*Case Report*

# Moxidectin/Imidacloprid Spot-On Plus Doxycycline Treatment for Lymphatic Filariasis in a Dog: A Case Report

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## Abstract

**Lymphatic filariasis, a zoonotic problem found in many countries, involves *Brugia pahangi* as one of the causative agents, with domestic dogs and cats serving as the definitive hosts. In this case study, an 8-month-old American Pitbull dog presented with clinical signs of left hindlimb pitting edema and lymphadenopathy. *Brugia* spp. microfilariae were identified using the Modified Knott's test and buffy coat smear with staining technique. Species identification confirmed the presence of *Brugia pahangi* through acid phosphatase enzyme activity and PCR analysis. Histological examination of the lymph nodes revealed marked lymphoid follicular hyperplasia with medullary fibrosis, sinus histiocytosis, and evidence of old hemorrhage. For a period of 5 months, the dog received a monthly spot-on combination of moxidectin and imidacloprid. Additionally, doxycycline and prednisolone were prescribed during the first month of treatment. Microfilariae were monitored using the Modified Knott's test and buffy coat smear at intervals of 1 to 4 weeks, up to 22 weeks. After 2 weeks of treatment, there was no evidence of left hindlimb edema or left popliteal lymphadenopathy. However, at the 6$^{th}$ and 10$^{th}$ week, the edematous sign in the left hindlimb recurred following the completion of the prednisolone course and after the biopsy procedure, respectively. Microfilaremia became negative at 3 weeks after the initiation of treatment and remained negative thereafter. In conclusion, this case study demonstrates the effectiveness of the combination of moxidectin and imidacloprid with doxycycline for the treatment of *Brugia pahangi* infection in dogs.**

**Keywords:** *Brugia pahangi*, Doxycycline, Lymphatic filariasis, Moxidectin

# การใช้ยาหยดม็อกซิเด็กตินและอิมิดาโคลพริดร่วมกับด็อกซีไซคลิน ในการรักษาโรคพยาธิฟิลาเรียในระบบน้ำเหลืองในสุนัข: รายงานสัตว์ป่วย

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## บทคัดย่อ

โรคพยาธิฟิลาเรียในระบบน้ำเหลืองเป็นโรคสัตว์สู่คนที่รู้จักกันดีในหลายประเทศ โดยมีพยาธิ ***Brugia pahangi*** เป็นหนึ่งในสาเหตุของเชื้อก่อโรคในสุนัขและแมวซึ่งเป็นโฮสต์จำเพาะ สุนัขพันธุ์อเมริกันพิทบูล อายุ 8 เดือน มีอาการขาหลังซ้ายบวมร่วมกับต่อมน้ำเหลืองโต สุนัขได้รับการวินิจฉัยตรวจพบไมโครฟิลาเรียด้วยวิธีทดสอบโมดิฟายน็อตและวิธีการย้อมสีบัฟฟี่โค้ท และได้รับการยืนยันจำแนกชนิดพยาธิด้วยการย้อมสีทางฮิสโตเคมีดูปฏิกิริยาของเอนไซม์แอซิดฟอสฟาเทส และปฏิกิริยาลูกโซ่พอลิเมอเรส ระบุเป็นพยาธิชนิด ***Brugia pahangi*** ผลตรวจทางจุลพยาธิวิทยาของต่อมน้ำเหลืองทั้งสองข้างพบการเพิ่มจำนวนขึ้นเป็นอย่างมากของกลุ่มเซลล์ลิมโฟไซต์ร่วมกับการสะสมของพังผืดในชั้นเมดัลลา การเพิ่มจำนวนเซลล์ฮิสทิโอไซต์ในโพรงเลือด และร่องรอยการเกิดเลือดออก สุนัขตัวนี้ได้รับการรักษาด้วยยาหยดที่มีส่วนประกอบของม็อกซิเด็กตินและอิมิดาโคลพริดทุกเดือนเป็นเวลา 5 เดือน ร่วมกับยากินด็อกซีไซคลิน และเพรดนิโซโลนในช่วงเดือนแรก หลังจากนั้นสุนัขได้รับการตรวจติดตามหาไมโครฟิลาเรียทุก 1 ถึง 4 สัปดาห์ จนถึง 22 สัปดาห์ของการรักษาด้วยวิธีทดสอบโมดิฟายน็อตและวิธีการย้อมสีบัฟฟี่โค้ท พบว่าอาการขาหลังซ้ายบวมและภาวะต่อมน้ำเหลือโตหายดีหลังการรักษาเพียง 2 สัปดาห์ แต่กลับมาพบอาการขาบวมอีกครั้งในสัปดาห์ที่ 6 และ 10 ซึ่งเป็นช่วงหลังการหยุดยาเพรดนิโซโลนและหลังการผ่าตัดชิ้นเนื้อเพื่อการวินิจฉัยตามลำดับ ในขณะที่ไมโครฟิลาเรียในกระแสเลือดให้ผลเป็นลบในสัปดาห์ที่ 3 ของการรักษาและตลอดจนจบการศึกษา จากการศึกษานี้พบว่า การใช้ยาหยดม็อกซิเด็กตินและอิมิดาโคลพริดร่วมกับด็อกซีไซคลินสามารถนำมาใช้ในการรักษาสุนัขที่เป็นโรคพยาธิฟิลาเรียในระบบน้ำเหลืองได้อย่างมีประสิทธิภาพ

**คำสำคัญ :** *Brugia pahangi* ด็อกซีไซคลิน โรคพยาธิฟิลาเรียในระบบน้ำเหลือง ม็อกซิเด็กติน

## Introduction

Lymphatic filariasis is a significant public health concern commonly referred to as elephantiasis in humans (CDC 2018). This condition is primarily caused by infection with parasitic nematodes belonging to the Filarioidea family, including *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (CDC 2018; Kaikuntod et al., 2018). Lymphatic filariasis has exhibited a widespread presence across numerous countries spanning the tropics and sub-tropics of Asia, Africa, the Western Pacific, as well as select regions in the Caribbean and South America. (CDC 2018; Kaikuntod et al., 2018). Lymphatic filariasis is particularly prevalent in Southeast Asian countries, with Thailand being among the commonly affected regions. (Kaikuntod et al., 2021). *Brugia* spp., specifically *Brugia malayi* and *Brugia pahangi*, have been identified as causative agents of infection in dogs and cats. Additionally, both dogs and cats can serve as reservoir hosts for *B. malayi* in regions such as Pattani, Surat Thani, Nakhon Si Thammarat, Phattalung, and Narathiwat (Saeung et al., 2013). *B. pahangi*, on the other hand, has been reported in Narathiwat, Chiang Mai, Chanthaburi, Samut Sakhon, and Bangkok. (Junhom et al., 2006; Satjawongvanit et al., 2019; Loymek et al., 2021).

Both *B. malayi* and *B. pahangi* are transmitted through the bites of infected mosquitoes belonging to the genera *Aedes, Anopheles, Culex,* and *Mansonia*. (Laojun et al., 2022). During a blood meal, the third-stage infective larvae enter the bloodstream and subsequently migrate to the lymphatic system, where they undergo development into adult parasites. (CDC 2018). The molting process, transforming the larvae into adults, typically occurs within a time frame of approximately 23 to 27 days. (Schacher 1962). Subsequently, microfilariae are generated and released into the bloodstream, typically occurring within a period of 40 to 60 days or up to 3 months following the initial infection. (Edeson et al., 1960; Ewert and Singh 1969; Denham and Rogers 1975). In the case of *B. pahangi*, dogs and cats serve as definitive hosts, while humans are considered accidental hosts. Clinical manifestations in animals can vary from asymptomatic cases to the development of lymphadenopathy and lymphedema. (Kaikuntod et al., 2018). According to a study, the prevalence of *B. pahangi* infection in dogs was found to be 12.25% in Chanthaburi and 2.3% in Samut Sakhon. (Loymek et al., 2021). In a separate study conducted in Chiang Mai, the prevalence of *B. pahangi* infection in dogs was reported to be 8.31%. (Kaikuntod et al., 2021). Additionally, a study conducted in Selangor state, Malaysia, revealed a prevalence of 23.5% for *B. pahangi* infection among cats. (Al-Abd et al., 2015). Furthermore, there has been a reported case of zoonotic filariasis caused by *B. pahangi* in Thailand, affecting a human individual. (Thongpiya et al., 2021).

According to the guidelines set forth by the Tropical Council for Companion Animal Parasites, the recommended treatments for lymphatic filariasis include moxidectin, selamectin, doramectin, and ivermectin (TroCCAP 2019). Ivermectin is extensively utilized for the treatment of *B. pahangi*-infected and *B. malayi*-infected cats, proving to be an effective therapeutic option. (Taweethavonsawat and Chungpivat 2013; Khowawisetsut et al., 2017). In one study, topical selamectin was administered as a treatment for naturally infected cats, demonstrating its potential as an effective therapeutic approach. (Sarasombath et al., 2019). Limited studies are available regarding the treatment of lymphatic filariasis in dogs. However, a recent discovery of *Wolbachia* endosymbiont rickettsia-like bacteria in filarial nematodes has shed light on their role in promoting filarial fertility

and larval development. This finding holds promise for potential future interventions and treatment strategies in dogs affected by lymphatic filariasis (Bandi et al., 1999). As a result, targeting *Wolbachia* through the use of doxycycline has been considered to enhance the effectiveness of therapy in animals infected with *Brugia*. In this particular study, we present the first report on the therapeutic efficacy of a combination treatment involving topical moxidectin and doxycycline for the treatment of *B. pahangi* infection in a clinical case of lymphatic filariasis in a dog.

## Case description

### History and physical examination

On 7th November 2022, a female American Pitbull dog, weighing 31.7 kg and aged 8 months, was presented at the Prasu Arthorn Veterinary Teaching Hospital, Mahidol University, Nakhon Pathom, Thailand. The dog exhibited recurrent left hindlimb edema. The symptom initially appeared one month prior and had temporarily resolved following treatment at a private clinic with prednisolone, enrofloxacin, and furosemide, although the specific dosage and duration of the treatment were unspecified. The dog had an incomplete vaccination history but received monthly afoxolaner (NexGard®; Boehringer Ingelheim). According to the owners, the dog showed normal weight bearing and displayed good vital signs. During the physical examination, the dog appeared alert and responsive, with a rectal temperature of 102.8°F. Normal heart and lung sounds were detected upon thoracic auscultation. Pitting edema was observed from the hock joint to the digit of the left hindlimb, accompanied by lymphadenopathy of the left popliteal lymph node (Figure 1).

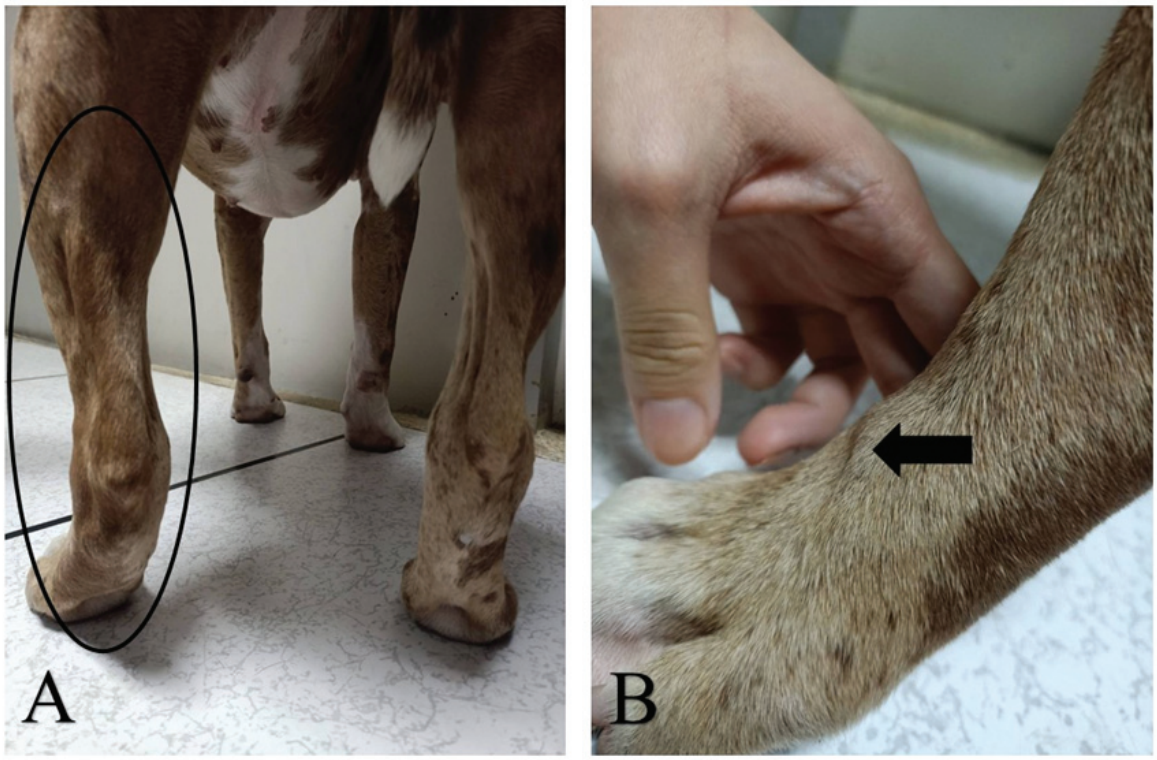


**Figure 1.** (A) The dog exhibited left hindlimb edema (circled area), indicating swelling in the affected limb, and (B) upon applying pressure to the metatarsal area, a persistent dimple was observed in the skin (arrow), indicating the presence of pitting edema.

**Initial diagnosis**

The complete blood count and serum chemistry profile results, including alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, albumin, total protein, and glucose, fell within the reference ranges, except for neutrophilia observed at 12.66 x $10^3$/μL (reference range: 3.00-11.5 x $10^3$/μL). The dog tested negative for *Dirofilaria immitis* antigen, as well as antibodies for *Anaplasma platys, Anaplasma phagocytophilum, Borrelia burgdorferi, Ehrlichia canis,* and *Ehrlichia ewingii*, based on an enzyme-linked immunosorbent assay (SNAP 4Dx Plus; IDEXX). Radiographs of the hindlimbs showed evidence of soft tissue swelling on the left side. Additionally, microfilariae were detected during the Modified Knott's test.

**Morphology identification**

Approximately sixty microliters of EDTA whole blood were processed for capillary tube centrifugation. The buffy coat thin blood smear was then prepared and subjected to Giemsa staining for the identification of microfilaria morphology. The microfilarial sheath and two-terminal nuclei were detected, indicating the presence of *Brugia* spp. (Figure 2A).

In addition, a thick smear consisting of three lines of 20 μL EDTA whole blood was prepared on a glass slide. The slide was air-dried overnight and subsequently underwent histochemical staining to investigate the species identification of the microfilariae based on acid phosphatase enzyme activity (Omar, 1977; Ravindran et al., 2014) at the Parasitology unit, Faculty of Veterinary Science, Chulalongkorn University, Thailand. The microfilaria exhibited significant and widespread acid phosphatase activity throughout its entire body, particularly in the amphid (AM), excretory vesicle (EV), anal vesicle (AV), and phasmids (PM) regions, indicating the presence of *B. pahangi* (Figure 2B).

**Molecular identification and sequencing**

Microfilarial DNA was extracted from a 200 μL EDTA whole blood sample using the PureLinkTM Genomic DNA Mini Kit (Thermo Fisher Scientific,

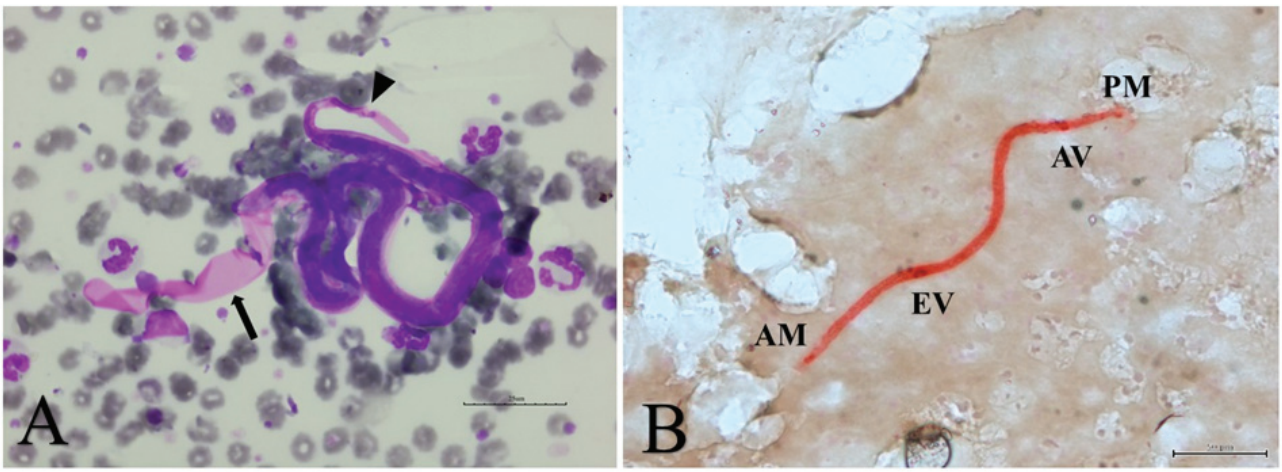


**Figure 2.** (A) Microfilaria stained with Giemsa, revealing the presence of the microfilarial sheath (arrow) and two-terminal nuclei (arrowhead), observed at 1,000x magnification (scale bar = 25 μm). (B) Acid phosphatase staining of *B. pahangi* microfilaria, demonstrating diffuse staining throughout the entire body, visualized at 400x magnification (scale bar = 50 μm).

Waltham, MA). For amplification of the *B. pahangi* 5.8S-ITS2-28S gene, primers DIDR-F1 (5'-AGTGCGA ATTGCAGACGCATTGAG-3') and DIDR-R1 (5'-AGCGGGTAATCACGACTGAGTTGA-3') (Macrogen laboratory, Seoul, South Korea) were employed. These primers are capable of amplifying any filarial 5.8S-ITS2-28S gene (Rishniw et al., 2006), and approximately 600 base pairs (bp) of the *B. pahangi* 5.8S-ITS2-28S gene were targeted (Figure 3A). As a positive control, *D. immitis* microfilariae DNA was included, confirmed through DNA sequencing of the filarial 5.8S-ITS2-28S gene, with a PCR product size of 550 bp.

PCR amplifications were performed in a T100TM Thermal Cycler (Bio-Rad, Hercules, CA, USA) following the specified conditions: an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step of 5 minutes at 72°C concluded the amplification process. DNA sequencing was performed using the 5.8S-ITS2-28S filaria-specific primers, DIDR-F1, and DIDR-R1. The nucleotide sequence accession number for *B. pahangi* is OQ352833 (Figure 3B).

**Bioinformatics and Phylogenetic analyses**

The obtained *B. pahangi* 5.8S-ITS2-28S sequence was subjected to analysis using various web-based programs. To compare the sequence with existing sequences in the GenBank database, the Basic Local Alignment Search Tool (BLAST) was employed (accessed on 31 January 2023). Multiple sequence alignments were performed using the ClustalW web-based tool (accessed on 31 January 2023), as described by Larkin et al. (2007). For phylogenetic analysis, maximum likelihood trees with bootstrapping (100 replications) were constructed

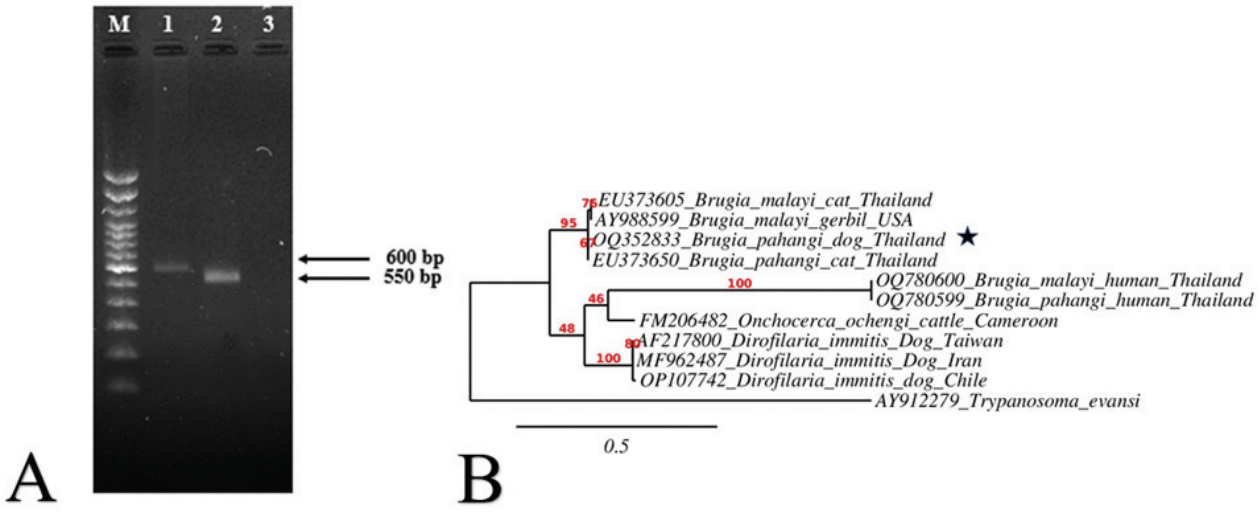


**Figure 3.** (A) Gel electrophoresis of PCR amplification targeting the 5.8S-ITS2-28S gene on a 2% agarose gel. Lane M represents the DNA ladder with a molecular weight of 100 bp. Lane 1 shows the PCR product from *B. pahangi* microfilaria (sample from this case report) with an expected size of approximately 600 bp. Lane 2 displays the PCR product from *D. immitis* microfilaria, serving as the positive control, showing a typical band size of approximately 550 bp. Lane 3 represents the negative control (nuclease-free water). (B) Phylogenetic tree analysis of *B. pahangi* based on nucleotide sequences from a fragment of the 5.8S-ITS2-28S gene using the maximum likelihood method. The sequence analyzed in this study is marked with a star.

using the advanced mode of the Phylogeny.fr web server (accessed on 31 January 2023), following the method described by Dereeper et al. (2008). In the phylogenetic analysis, *Trypanosoma evansi* was included as the outgroup for the 5.8S-ITS2-28S gene.

**Histopathological findings**

To further investigate the left popliteal lymphadenopathy, fine needle aspiration was performed on the day of presentation, revealing predominantly round cells with scant cytoplasm, raising suspicion of a round cell tumor. In order to confirm the diagnosis, a biopsy of both popliteal lymph nodes was conducted seven weeks later. The dog was premedicated with intramuscular morphine (0.3 mg/kg) and intravenous diazepam (0.5 mg/kg), followed by induction using intravenous propofol (3.5 mg/kg). The dog was intubated, and a sample was collected under isoflurane anesthesia. Prior to the procedure, a single preoperative dose of intravenous cefazolin (25 mg/kg) and subcutaneous carprofen (2.5 mg/kg) were administered. Postoperatively, the dog was prescribed oral cephalexin (26 mg/kg) twice daily for 7 days and oral carprofen (2.2 mg/kg) twice daily for 3 days. Histological examination revealed marked lymphoid follicular hyperplasia with medullary fibrosis, sinus histiocytosis, and evidence of old hemorrhage (Figure 4).

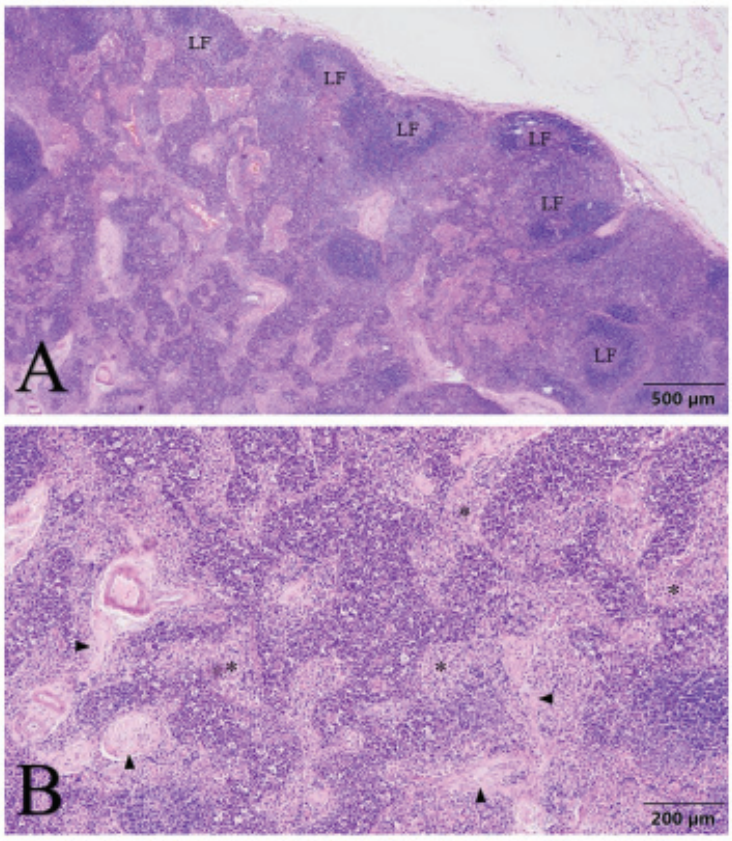


**Figure 4.** (A) Histological examination of lymph nodes revealing marked expansion of lymphoid follicles (LF). These follicles consist of central large lymphocytes surrounded by a distinct marginal zone, maintaining their polarity. Magnification: 100x (scale bar = 500 µm). (B) The medullary region shows diffuse infiltration of dense collagenous connective tissue (arrowhead). The subcapsular and medullary sinuses contain numerous histiocytes, a high number of macrophages containing dense yellow-brown granular or globular pigment consistent with hemosiderin, and occasional erythrocytes (asterisk). Many plasma cells are observed within the medullary cords. Magnification: 400x (scale bar = 200 µm).

**Treatment and monitoring**

Subsequently, the dog received a diagnosis of lymphatic filariasis caused by *B. pahangi*. Throughout the study, the dog had been undergoing treatment with a monthly topical combination of moxidectin (dosage range 2.5-6.25 mg/kg) and imidacloprid (dosage range 10-25 mg/kg) (Advocate®; Bayer) for a period of 5 months. In addition, doxycycline was administered orally at a dose of 10 mg/kg twice daily for 30 days. Alongside the topical medication and antibiotics, the dog was prescribed oral prednisolone (0.5 mg/kg) twice daily for the first week, followed by a tapering dosage regimen of once daily in the second week and every other day in the third and fourth weeks.

During the initial month of treatment, the dog underwent weekly monitoring with blood sample collection. Once the clinical signs stabilized, blood samples were taken from the dog every one to two months. The blood samples were utilized for microfilariae detection using the Modified Knott's test and the buffy coat thin blood smear techniques. As early as two weeks post-treatment, there was no evidence of left hindlimb edema or left popliteal lymphadenopathy. The numbers of microfilariae detected from the buffy coat thin blood smear method were 50.00, 16.67, and 33.33 microfilariae/mL at weeks 0, 1, and 2, respectively (Fig. 5). The Modified Knott's test yielded negative results by the 2nd week. Three weeks after the first dose of topical moxidectin, both techniques demonstrated complete clearance of microfilariae, a trend that persisted until 22 weeks after treatment, as depicted in Figure 5 and Table 1. Unfortunately, left hindlimb edema recurred at the 6th and 10th weeks, which were before the biopsy procedure and after the removal of stitches, respectively. The dog was prescribed oral prednisolone at a dosage of 1 mg/kg/day for 5 days, followed by 0.5 mg/kg/day for an additional 5 days.

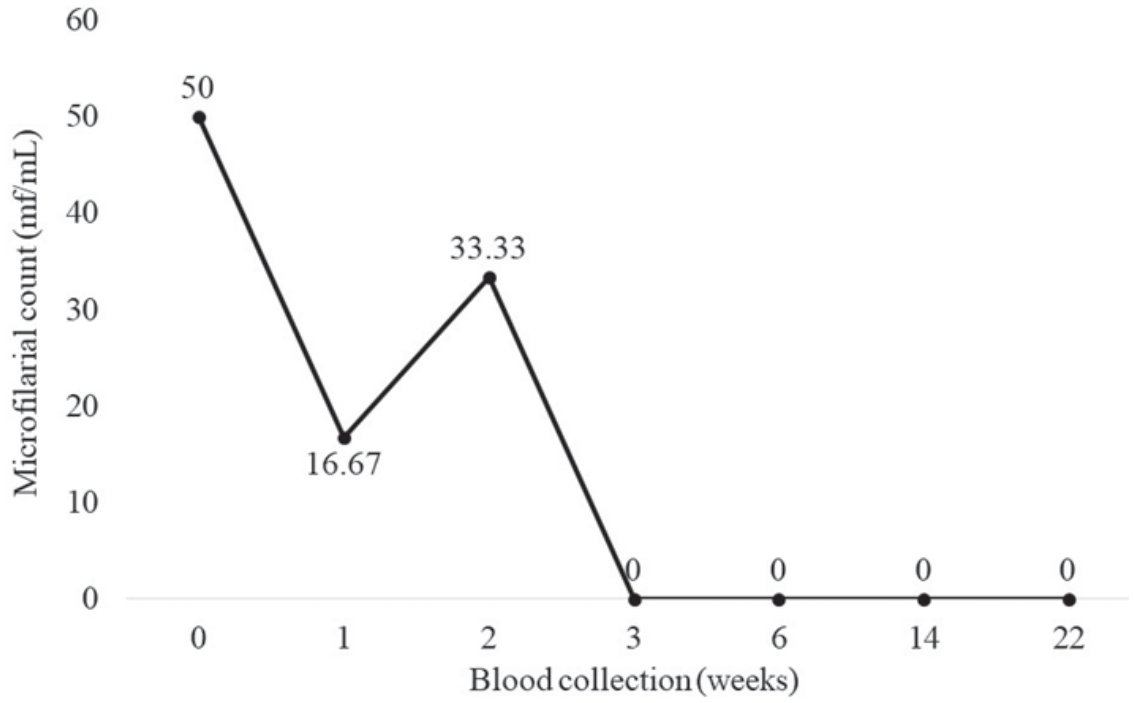


**Figure 5.** Graph depicting the microfilarial count (mf/mL) of *Brugia pahangi* from the buffy coat smear during the treatment period. The number of microfilariae per mL was estimated from a 60 μL blood sample.

**Table 1.** Modified Knott's test results during the treatment period.

| Week(s) | 0 | 1 | 2 | 3 | 6 | 14 | 18 | 22 |
|---|---|---|---|---|---|---|---|---|
| **Modified Knott's test** | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve |

## Discussion

In order to gain insight into the impact of filaria on lymphatic processes, previous studies have employed lymphagiographic techniques. One such technique, xeroradiographic lymphangiography, was introduced in 1986 to investigate lymphatic changes in *B. pahangi*-infected dogs. The study revealed that the main pathological changes observed were lymphatic occlusion and lymphatic leakage, which contributed to the development of pitting edema in the limbs (Snowden et al., 1986). Additionally, the production of inflammatory mediators has been identified as a key factor in edema formation. Histamine and/or prostaglandin E2 (PGE-2) have been associated with the development of edema (Ortan et al., 1998). The most common clinical signs reported in the study by Sadarama et al. (2019) were fever, observed in 40% of the cases, followed by lymphadenopathy (31.42%) and limb edema (22.85%). Other previously reported clinical symptoms include anorexia, vomiting, alopecia, dermatitis, scrotal edema, lameness, conjunctivitis, lacrimation, and corneal opacity (Ambily et al., 2011; Sadarama et al., 2019). Histological examination of lymph nodes in this case revealed similar findings to those of lymph nodes infected with parasites. The characteristic features included follicular hyperplasia infiltrated with mononuclear cells, as well as marked capsular and medullary fibrosis (Snowden and Hammerberg 1989).

Microscopic examination is a widely used conventional method for screening microfilariae, both in human filariasis and in the veterinary field. Techniques such as wet mount preparation, thick blood film, Modified Knott's concentration, capillary tube technique, and buffy coat thin blood smear are commonly employed (Goldsmid 1970). Among these methods, the Modified Knott's test and the buffy coat thin blood smear are recommended for microfilaria detection, with no significant difference in sensitivity (Mylonakis et al., 2004; Marcos et al., 2016). The buffy coat thin blood smear method offers advantages such as requiring less blood sample and facilitating the speciation of microfilariae (Mylonakis et al., 2004; Marcos et al., 2016). However, it is worth noting that the Modified Knott's test may lead to false negative results in cases of infestation with a low number of microfilariae (<25 microfilariae/mL) (Mylonakis et al., 2004). In the case of the Modified Knott's test result at 2 weeks post-treatment, it is possible that the false negative result was due to human error or individual skill, as the interpretations were performed by different technicians each week. An alternative approach for species identification is histochemical staining using acid phosphatase. Acid phosphatase activity can differentiate microfilariae of *D. immitis, D. repens, B. malayi,* and *B. pahangi* based on different staining patterns. In *B. pahangi* microfilaria, diffuse acid phosphatase activity is observed along the entire body length, whereas in *B. malayi* microfilaria, acid phosphatase activity is predominantly observed in the areas of amphid, excretory pore, anal pore, and phasmid (Chungpivat and Taweethavonsawat 2008). Polymerase chain reaction (PCR) is a molecular technique that utilizes specific primers for amplification. Gene

targets such as ITS1 and 5.8S-ITS2-28S have been previously employed in PCR assays, revealing different band sizes for each species (Kaikuntod et al., 2021). In this study, the obtained 5.8S-ITS2-28S gene sequence showed 100% identity to the previously recorded sequence (EU373650) of *B. pahangi* from a cat in Thailand, whereas the sequence of the 5.8S-ITS2-28S gene from *B. malayi* (EU373605) showed 97% identity. These findings further support the accurate species identification of the filarial infection.

The results of ivermectin treatment in infected cats have shown successful outcomes, with negative microfilariae detection achieved within 1-2 months of treatment (Taweethavonsawat and Chungpivat 2013; Khowawisetsut et al., 2017). Additionally, there was a significant reduction in microfilariae levels within one month of ivermectin treatment (Taweethavonsawat and Chungpivat 2013). However, it is important to note that 25% of cats experienced a recurrence of microfilaraemia after 3 to 5 months (Khowawisetsut et al., 2017). In the case of topical selamectin treatment for brugian filariasis in cats, a study demonstrated 100% effective elimination of microfilariae at various time points (Sarasombath et al., 2019). A study conducted by Sadarama et al. (2019) is the only available report on infected dogs, and it revealed that the use of ivermectin, diethylcarbamazine, or a combination of both can reduce the microfilarial concentration. However, none of the treatments achieved complete elimination of microfilariae within a shorter 21-day period. These findings highlight the efficacy of ivermectin and selamectin in reducing microfilariae levels in cats, although recurrence of microfilaraemia can occur. The available study on infected dogs suggests that current treatments may not achieve complete amicrofilaremic status within a shorter timeframe. Further research is needed to explore more effective treatment options for lymphatic filariasis in both cats and dogs.

Moxidectin is a macrocyclic lactone formulation that specifically targets glutamate-gated chloride ion channels (GluCls) found in the excretory-secretory pore of microfilaria and the reproductive tissue of adult *B. malayi* worms (Moreno et al., 2010; Li et al., 2014). This binding action leads to the clearance of microfilariae and inhibition of their production (Li et al., 2014). Moxidectin has shown higher microfilaricidal efficacy compared to other macrocyclic lactones (Bowman and Mannella 2011; Paran and Svobodová 2011; Regalbono et al., 2016). Doxycycline is effective against *Wolbachia* endosymbiotic bacteria that have been detected in various filarial nematodes (Bandi et al., 1998). Studies by Bandi et al. (1999) have shown that tetracycline-treated worms did not have detectable *Wolbachia* in the reproductive tract when examined using transmission electron microscopy. Additionally, PCR results have revealed that *Wolbachia* expression is limited to the caudal end of the reproductive tract. Recent literature has also demonstrated the efficacy of topical moxidectin combined with oral doxycycline as a treatment protocol for adulticide therapy in canine heartworm infection (Chandrasakha et al., 2021; Jacobson and DiGangi 2021). Therefore, a topical formulation containing moxidectin along with oral doxycycline was chosen for the treatment of this patient.

In our study, the levels of microfilariae in the buffy coat smear decreased at 1 week and reached zero at 3 weeks after the administration of topical moxidectin. Subsequently, there was no recurrence of microfilaraemia in the dog throughout the study period. The recurrence of left limb edema at the $6^{th}$ and $10^{th}$ week, despite the dog being amicrofilaremic, can be explained by previous studies. Snowden and Hammerberg (1989) suggested that

episodes of lymphadenopathy or limb edema in observed dogs were not necessarily associated with parasite exposure and could last for months. Therefore, the early recovery symptoms observed in this dog are likely due to the effect of prednisolone. The development of limb edema following the discontinuation of prednisolone after one week further supports this assumption. The later episode of limb edema may be related to the inflammatory response resulting from the post-surgical procedure and the dog's self-mutilation.

## Conclusion

In conclusion, this case study highlights the effectiveness of a monthly topical combination of moxidectin and imidacloprid along with doxycycline in treating *B. pahangi* infection in dogs. Considering the limited treatment guidelines currently available for lymphatic filariasis in dogs, this study not only provides valuable insights but also serves as a treatment guideline for brugian lymphatic filarial infection in dogs. The successful management of the infection, as demonstrated in this case, emphasizes the importance of a comprehensive and targeted treatment approach involving both microfilaricidal and adulticidal agents, along with the use of doxycycline to target *Wolbachia* endosymbionts. Further research and clinical studies are warranted to validate and refine these treatment protocols for the effective management of lymphatic filariasis in dogs.

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