately three months (Krajaejun et al., 2006). Necropsy findings of vascular dilation and intraluminal masses within the abdominal aorta were also consistent with those described in human cases (Schloemer et al., 2013; Thongsuk et al., 2021). Additionally, CT lymphangiography demonstrated obstruction of the lymphatic vessels, a finding that was critical for diagnosis and parallels the use of CT imaging in humans to identify characteristic lymphatic involvement.

This consideration is particularly important for patients who do not respond to standard antibiotic therapy (Keoprasom et al., 2013). Although cytological examination did not reveal the classical fungal hyphae described in previous reports (Chindamporn et al., 2020), it demonstrated marked inflammation characterized by pyogranulomatous and eosinophilic responses, along with the

presence of fibrin, keratin and red blood cells. These findings likely reflect the severity of the lesions and the extent of tissue necrosis. Histopathological examination further confirmed hyphal invasion of the arterial walls, revealing broad, ribbon-like, sparsely septate hyphae, measuring 4–10 µm in width, with irregular, non-dichotomous branching and non-parallel walls. The hyphae stained positively with Grocott's methenamine silver (GMS), consistent with *Pythium insidiosum* (Camus et al., 2004; Salipante et al., 2012). An unusual finding was a granulomatous invasion of the caudal vena cava, a feature rarely reported in human cases, which may represent a species-specific difference or late-stage extension from adjacent tissues.

Accurate diagnosis is critical for improving prognosis, as pythiosis is a life-threatening condition (Gaastra et al., 2010). Diagnosis relies on an integrated approach combining clinical findings, imaging studies and histopathological evaluation, and is confirmed by fungal culture and serological testing (Sukanan et al., 2022). However, diagnosis of *Pythium insidiosum* based solely on morphological features is often presumptive because of its resemblance to other filamentous fungi, and histological visualization may be challenging; therefore, additional diagnostic modalities are typically required for definitive identification. Grocott's methenamine silver (GMS) staining is generally more effective for highlighting hyphal structures. Molecular techniques, such as PCR, offer greater sensitivity by detecting pathogen DNA regardless of hyphal integrity (Elshafie et al., 2022), while the immunochromatographic test (ICT) enables rapid diagnosis (Rotchanapreeda et al., 2021). Accordingly, a combination of histopathological examination and nested PCR has been recommended to enhance diagnostic accuracy (Elshafie et al., 2022). In the present case, a multimodal diagnostic approach—including histopathology with GMS staining, ICT, fungal culture and ITS-PCR—was employed to confirm the presence of *P. insidiosum*.

Surgical excision remains the treatment of choice for pythiosis in both animals and humans. In cases of cutaneous pythiosis involving the extremities, amputation is recommended to achieve complete removal of infected tissue. Owing to the absence of ergosterol in the cytoplasmic membrane of *Pythium insidiosum*, most conventional antifungal agents, including azoles, exhibit limited efficacy. Nevertheless, antifungal drugs such as itraconazole, terbinafine and amphotericin B may partially disrupt cytoplasmic membrane permeability, thereby providing modest antifungal activity (Samanta, 2015; Sukanan et al., 2022). Consequently, a combination regimen of oral terbinafine and itraconazole administered for two to three months following surgery has been advocated. Immunotherapy has also been reported as a successful adjunctive treatment in human cases of vascular pythiosis (Khunkhet et al., 2015). In humans, favorable outcomes have been achieved using a multimodal approach combining surgical intervention with oral antimicrobial agents, including itraconazole, azithromycin and doxycycline (Norasetthada, 2025). Additionally, serum β-D-glucan levels and *P. insidiosum*-specific antibody titers have been proposed as potential biomarkers for monitoring disease activity in human vascular pythiosis (Worasilchai et al., 2018). Unfortunately, in advanced veterinary cases that are refractory to treatment, euthanasia may represent a humane and ethical option to alleviate further suffering.

Despite increasing recognition of pythiosis as a significant infectious disease in animals, there have been no documented case reports of vascular or ocular pythiosis to date (Yolanda and Krajaejun, 2022). This apparent absence may reflect underdiagnosis rather than true rarity, potentially attributable to atypical clinical presentations and inherent diagnostic challenges that contribute to misdiagnosis or delayed recognition of the disease.

CONCLUSIONS

This report documents the first confirmed case of vascular pythiosis in a dog, representing an extremely rare disease manifestation in animals. This case highlights the severity of the disease and demonstrates a pathogenesis that closely resembles the human form. Despite aggressive treatment, the infection progressed rapidly, resulting in extensive tissue destruction, vascular occlusion, and systemic inflammation. Postmortem examination confirmed widespread granulomatous inflammation, necrosis and invasion of major arteries, veins and internal organs by fungal hyphae. These findings emphasize the critical need for early recognition and a multimodal diagnostic approach in dogs presenting with chronic, treatment-refractory cutaneous lesions, particularly in endemic areas. Given the poor prognosis associated with this disease presentation, prompt and accurate diagnostic confirmation is essential to improve patient outcomes. Moreover, this report underscores the value of the canine model as a relevant translational system for investigating complex human pathologies.

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AUTHOR CONTRIBUTIONS

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