JOURNAL OF
APPLIED ANIMAL SCIENCE

ISSN 1906-2257 VOL.16 NO.1 JANUARY-JUNE 2023

"Journal of Applied Animal Science" (JAAS)

Scope of the Journal

The philosophy of the Faculty of Veterinary Science, Mahidol University, is *"One Health"*, i.e., to interweave the disciplines of veterinary sciences with medical sciences for extreme advantages to human, animals and environment. The *Journal of Applied Animal Science (JAAS)*, is a peered review journal which published 2 numbers (January-June, July-December) a year by Faculty of Veterinary Science, Mahidol University, accepts manuscripts presenting information for publication with this philosophy in mind. Articles published in *JAAS* include a broad range of research topics in veterinary science, animal science, animal husbandry, animal production and fundamental aspects of genetics, nutrition, physiology, and preparation and utilization of animal products. Articles typically report research with cattle, companion animals, goats, horses, pigs, and sheep; however, studies involving other farm animals, aquatic and wildlife species, and laboratory animal species that address fundamental questions related to livestock and companion animal biology will be considered for publication.

Journal Management

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โทร. 0-2441-5242 ต่อ 1308 โทรสาร 0-2441-0937

Website http://www.vs.mahidol.ac.th/jaas Editor-in-Chief: Assoc.Prof. Dr.Tanasak Changbunjong, E-mail address: editor.jaas2020@gmail.com

Journal manager: Natcha Kaewkrajang

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“Journal of Applied Animal Science” (JAAS)

สารจากคณบดี

ในวารสาร Journal of Applied Animal Science (JAAS) Vol.16 No. 1 (2023): January-June นี้ มีมีเนื้อหาที่น่าสนใจและหลากหลายด้านที่จะสร้างความรู้และแนวทางในการประยุกต์ใช้ในวงการสัตวแพทย์ ซึ่งมีเนื้อหาสาระที่เป็นการอัพเดทความรู้ในเรื่องการวินิจฉัยและการรักษาใหม่ๆ และขอถือโอกาสนี้ขอบคุณเนื้อหาจากผู้ประพันธ์ที่ได้มีส่วนร่วมในฉบับนี้ ซึ่งเป็น case report จำนวน 3 เรื่อง ดังนี้

1. “A Case Report of Canine Pure Red Cell Aplasia in Middle-Aged Male Pomeranian”
2. “Moxidectin/Imidacloprid Spot-On Plus Doxycycline Treatment for Lymphatic Filariasis in a Dog: A Case Report”
3. “Partial Cystectomy and Ureteroneocystostomy in a Dog with Urinary Bladder Squamous Cell Carcinoma”

นอกจากนี้ ยังมี research article เรื่อง “Effects of a Standardized Extract of Centella asiatica ECa 233 on Clinical Parameters and Radiography Using Computed Tomography in Dogs with Osteoarthritis”

บทความทั้งหมดนี้ผ่านกระบวนการ peer review ซึ่งนำทีมโดยบรรณาธิการวารสาร รศ.ดร.น.สพ.ธนศักดิ์ ช่างบรรจง และผู้รับผิดชอบงานวารสารทุกท่าน ที่ได้สละเวลาอันมีค่าจัดทำเล่มวารสาร ประสานงานและอำนวยความสะดวกกับผู้เขียนบทความตั้งแต่เริ่มต้นจนแล้วเสร็จเพื่อให้แน่ใจว่ามีคุณภาพและสอดคล้องกับมาตรฐานทางวิชาการ ทีมงานได้ให้ความสำคัญกับความมุ่งมั่นในการนำวารสารเข้าสู่ฐานข้อมูลในระดับนานาชาติเพื่อเผยแพร่องค์ความรู้ในวงกว้าง

รองศาสตราจารย์ ดร.สัตวแพทย์หญิงวลาสินี ศักดิ์คำดวง
คณบดีคณะสัตวแพทยศาสตร์
มหาวิทยาลัยมหิดล

"Journal of Applied Animal Science" (JAAS)

ปีที่ 16 ฉบับที่ 1 มกราคม-มิถุนายน 2566 Vol. 16 No. 1 January-June 2023

สารบัญ

คำแนะนำสำหรับผู้แต่ง

"Journal of Applied Animal Science" (JAAS)

วารสารสัตวศาสตร์ประยุกต์เป็นวารสารวิชาการราย 6 เดือน (2 ฉบับต่อปี เดือนมกราคม-มิถุนายน และเดือนกรกฎาคม-ธันวาคม) ของคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล เผยแพร่ผลงานวิจัยครอบคลุมสหสาขาวิชาทั้งสัตวแพทยศาสตร์ และสัตวศาสตร์ ตั้งแต่พื้นฐานถึงระดับโมเลกุล รวมถึงรายงานทางคลินิก บทความที่ได้รับการตีพิมพ์ในวารสารต้องผ่านการประเมินโดยผู้ทรงคุณวุฒิอย่างน้อย 3 ท่าน ในรูปแบบ double-blind peer review

ผู้สนใจส่งบทความเพื่อตีพิมพ์ในวารสารสัตวศาสตร์ประยุกต์กรุณาปฏิบัติตามข้อแนะนำและส่งพร้อมจดหมายนำ

1. ประเภทบทความ ที่รับพิจารณาได้แก่ รายงานการวิจัย รายงานฉบับย่อ บทความปริทัศน์และรายงานทางคลินิกเขียนด้วยภาษาไทยหรือภาษาอังกฤษ แต่บทคัดย่อต้องมีทั้งภาษาไทยและภาษาอังกฤษ

2. การส่ง ส่งต้นฉบับพร้อมสำเนา 4 ชุด และไฟล์ดิจิตอลทางไปรษณีย์ ไฟล์ดิจิตอลต้องสร้างด้วยโปรแกรม MS-Word หรือซอฟต์แวร์ที่ใช้แทนกันได้ อาจส่งต้นฉบับผ่านอีเมลโดยไม่มีสำเนาได้

3. รูปแบบ ขนาดกระดาษเอ 4 พิมพ์หน้าเดียว เว้นระยะ 1 บรรทัด ขอบกระดาษ 2.54 ซม. (1 นิ้ว) ฟอนต์ Angsana New หรือ TH SarabunPSK 16 พอยต์

4. ส่วนประกอบ รายงานการวิจัยต้องประกอบด้วยหน้าแรก (ได้แก่ ชื่อเรื่อง ชื่อผู้แต่ง สถานที่ทำงานและที่อยู่ชื่อผู้แต่งหลักพร้อมที่อยู่ที่ติดต่อได้และอีเมล พิมพ์ทั้งภาษาไทยและภาษาอังกฤษ) บทคัดย่อ (สั้นกระชับได้ใจความและคำสำคัญ 3-4 คำ) บทนำ อุปกรณ์และวิธีการ ผลการวิจัย วิจารณ์ กิตติกรรมประกาศและเอกสารอ้างอิง

ก. รายงานฉบับย่อและรายงานทางคลินิก อาจเขียนโดยไม่แยกหัวข้อ หรืออาจรวมส่วนผลการวิจัยและวิจารณ์เป็นหัวข้อเดียว

ข. บทความปริทัศน์ ควรเริ่มด้วยบทนำ แล้วบรรยายโดยแยกตามหัวข้อที่ต้องการนำเสนอ พร้อมบทสรุป

5. ตาราง-รูปภาพ ตารางและรูปภาพให้แทรกไว้ท้ายสุดของบทความ คำบรรยายตารางพิมพ์ด้านบน คำบรรยายรูปภาพพิมพ์ใต้ภาพ และมีหมายเลขอาระบิกกำกับตามลำดับการอ้างถึง ตารางควรเข้าใจได้ง่าย ให้ส่งรูปภาพความละเอียดสูงแยกต่างหากมาพร้อมด้วย

6. การอ้างอิง ผู้แต่งต้องปฏิบัติตามรูปแบบการอ้างอิงของวารสาร การอ้างอิงในเนื้อหาใช้ระบบนาม-ปี เช่น (คัมภีร์ กอธีระกุล และคณะ 2530) หรือ คัมภีร์ กอธีระกุล และคณะ (2530) การเขียนรายการเอกสารอ้างอิงให้เขียนไว้หลังกิตติกรรมประกาศ โดยพิมพ์เอกสารภาษาไทยก่อนแล้วตามด้วยเอกสารภาษาอังกฤษ สำหรับการเขียนเอกสารอ้างอิงภาษาอังกฤษให้ดูจากส่วนแนะนำภาษาอังกฤษ

คัมภีร์ กอธีระกุล, เทิด เทศประทีป, วรา พานิชเกรียงไกร, โสมทัต วงศ์สว่าง, วราภรณ์ แซ่ลี้, สมศักดิ์ ภัคีศิริภรณ์. การสำรวจพบเชื้อ *อี.โคไล* ซีโรไทป์ K88 จากลูกสุกรวัยดูดนมและหลังหย่านม. เวชชสารสัตวแพทย์. 2530; 17(1): 21-7.

7. ชื่อวิทยาศาสตร์ ให้พิมพ์เป็นภาษาอังกฤษตามประมวลนามศัพท์สากลและทำให้เด่นแตกต่างจากเนื้อหา

8. การถอดคำไทยเป็นภาษาอังกฤษ ใช้หลักเกณฑ์การถอดอักษรไทยเป็นอักษรโรมันแบบถ่ายเสียงของราชบัณฑิตยสถาน

9. อักษรย่อและสัญลักษณ์ หากเป็นที่รับรู้โดยทั่วกันอนุโลมให้ใช้ได้โดยไม่ต้องพิมพ์ตัวเต็มก่อน

สำหรับรายละเอียดเพิ่มเติมและแม่แบบต้นฉบับ ให้ไปที่เว็บไซต์ของวารสาร https://he02.tci-thaijo.org/index.php/jaas_muvs

อีเมลบรรณาธิการวารสาร editor.jaas2020@gmail.com

ที่อยู่ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล 999 ถนนพุทธมณฑล สาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

Instructions to Authors

"Journal of Applied Animal Science" (JAAS)

Journal of Applied Animal Science is a peer-review journal (2 issues/year; January-June and July-December) which publishes papers that report on original research covering broadly interdisciplinary of veterinary and animal sciences with results of more than local regard. *JAAS* invite and welcome submissions on existing new research from basic to molecular. Articles published under our journal are double-blind peer reviewed by at least 3 reviewers.

The author should follow the instructions below for manuscript preparation and submit with covering letter.

1. Categories: *JAAS* accepts varieties of article, including research articles, short communications, reviews and also clinical reports.

2. Language: English articles are preferable; however, both Thai and English manuscripts are acceptable, with Thai and English abstracts.

3. Submission: Submission via email is our most preferable way. However, submission of the manuscript is acceptable by either paper (4 copies) or digital format (email). Finally, digital format must be submitted. The submission file is in MS-Word format or compatible software.

4. Format: The manuscript should be used A4 size with margin of 2.54 cm (1 in), double spacing and indentions by using tabs. Times New Roman font 12 points is favored for English and Angsana New or TH SarabunPSK 16 points is desirous for Thai.

5. Components: The research manuscripts should have sequential components as title page, abstract and 3-4 keywords, introduction, materials and methods, results, discussion, conclusion, acknowledgements and references. Title page, in both Thai and English, includes title, author(s) and affiliation(s) for each author. Corresponding author must provide full contact address and email.

a. Short communications or clinical reports: These could be written as no sections, combination of results and discussion or introduction and followed by several presentation sections.

b. Reviews: The manuscript should start with introduction and followed by demonstration sections and conclusion.

6. Tables-Figures: Tables and figures must be numbered by using Arabic numbers. The caption must be written on the top of table or the bottom of figure. Tables and figures should be put at the end of article. All tables should be understandable by itself. All figures with high quality should be prepared in black and white as separate files.

7. References: Authors must be careful for the reference formats of both in-text citations and bibliography. In-text citations use author(s)-year in parentheses, the proper format is (Smith 2008; Kennedy and Smith 2009; John et al., 2010a, 2010b) or Smith (2008).Two authors use "and" in between. Using "et al.," when there are more than 2 authors. Multiple citations in a sentence must be in chronological order first, then alphabetical order. Bibliography should be in the last part of article and arranged alphabetically by authors or title. List first 6 authors and followed by "et al." when there are more than 6 authors. The title is followed the last author. Abbreviated journals are according to the conventional ISO abbreviations used by PubMed. One-word journal title must be spelled out. Year of publication, volume, issue in parentheses, and begin and end pages. These are examples of bibliography.

Barker K. At the Bench: A laboratory navigator. New York: Cold Spring Harbor Laboratory Press; 1998.

Fairbrother JM, Gyles CL. Escherichiacoliinfections. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, editors. Diseases of swine. 9th ed. Iowa: Blackwell Publishing; 2006. p. 639-74.

Laohasinnarong D, Kaeoket K, Prasitphon B. Estrus synchronization in gilts with altrenogest by different given time. Proceedings of the 19th IPVS Congress. Copenhagen, Denmark: Narayana Press; 2006. p. 118.

Meng X-J, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, et al. A novel virus in swine is closely related to the human hepatitis E virus. Proc Natl Acad Sci. 1997;94(18):9860-5.

WHO media centre. African trypanosomiasis (sleeping sickness) [Internet]. WHO. 2010 [cited 2011 Oct 29]. Available from: http://www.who.int/mediacentre/factsheets/fs259/en/.

8. Scientific terms should use the update and follow the International Code of Nomenclature, written by emphasis.

9. Standard abbreviations and symbols are acceptable without definition.

Please visit *JAAS* website for more information and manuscript template, https://he02.tci-thaijo.org/index.php/jaas_muvs

Editor-in-Chief email: editor.jaas2020@gmail.com

Address: Faculty of Veterinary Science, Mahidol University, 999 Phuttamonthon Sai 4, Salaya, Phuttamonthon, Nakornphatom 73170 Thailand.

Editor Note

สวัสดีครับท่านผู้อ่านและสมาชิกวารสาร Journal of Applied Animal Science (JAAS) ทุกท่าน วารสารฉบับนี้เป็นวารสารฉบับแรก ของปีที่ 16 ซึ่งนับว่าเป็นปีที่ 16 ที่วารสารได้เผยแพร่ในลักษณะรูปเล่มและเป็นปีที่ 2 ที่วารสารเผยแพร่ในรูปแบบออนไลน์ (เว็บไซต์) ภายใต้หมายเลข electronic ISSN ของวารสาร 2985-1211

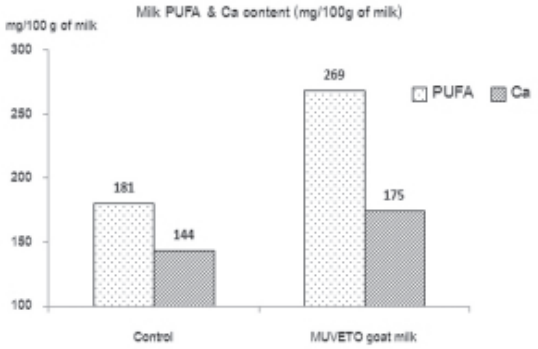
ภายในวารสารฉบับนี้ประกอบด้วยบทความที่น่าสนใจหลายเรื่อง ได้แก่ บทความวิจัย 1 เรื่อง **"ผลของสารสกัดมาตรฐานบัวบกอีซีเอ 233 ต่อการประเมินทางคลินิกและภาพทางรังสีโดยใช้เครื่องเอกซเรย์คอมพิวเตอร์ในสุนัขที่มีภาวะข้อเสื่อม"** และรายงานสัตว์ป่วย 3 เรื่อง **"ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดงในสุนัขโตเต็มวัย พันธุ์ปอมเมอเรเนียน เพศผู้" "การใช้ยาหยดม็อกซิเด็กตินและอิมิดาโคลพริดร่วมกับด็อกซีไซคลินในการรักษาโรคพยาธิฟิลาเรียในระบบน้ำเหลืองในสุนัข: รายงานสัตว์ป่วย"** และ **"การตัดกระเพาะปัสสาวะออกบางส่วนร่วมกับเปลี่ยนย้ายรูเปิดท่อไตบริเวณกระเพาะปัสสาวะในสุนัขที่เป็นมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์"**

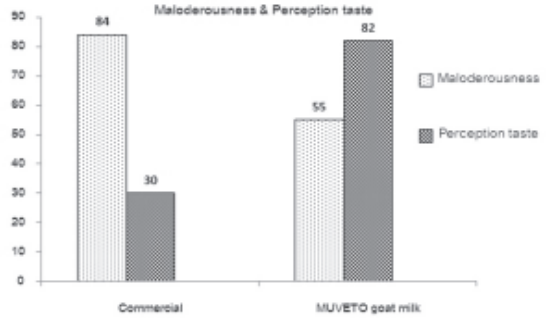
บทความที่ได้รับการตีพิมพ์ในวารสาร Journal of Applied Animal Science (JAAS) ทุกเรื่องได้ผ่านการประเมินและพิจารณาจากผู้ทรงคุณวุฒิที่มีความเชี่ยวชาญในสาขานั้นๆ อย่างน้อย 3 ท่าน จึงเป็นการการันตีได้ถึงคุณภาพของบทความที่ได้รับการเผยแพร่ในวารสารของเรา นอกจากนี้ทางวารสารยังมีระยะเวลาในพิจารณาบทความและระยะเวลาเผยแพร่ที่รวดเร็วมากยิ่งขึ้น ดังนั้นจึงขอเชิญผู้ที่สนใจจะเผยแพร่ผลงานงานวิจัยและองค์ความรู้ต่าง ๆ ด้านสัตวแพทยศาสตร์และสัตวศาสตร์ ส่งบทความได้ที่ https://he02.tci-thaijo.org/index.php/jaas_muvs/about/submissions หรือติดต่อสอบถามเพิ่มเติม โทรศัพท์ 0-2441-5242 ต่อ 1308

รองศาสตราจารย์ ดร.นายสัตวแพทย์ธนศักดิ์ ช่างบรรจง

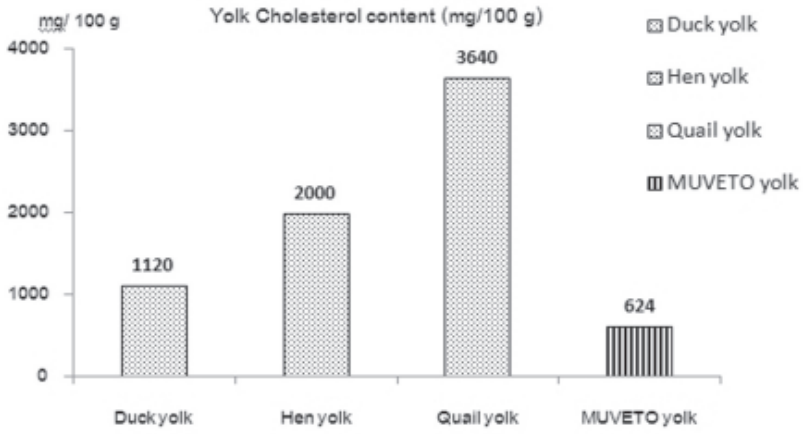
บรรณาธิการ (Editor-in-Chief)

MUVETO





Functional goat milk: Naturally high PUFA,Ca and maloderousness



Functional egg: Low cholesterol

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Case Report

A Case Report of Canine Pure Red Cell Aplasia in Middle-Aged Male Pomeranian

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Received: 2 March 2023; Revised: 13 April 2023; Accepted: 24 April 2023

Abstract

Canine pure red cell aplasia (PRCA) is a rare pathological condition in dogs related to a lack of erythroid precursor linages that cause a failure of red blood cell production, leading to severe anemia. Middle-aged dogs, especially females, have a great chance of developing acquired pure red cell aplasia. The clinical signs show pale mucous membrane, systolic murmur, tachypnea, tachycardia, weakness, syncope, and seizure. For diagnosis, the clinical pathological findings show the normocytic normochromic non-regenerative anemia, and the bone marrow pathological findings show a lack of erythroid precursor cells. According to the responsiveness of the immunosuppressive therapy, PRCA is proposed as one form of the immune-mediated anemia. Most PRCA are secondary diseases associated with recombinant human erythropoietin (rhEPO) treatment, infectious disease including parvoviral infection or modified-live parvovirus vaccination, or bone marrow cancer. In case of poor prognosis, the histopathology shows collagenous fibrosis in the bone marrow, and recurrent diseases after withdrawing the treatment commonly occur. In this case study, an 11-year-old non-castrated male Pomeranian dog was presented with a history of syncope. The non-regenerative anemia was found with a decrease of erythroid precursor in the bone marrow. The dog has been successfully treated with prednisolone 2 mg/kg once daily and tapered down every two weeks. The clinical signs of syncope and other blood parameters improved after 2 weeks of treatment.

Keywords: Pure red cell aplasia, non-regenerative anemia, syncope

ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดงในสุนัขโตเต็มวัย พันธุ์ปอมเมอเรเนียน เพศผู้

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Received: 2 March 2023; Revised: 13 April 2023; Accepted: 24 April 2023

บทคัดย่อ

ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดง (Pure red cell aplasia: PRCA) เป็นภาวะที่พบได้ไม่บ่อย ส่งผลให้สัตว์มีภาวะเลือดจางรุนแรง มักพบในสุนัขโตเต็มวัยโดยเฉพาะเพศเมีย สุนัขมักมาด้วยอาการเยื่อเมือกซีด หายใจเร็ว หัวใจเต้นเร็ว หรือมีภาวะหมดสติ เมื่อตรวจเลือดมักพบภาวะเลือดจางชนิดที่ไม่มีการตอบสนองจากไขกระดูก เม็ดเลือดแดงมักมีขนาดปกติ โดยคาดว่ามีความเกี่ยวข้องกับระบบภูมิคุ้มกันเนื่องจากในการรักษามีการตอบสนองต่อยากดภูมิคุ้มกันได้ดี มักเป็นโรคที่เกิดตามมาจากการใช้ฮอร์โมนอิริโทโพอิติน (rhEPO) กระตุ้นการสร้างเม็ดเลือด การติดเชื้อ เช่น พาร์โวไวรัส พยาธิเม็ดเลือด หรือ เป็นมะเร็งกระดูก เป็นต้น ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดงมีพยากรณ์โรคที่ไม่ดี โดยเฉพาะอย่างยิ่งในกรณีที่ไขกระดูกมีการสร้างคอลลาเจนมาแทนที่แล้ว และมักกลับมาเป็นซ้ำหลังจากหยุดยากดภูมิคุ้มกัน สุนัขพันธุ์ปอมเมอเรเนียน อายุ 11 ปี มาโรงพยาบาลสัตว์ด้วยอาการหมดสติเป็นครั้งคราว หลังตรวจพบว่ามีภาวะเลือดจางรุนแรงโดยไม่พบการตอบสนองของไขกระดูก จึงมีการเก็บตัวอย่างไขกระดูก พบภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดง และพบว่ามีการตอบสนองต่อการรักษาด้วยยากดภูมิคุ้มกันชนิดเพรดนิโซโลน ขนาด 2 มิลลิกรัมต่อน้ำหนักตัว 1 กิโลกรัมได้ดี ภายหลังการรักษา 2 สัปดาห์ ไม่พบอาการหมดสติและมีผลเลือดอยู่ในเกณฑ์ปกติ

คำสำคัญ : ภาวะไขกระดูกขาดเซลล์ต้นกำเนิดเม็ดเลือดแดง ภาวะเลือดจางที่ชนิดไม่มีการตอบสนองของไขกระดูก ภาวะหมดสติ

Introduction

Pure red cell aplasia (PRCA) in dogs is associated with the disease of erythroid precursor and progenitor leading to a secondary failure of the erythropoiesis (Lucidi 2022). The clinical signs are pale mucous membrane, systolic murmur, tachypnea, or tachycardia (Means 2016). Most PRCA has a greater incidence in middle-aged female dogs and has a common clinical sign of severe anemia (Lucidi 2022). The information related to breed predisposition is unknown (Brooks et al., 2022).

Anemia is the most common hematological abnormality defined as a reduced red blood cell (RBC) mass and is recognized when the hematological values of the red blood cell panels, including the packed cell volume (PCV), hematocrit (Hct), hemoglobin concentration (Hb), or RBC counts, are below reference intervals (Ettinger and Feldman 2000). Anemia contributes to the development of pale mucous membrane, tachypnea, tachycardia, anorexia, weight loss, fatigue, lethargy, depression, weakness, syncope, or seizures (Ettinger et al., 2017). The two main pathophysiological mechanisms, regenerative anemia and non-regenerative anemia, are the causes of pathological anemia and divided into hemorrhage, hemolysis, and decreased red blood cell production (Ettinger and Feldman 2000). To find the causes of anemia, distinguishing between regenerative and non-regenerative anemia is essential for clinical diagnosis (Means 2016). The cause of regenerative anemia is likely to be hemolysis or hemorrhage (Ettinger et al., 2017). Meanwhile, the non-regenerative anemia is due to the decreased red blood cell production, which can be related to an abnormal function of extramedullary or intramedullary diseases including immune-mediated diseases (Weiss 2008). In the persistent chronic non-regenerative anemia in dogs, the clinical information shows the ineffective erythropoiesis for at least five days for the clinical duration (Lucidi 2022). The clinical pathological findings show normocytic normochromic anemia correlated to the reduced erythrocytic production and the low number of erythrocytic precursors in the bone marrow (Brooks et al., 2022).

The immune-mediated non-regenerative anemia is associated with a persistent anemia with a lack of a reticulocyte (Lucidi 2022). The symptom can be classified by the stage of targeted erythroid cell in bone marrow into three diseases including pure red cell aplasia (PRCA), precursor-targeted immune-mediated anemia (PIMA) and immune-mediated hemolytic anemia (IMHA) (Lucidi 2022; Brooks et al., 2022).

For the diagnosis of PRCA, the hematological findings are usually severe non-regenerative anemia with normocytic normochromic RBCs; therefore, the canine Coomb's test and direct antiglobulin test (DAT) which detects antibodies or complement bound to RBC membranes, are expected to be negative, but positive results have been occasionally reported; consequently, characteristic of bone marrow is very useful to clarify the diseases (Lucidi 2022). Not only PIMA but also IMHA and erythroid hypoplasia are often misunderstood due to the similarity in clinical conditions (Lucidi et al., 2017). PRCA affects erythroid progenitors and the presence of only rare erythroid progenitors in bone marrow (Brooks et al., 2022). PIMA affects early erythroid precursors and rubiblasts through metarubicytes respectively, and IMHA directly affects the number of red blood cells; meanwhile, erythroid hypoplasia presence marks red blood cell disorders (Lucidi 2022).

PRCA usually found as an acquired disease that can be both a primary disorder or secondary to some other

disorder (Means 2016) and has been associated with recombinant human erythropoietin (rhEPO) treatment (Fiocchi et al., 2017), parvoviral infection, or modified-live parvovirus vaccination, *Ehrlichia canis* infection, myelodysplastic syndromes, and myelofibrosis (Lucidi 2022).

The therapeutic strategy for PRCA in dogs is directly involved to the using of the immunosuppressive therapy (To et al., 2021). Glucocorticoids are the drug of choice for treating the canine PRCA, including prednisone, prednisolone, or dexamethasone administration (Brooks et al., 2022). Prednisolone is an intermediate acting corticosteroid and well absorbed after oral administration; and peak plasma concentration is increased in 0.5-3 hours (Schijvens et al., 2019) with half-life of 12-36 hours (Plumb 2011). It is a lipophilic molecule that can easily diffuse across the cell membrane and bind to the glucocorticoid receptors in the cytoplasm then the glucocorticoid complex is formed and transferred into the nucleus (Plumb 2011). After entering the nucleus, the activated glucocorticoid complex binds to the DNA resulting in increased an expression of anti-inflammatory genes and decreased an expression of pro-inflammatory genes (Schijvens et al., 2019). Prednisolone is metabolized in the liver and excreted by the kidney (Plumb 2011). Adverse effects of long-term administration exogenous corticosteroids with immunosuppressive dose are usually common and severe including dry hair coat, weight gain, panting, vomiting, diarrhea, elevated liver enzymes associated with steroid hepatopathy, pancreatitis, gastrointestinal ulceration, lipemia, activation of diabetes mellitus, iatrogenic hyperadrenocorticism, and thromboembolism; also the dog may be seen with polydipsia, polyuria, and polyphagia (Plumb 2011). Moreover, the dogs with thromboembolism significantly have an approximately 25 times shorter survival time than the dogs without thromboembolism (Ettinger and Feldman 2000).

Case description

An 11-years-old non-castrated male Pomeranian dog, 4.1 kilograms in body weight, was presented at the Prasuarthorn animal hospital, Faculty of Veterinary Science, Mahidol University, with a history of syncope twice for 10 minutes on the day before presented. He was fully vaccinated and regularly checked by a parasitic prevention program. Any abnormal medical conditions were not previously noticed by owners, and the dog has no history of toxin ingestion.

On initial physical examination, the dog was bright, alert, and responsive. The heart murmur was noticed, but the lung sound was normal. The heart rate was 130 bpm with a systolic blood pressure of 110 mmHg, and the respiratory rate was 28 bpm. The mucous membrane was pale with CRT < 2 sec. The moderate dental tartar was observed from the oral examination. There were no ocular and nasal discharges. The uncomfortable clinical signs such as abdominal cramp, or discomfort were not found. The rectal temperature was 101°F.

For the clinical diagnosis, the disease investigations are shown in Figure 1. A hematological examination was completed by the Mindray BC-5300 Vet, an auto hematology analyzer, to rule out systemic diseases and showed severe normocytic normochromic anemia with non-regenerative. Blood smear shows few polychromasia, hypochromasia, poikilocytosis with macrocyte and microcyte which are associated to an iron deficiency. The serum biochemistry was tested by the Beckman Coulter AU-480 chemistry analyzer and resulted in unremarkable. The reticulocyte percentage was corrected

with a normal PCV (45%), and the corrected reticulocyte percentage is 0.033% indicating non-regenerative anemia. The full results of the blood examination are shown in Table 1. The repeated complete blood count was examined three days after the first visit are shown in Table 2. The hematological examination showed the lower of all red blood cells, hemoglobin, and hematocrit than the result from the first visit. The second corrected reticulocyte percentage is 0.06% interpreted as non-regenerative anemia. All blood smear test results stay consisted; however thrombocytopenia occurring from decrease production, increase consumption, increase destruction, and increase sequestration was found, so the test kit for *Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi* antibody, and *Dirofilaria immitis* antigen was performed to investigate the blood parasites (SNAP 4Dx test kit®, IDEXX, Thailand). The Polymerase Chain Reaction (PCR) was also done to confirm *Ehrlichia canis*, *Anaplasma* spp., *Babesia* spp., and *Hepatozoon canis* infection. The PCR results were all negative. The flow cytometric assay for detecting the platelet-IgG was also utilized to rule out IMT and the result was negative.

The immune-mediated hemolytic anemia (IMHA) was investigated by the direct canine Coomb's test and the flow cytometric assay for detecting the RBC-IgG. Both assay results were negative.

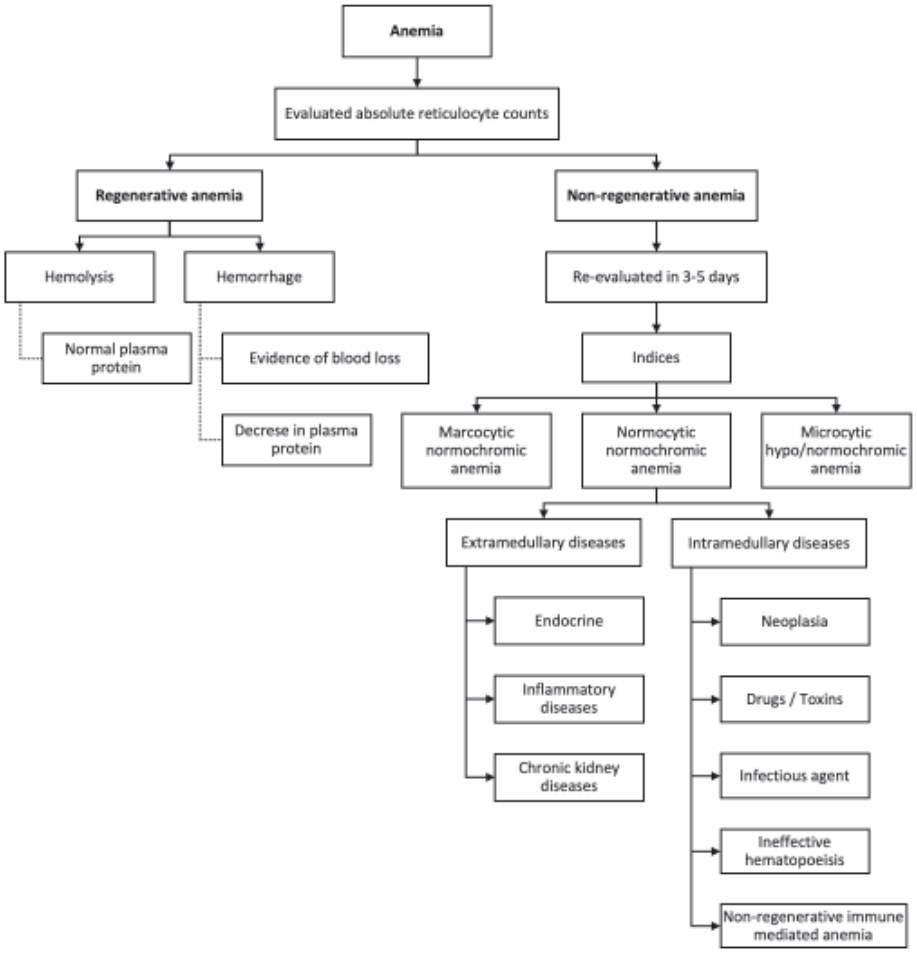

Figure 1. Flow chart of disease investigation. Anemia can be divided into regenerative and non-regenerative by absolute reticulocyte counts, which should be re-evaluated in 3 to 5 days. The non-regenerative anemia can be separated by indices to investigate causes of anemia.

Table 1. Results of blood examination on 14 July 2022, the first day of the visit.

Parameters	14/07/2022	Reference	Unit
Complete blood count			
WBC	8.28	6.0-17.0	10^3/uL
Monocytes	0.66	0.15-1.35	10^3/uL
Neutrophils	5.96	3.00-11.50	10^3/uL
Band neutrophils	0	0-3	%
Lymphocytes	1.57	1.00-4.80	10^3/uL
Eosinophils	0.08	0.10-1.25	10^3/uL
Basophils	0	0.00-0.10	10^3/uL
RBC	2.18	5.0-9.0	10^6/uL
Hb	4.8	10.0-18.0	g/dL
Hct	14.9	35-55	%
MCV	68.0	60-77	fL
MCH	22.0	20-25	pg
MCHC	32.3	32-36	g/dL
PLT	226	200-500	10^3/uL
RDW	20.6	12-15	%
Plasma protein	6.6	6.0-7.5	g/dL
Platelet smear	Adequate		
Reticulocytes	0.1	0-1.5	%
Blood morphology			
Hypochromia	2+		
Polychromasia	Few		
Anisocytosis	2+		
Macrocyte	1+		
Microcyte	1+		
Blood chemistry			
BUN	16	7-27	mg/dL
Creatinine	1.11	0.5-1.8	mg/dL
ALT	88	10-100	U/L
ALP	66	23-212	U/L
Total protein	6.4	5.2-8.2	g/dL
Albumin	2.9	2.7-3.8	g/dL

Table 2. Results of blood examination on 16 July 2022, the second day of the visit.

Parameters	16/07/2022	Reference	Unit
Complete blood count			
WBC	9.14	6.0-17.0	10^3/uL
Monocytes	0.09	0.15-1.35	10^3/uL
Neutrophils	6.76	3.00-11.50	10^3/uL
Band neutrophils	0	0-3	%
Lymphocytes	2.28	1.00-4.80	10^3/uL
Eosinophils	0	0.10-1.25	10^3/uL
Basophils	0	0.00-0.10	10^3/uL
RBC	2.08	5.0-9.0	10^6/uL
Hb	4.8	10.0-18.0	g/dL
Hct	13.9	35-55	%
MCV	67.1	60-77	fL
MCH	23.1	20-25	pg
MCHC	34.4	32-36	g/dL
PLT	170	200-500	10^3/uL
RDW	21.8	12-15	%
Plasma protein	7	6.0-7.5	g/dL
Platelet smear	Adequate		
Reticulocytes	0.2	0-1.5	%
Blood morphology			
Hypochromia	2+		
Polychromasia	Few		
Anisocytosis	1+		
Macrocyte	1+		
Microcyte	Few		

Thoracic radiography was conducted by the Philips Optimus 65 had shown in Figure 2, and abdominal radiography had shown in Figure 3 to rule out cardiopulmonary diseases and internal abdominal mass. Thoracic radiography illustrated unremarkable pulmonary diseases and mild cardiomegaly in the vertebral heart score (VHS) of 10.5 with a pulmonary knob, echocardiography should be further investigated. For abdominal radiography, calcification was found in both renal pelvises. There is no evidence of renomegaly, hepatomegaly, or splenomegaly.

Ultrasonography was executed by the GE Healthcare Logiq E10S to investigate renal blood flow and rule out abdominal mass. The ultrasonography result is homogeneous diffuse hypoechoic parenchyma with a prominent portal vein and rounding of the caudal tip of the liver as shown in Figure 4. The hepatic vein was dilated by 4 mm in Figure 5. Hyperechoic of the renal cortex and decrease of corticomedullary distinction with normal color blood flow at interlobar vessels of both kidneys within normal kidney size in length 3.7 cm in Figure 6. Gall bladder, spleen, adrenal glands, pancreas,

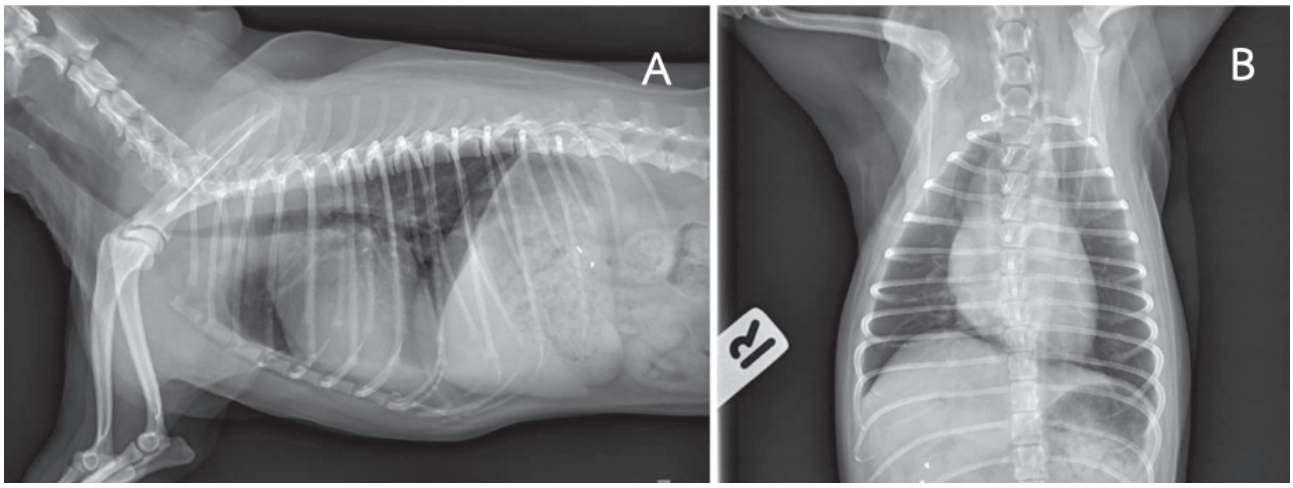

Figure 2. Lateral (A) and ventrodorsal (B) view of thoracic radiography suggested mild cardiomegaly in a vertebral heart score of 10.5.

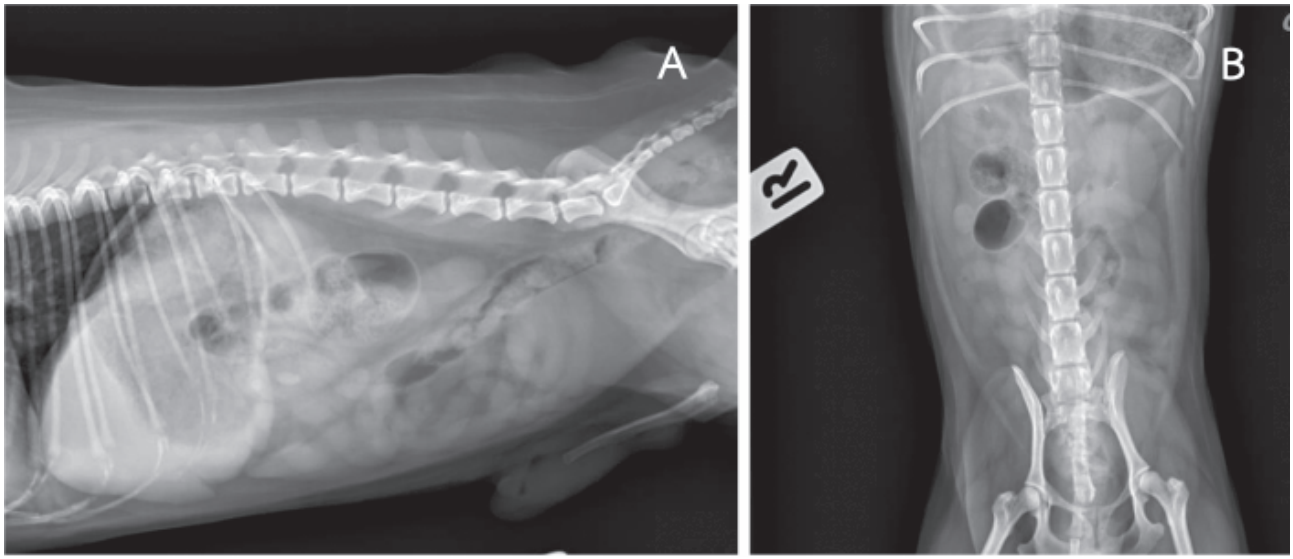

Figure 3. Lateral (A) and ventrodorsal (B) view of abdominal radiography which is unremarkable.

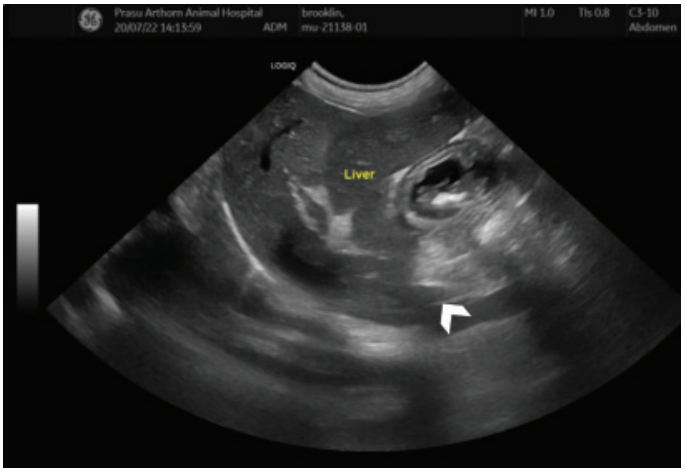

Figure 4. Rounding of the hepatic caudal tip showed a hepatic congestion (white arrowhead).

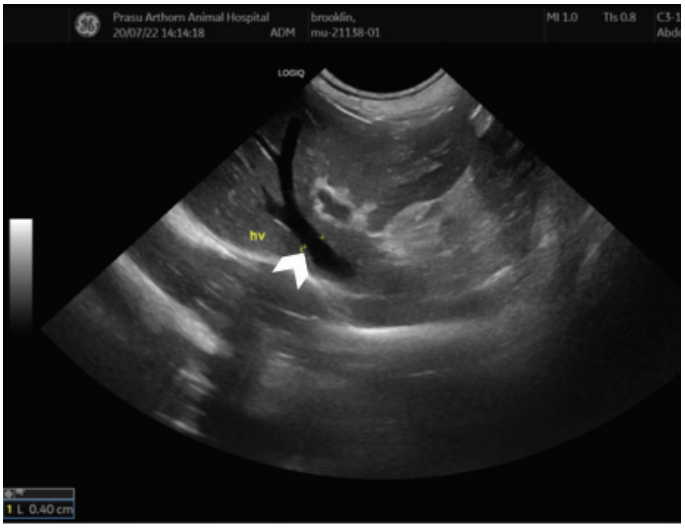

Figure 5. Hepatic vein was dilated in diameter of 4 mm specified a hepatic congestion (white arrowhead).

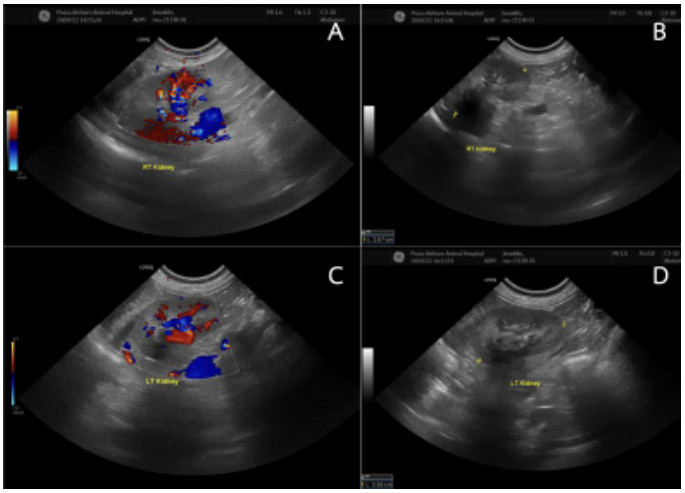

Figure 6. Right kidney with color flow (A), right kidney with no color flow (B), left kidney with color flow (C), and left kidney with no color flow (D) are decrease of corticomedullary distinction with normal color blood flow at interlobar vessels of both kidneys within normal kidney size both in length 3.7 cm that indicates an early stage of CKD.

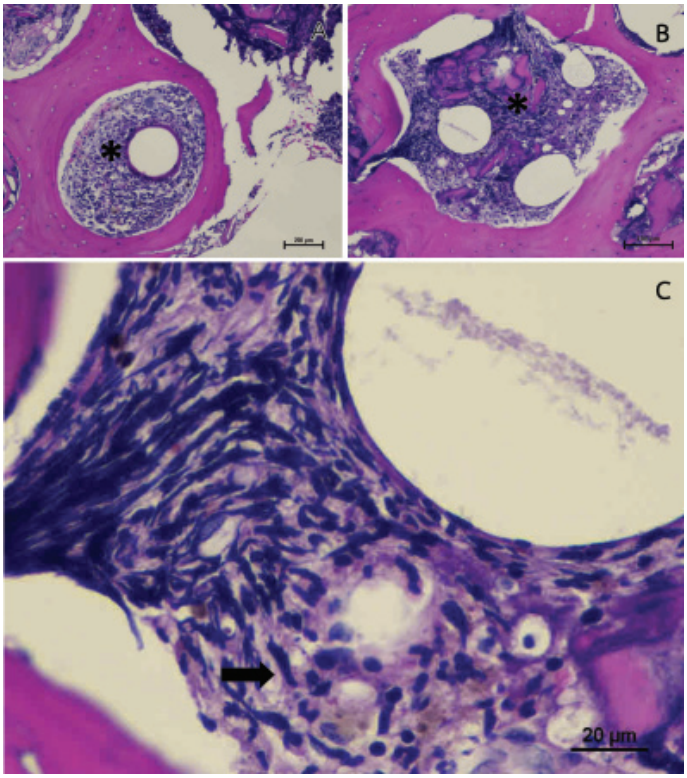

Figure 7. Bone marrow biopsy with H&E stain 4x (A), 10x (B), and 40x (C) respectively. Osteodystrophy The progenitor cells were replaced as fibrous tissues produced by fibroblasts, (asterisk). The fibroblastic spindle-shaped cells proliferated in the marrow cavity (arrow).

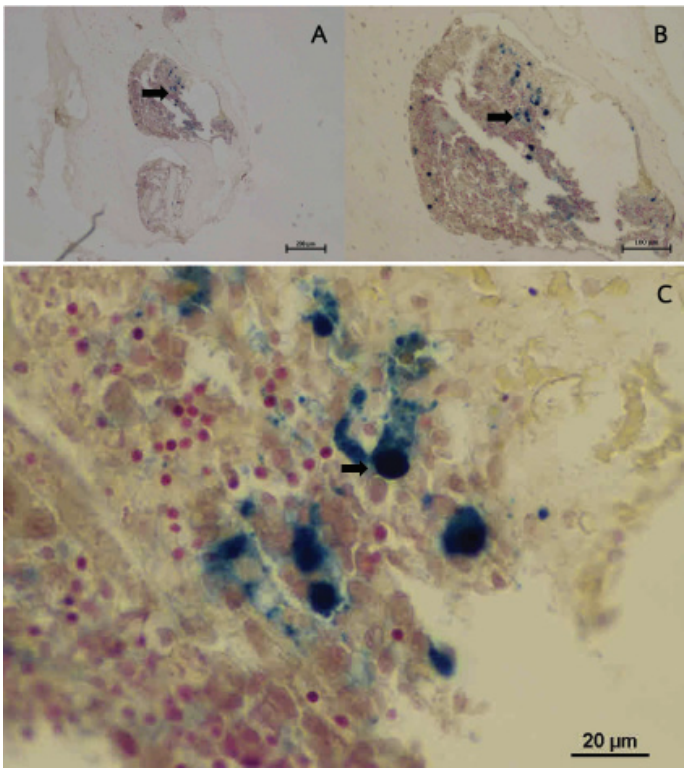

Figure 8. Bone marrow biopsy with Prussian blue 4x (A), 10x (B), and 40x (C) respectively. The iron-storage cells were stained to identify the iron-storage resources in the bone marrow. The blue-stained positive cells were depleted in the bone marrow cavity (arrows).

urinary bladder, and abdominal lymph node are no remarkable lesions. According to the ultrasonographic findings, passive congestion and renal nephropathy with early stage of chronic kidney disease (CKD) was diagnosed, there are no remarkable organomegaly, or neoplasia were found. To confirm an organomegaly or internal mass, a whole-body CT scan was done and resulted in unremarkable.

To investigate the cardiogenic diseases, echocardiography was performed by the Mindray Merge DC-70. The 2D measurement found that left atrial and aortic (LA: Ao) ratio is 1.34, and the normalized left ventricular internal dimension in diastole (LVIDDN) is 1.08. Therefore, both ratios are in a normal range. Doppler measurement found a mitral valve thickening and mild regurgitation without mitral valve prolapses. Marked tricuspid regurgitation (Max 3.8 m/s) and trivial pulmonic regurgitation was reported. Aorta, Pulmonary artery, and vein were not congested. The systolic and diastolic LV functions were non-remarkable lesions, with no aortic regurgitation. The diagnosis for echocardiography is myxomatous mitral valve disease stage B1 (MMVD B1) with intermediated pulmonary hypertension, cardiogenic syncope should be considered.

According to the diagnosis result above, there are no significant causes of severe non-regenerative anemia. A core bone marrow biopsy was collected from the left proximal humerus taken to investigate erythrocytic progenitors and precursors. The biopsy was processed as formalin-fixed embedded samples, sectioned, and stained with hematoxylin and eosin stain (H&E) and Perls Prussian blue stain. The bone marrow has a low number of erythroid lineages that were replaced by fibrous tissues and adipocytes, indicating osteodystrophy in Figure 7. The Perls Prussian blue was performed to identify non-heme iron deposition, and there was a non-heme iron deposition such as ferritin and hemosiderin in the bone marrow parenchyma in Figure 8. There are no remarkable abnormalities in cells such as leukemia or neoplasm found in this case, the bone marrow biopsy results in leading to the definitive diagnosis as pure red cell aplasia (PRCA).

For the treatment, a blood transfusion was completed while the diagnosis was in process and brought hematocrit from 13.9% to 32.9%. After the blood transfusion, the level of the hematocrit still decreased to 26.1% in two weeks. The prednisolone administration at 2 mg/kg per oral once a day was started with a repeated blood examination after the two weeks of the treatment, and the dog has a good response. Hematocrit was increased to 35.3%. After that, prednisolone was tapered down 25% every two weeks and recheck hematocrit. Hematocrit is still in reference interval. The comparison of hematocrit between before and after the treatment of prednisolone are shown in Table 3.

Table 3. Comparing of blood examination results between before and after the treatment.

Parameters	20/07/22*	4/8/22**	18/8/22***	7/9/22****	Reference	Unit
Complete blood count						
WBC	8.45	13.42	8.63	10.88	6.0-17.0	10^3/uL
Monocytes	0.52	0.8	0.43	0.32	0.15-1.35	10^3/uL
Neutrophils	6.16	10.1	6.12	8.26	3.00-11.50	10^3/uL
Band neutrophils	0	0	0	0	0-3	%
Lymphocytes	1.77	1.98	1.98	2.06	1.00-4.80	10^3/uL
Eosinophils	0	0	0.08	0.21	0.10-1.25	10^3/uL
Basophils	0	0	0	0	0.00-0.10	10^3/uL
RBC	4.62	3.64	4.46	5.81	5.0-9.0	10^6/uL
Hb	10.9	8.8	10.7	14.8	10.0-18.0	g/dL
Hct	32.9	26.1	35.3	45.3	35-55	%
MCV	71.1	71.8	79.1	78	60-77	fL
MCH	23.6	24.2	24	25.5	20-25	pg
MCHC	33.2	33.7	30.3	32.7	32-36	g/dL
PLT	153	190	308	443	200-500	10^3/uL
RDW	14.8	15.1	15.3	14.2	12-15	%
Plasma protein	8.6	7	8	7	6.0-7.5	g/dL
Platelet smear	Decrease	Adequate	Adequate	Adequate		
Hypochromia	-	Few	Few	-		
Polychromasia	-	Few	-	-		
Anisocytosis	Few	Few	1+	1+		
Macrocyte	Few	Few	1+	1+		
Microcyte	-	-				

*After blood transfusion, **Monitor in 2 weeks during the diagnosis, the hematocrit was decreasing, prednisolone was started after the blood examination, *** 2 weeks after start prednisolone 2 mg/kg, **** 2 weeks after tapering prednisolone down prednisolone 25%.

Discussion

Our case study is a middle-aged male dog that PRCA commonly affected (Lucidi 2022), presented at the animal hospital with a history of syncope associated with both cardiovascular system and severe anemia. For physical examination, the initial problem lists including a pale mucous membrane and systolic cardiac murmur in accordance with clinical signs of PRCA (Means 2016). From echocardiography results, the diagnosis is MMVD stage B1 with intermediate pulmonary hypertension and cardiogenic syncope was indicated, and it is probably associates to respiratory disease or hypoxia due to the tricuspid regurgitation that is related to systolic cardiac murmur (Reinero et al., 2020), consequently, cardiogenic syncope may not be the primary cause. The result of blood examination shows severe non-regenerative anemia which has two main causes dividing into extramedullary disease including endocrine disease, chronic kidney disease, inflammation, and neoplasia, and the other is intramedullary disease (Ettinger et al., 2017). According to the history taking and all diagnosis, extramedullary disease can be ruled out. The blood biochemistry shows unremarkable to renal and liver functions in the line with radiographic results have no significant of neoplasia lesion or abnormal shape of internal organ. Although, the ultrasonography result shows an early stage of CKD. 60% of CKD has particularly common in developed an anemia in dogs at IRIS stages 3 and 4 and frequently increase in end stage of renal disease (Lippi et al., 2021). For intramedullary disease, the bone marrow is characterized by complete lack of erythroid precursors typically as in PRCA (Means 2016) leading to our diagnosis.

According to a study of Brooks et al., the result demonstrated that the level of IgG fraction of serum of a dog with PRCA inhibited the generation of erythroid precursors in vitro correspond to the production of antibodies against membrane proteins expressed on erythroid progenitors and precursors, cytotoxic T-cell-mediated and molecules affecting the erythroid lineage and suppress erythropoiesis (Brooks et al., 2022). While the mechanism of primary PRCA remains unclear, most cases responsive to immunosuppressive therapy suggest that PRCA in dogs is immune-mediated anemia.

PRCA in dogs can be divided in to congenital and acquired. Most of them are acquired diseases, considering the limited data of young patients; it is difficult to identify congenital PRCA (To et al., 2021). As acquired PRCA can be secondary to a wide variety of conditions including blood parasite infections which can be ruled out from PCR and rule out rhEPO treatment by history taking, but parvovirus vaccination or infection remains one of the primary causes of PRCA. Some of puppies with parvovirus infection develop erythroid and myeloid hypoplasia, and the parvoviral infection or vaccination also causes PRCA in humans and affects erythropoietic precursor cells which can be tested by measuring IgG and IgM levels and by testing for the parvovirus DNA (Lucidi 2022). The limitation of this case is the lack of the test of parvovirus antigen detection due to the limitation of laboratory testing and the dog having no clinical signs associated with canine parvovirus. Parvoviral antigen from the bone marrow biopsy should be tested by fresh tissue sample while our sample is fixed in 10% formaldehyde which requires a specific primer. For blood examination, parvoviral antigen can be tested only if the dog is in viremia. If parvovirus infection can be ruled out, the cause of PRCA of this dog will be primary hematologic disorder, termed idiopathic PRCA. Furthermore, PRCA can be secondary to others disease such as mast cell

tumor (Akiyoshi 2021), or there is a study reported in human that PRCA are related to lymphoma (Choi et al., 2010).

For thrombocytopenia, the decrease production of thrombocytes can occur following an iron deficiency besides an iron supplement. There is a report in human studying about an iron supplement leading to thrombocytopenia (Eisa et al., 2021). Moreover, when the immune system affected to megakaryocyte-erythroid progenitor cells instead of erythroid progenitor cells, the production of thrombocytes will decrease and the destruction of it will increase by immune-mediated disease leading to severe thrombocytopenia termed immune-mediated thrombocytopenia (IMT). The thrombocytopenia of this dog has a better chance of being an iron deficiency according to mild thrombocytopenia and normocytic normochromic anemia were found.

The traditional immunosuppression approach in human began with an oral corticosteroid usually prednisone in doses 1 mg/kg. Overall, this approach appears to have a 40% response rate. (Means 2016). In most human cases, cyclosporine A is the first choice providing the best response rate but requires a concentration monitoring (150 to 250 ng/mL), and the second choice is cyclophosphamide (Lobbes 2023). In the meantime, the drug of choice for treating PRCA in dogs is corticosteroid comprising prednisolone (Brooks et al., 2022) with a combination of corticosteroids, adjuvant immuno-suppressive or immunomodulatory drugs that may be necessary in some cases. Nevertheless, it depends on the time it takes to respond to the treatment. Our case study uses prednisolone, a drug of choice for PRCA, 2 mg/kg per oral once daily then taper down 25% every two weeks; the dog has good response to the treatment. For long-term using corticosteroid, our case study was developed some of these predictable adverse effects which is steroid hepatopathy, polydipsia, polyuria, and weight gain from polyphagia; no thromboembolism were found during the treatment.

In addition, one or multiple blood transfusions may be essential therapy. The response time to the corticosteroid treatment of this dog is within 14 days and bringing the hematocrit back to the reference interval. The disease remission is within 128 days. The median initial response time was 38 days, and the time required for the PCV to return to the reference interval was 118 days; around 77% of dogs treated with immuno-suppressive therapy were in complete recovery (Brooks et al., 2022). The shorter response time than the median time may related to the blood transfusion before using corticosteroid treatment

An approximately 20% of remission dogs relapsed at a median of 302 days during weaning of immunosuppressive therapy (Brooks et al., 2022). The presence of collagenous fibrosis was significantly aggravating the disease. There is a study reported that the median survival time after PRCA was diagnosed without collagenous fibrosis is 1429 days, greater than two times of dogs with collagen myelofibrosis, whose median survival time is 578 days (Ettinger and Feldman 2000).

Conclusion

According to clinical signs of non-regenerative normocytic normochromic anemia, and lack of erythroid lineage from bone marrow biopsy, pure red cells aplasia has a high probability to be the final diagnosis. The dog responded well to the treatment of prednisolone 2 mg/kg per oral once daily and other clinical signs were clinically improved. Monitoring for relapse of this disease is still in progress. PRCA has a poor prognosis, especially with

collagenous fibrosis in the bone marrow, recurrent diseases after withdrawing the treatment commonly occur. The purpose of this case report is to make clinicians aware of possible causes of this disease and improve future definitive diagnosis.

Acknowledgments

The authors would like to express their sincerely gratitude to all staff at the Prasu-arthorn animal hospital, especially, Assistant Professor Rungrote Osathanon for being the consultant, Wachira Trakoolchaisri, DVM, the cardiologist for the diagnosis of cardiogenic disease, Pruksa Julapanthong, DVM, the ultrasonologist for the diagnostic ultrasound, and all the patient's support through collaboration.

References

Akiyoshi M. Precursor-targeted immune-mediated anemia in a dog with a stage IV mast cell tumor and bone marrow infiltration. Vet Clin Pathol. 2021;50:151-7.

Assenmacher TD, Jutkowitz LA, Koenigshof AM, Lucidi CdA, Scott MA. Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases (2004-2013). J Am Vet Med Assoc. 2019;255(3): 366-76.

Brooks MB, Harr KE, Seelig DM, Wardrop KJ, Weiss DJ. Schalm's Veterinary Hematology. 7th ed. Pondicherry: Wiley; 2022.

Casadevall N, Nataf J, Viron B, Kolta A, Kiladjian J-J, Martin-Dupont P, et al. Pure red cell aplasia and antierythropoeitin antibodies in patients treated with recombinant erythropoietin. N Engl J Med. 2002;346(7):469-75.

Choi JH, Oh YH, Park IK. A case of pure red cell aplasia associated with angioimmunoblastic T-cell lymphoma. Cancer Res Treat. 2010;42(2):115-7.

Eisa MS, Al-Tikrity MA, Babikikir MM, Yassin MA. Thrombocytopenia secondary to iron deficiency anemia responding to iron therapy. Clin Case Rep. 2021;9(7):1-4.

Ettinger SJ, Feldman EC. Textbook of veterinary internal medicine. 7th ed. St. Louis: Elsevier; 2000.

Ettinger SJ, Feldman EC, Cote E. Textbook of Veterinary Internal Medicine: Diseases of the dog and the cat. 8th ed. St. Louis: Elsevier; 2017.

Fiocchi EH, Cowgill LD, Brown DC, Markovich JE, Tucker S, Labato MA, et al. The Use of Darbepoetin to Stimulate Erythropoiesis in the Treatment of Anemia of Chronic Kidney Disease in Dogs. J Vet Intern Med. 2017;31(2):476-85.

Javinsky E. Hematology and Immune-Related Disorders. In: Little SE, editor. The Cat: Clinical medicine and management. St. Louis: Elsevier; 2012. p. 643-703.

Lippi I, Perondi F, Lubas G, Gori E, Pierini A, D'Addetta A, et al. Erythrogram Patterns in Dogs with Chronic Kidney Disease. Vet Sci. 2021;8(7):123.

Lobbes H. Pure red cell aplasia: Diagnosis, classification and treatment. Rev Med Interne. 2023;44(1):19-26.

Lucidi CA, de Rezende CLE, Jutkowitz LA, Scott MA. Histologic and cytologic bone marrow findings in dogs with suspected precursor-targeted immune-mediated anemia and associated phagocytosis of erythroid precursors. Vet Clin Pathol. 2017;46(3): 401-15.

Lucidi CA. Precursor-Targeted Immune-Mediated Anemia and Pure Red Cell Aplasia in Dogs and Cats. In: Brooks MB, Harr KE, Seelig DM, Wardrop KJ, Weiss DJ, editors. Schalm's Veterinary Hematology. 7th ed. Pondicherry: Wiley; 2022. p. 307-12.

Means J, Robert T. Pure red cell aplasia. Blood. 2016; 128(21):2504-9.

Parrish CR. Pathogenesis of feline panleukopenia virus and canine parvovirus. Baillidres Clin Haematol. 1995;8(1):57-71.

Plumb DC. Plum's Veterinary Drug Handbook. 7th ed. Wisconsin: Wiley; 2011. p. 2973-86.

Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, et al. ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. J Vet Intern Med. 2020;34(2):549-73.

Sawada K, Fujishima N, Hirokawa M. Acquired pure red cell aplasia: updated review of treatment. Br J Haematol. 2008;142(4):505-14.

Schijvens AM, Ter Heine R, de Wildt SN, Schreuder MF. Pharmacology and pharmacogenetics of prednisone and prednisolone in patients with nephrotic syndrome. Pediatr Nephrol. 2019;34(3):389-403.

Stokol T. Idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in dogs: 43 cases (1998-1999). J Am Vet Med Assoc. 2000; 216(9):1429-36.

To I, Boelsterli PR, Walter JJ, Rutland BE. Suspected primary pure red cell aplasia in a 4-month-old intact male mixed Bernese moutain dog. Can Vet J. 2021;62:408-12.

Weiss DJ. Bone marrow pathology in dogs and cats with non-regenerative immune-mediated haemolytic anaemia and pure red cell aplasia. J Comp Pathol. 2008;138(1):46-53.

Weiss DJ, Wardrop KJ. Schalm's Veterinary Hematology. 6th ed. Iowa: Wiley; 2011.

Case Report

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

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Received: 3 June 2023; Revised: 10 July 2023; Accepted: 17 July 2023

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 6th and 10th 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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Received: 3 June 2023; Revised: 10 July 2023; Accepted: 17 July 2023

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).

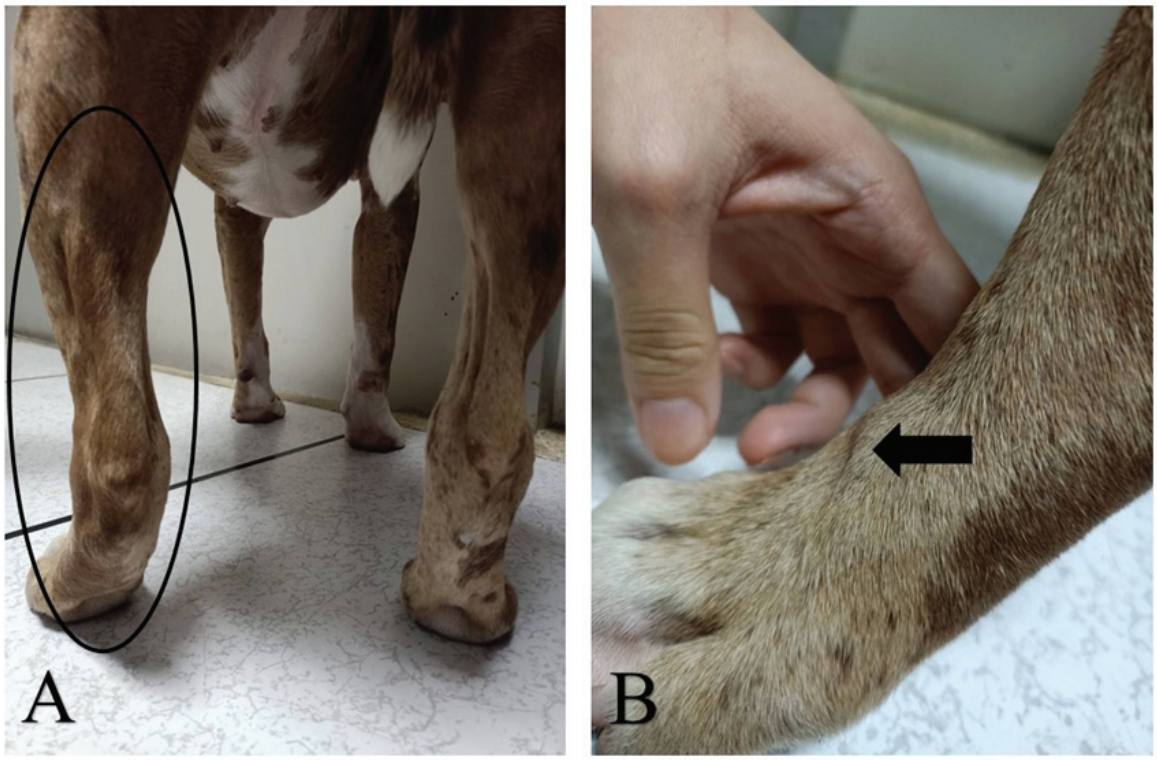

Figure 1. (A) The dog exhibited left hindlimb edema (circled area), indicating swelling in the affected limb, and (B) upon applying pressure to the metatarsal area, a persistent dimple was observed in the skin (arrow), indicating the presence of pitting edema.

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,

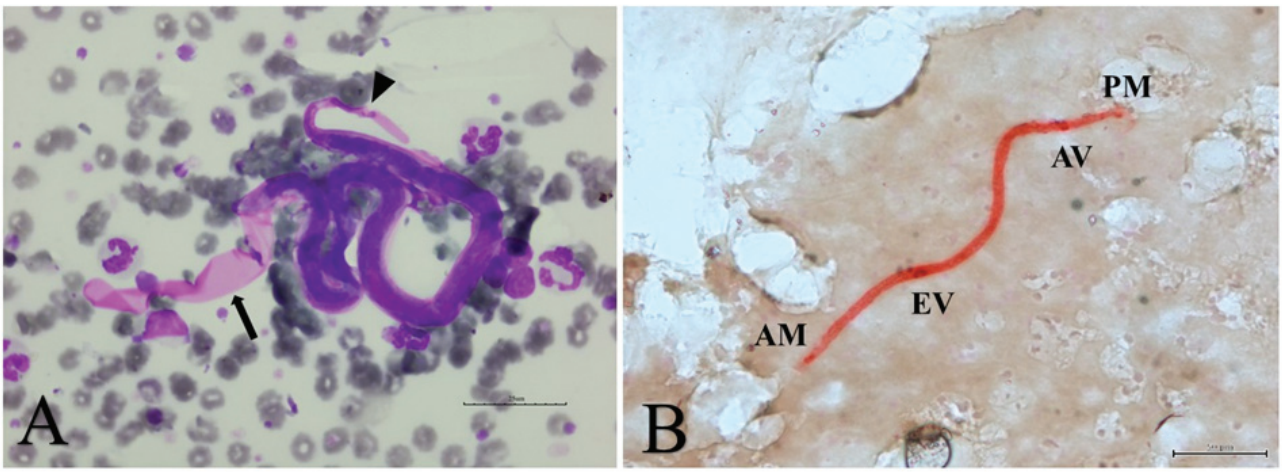

Figure 2. (A) Microfilaria stained with Giemsa, revealing the presence of the microfilarial sheath (arrow) and two-terminal nuclei (arrowhead), observed at 1,000x magnification (scale bar = 25 μm). (B) Acid phosphatase staining of *B. pahangi* microfilaria, demonstrating diffuse staining throughout the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

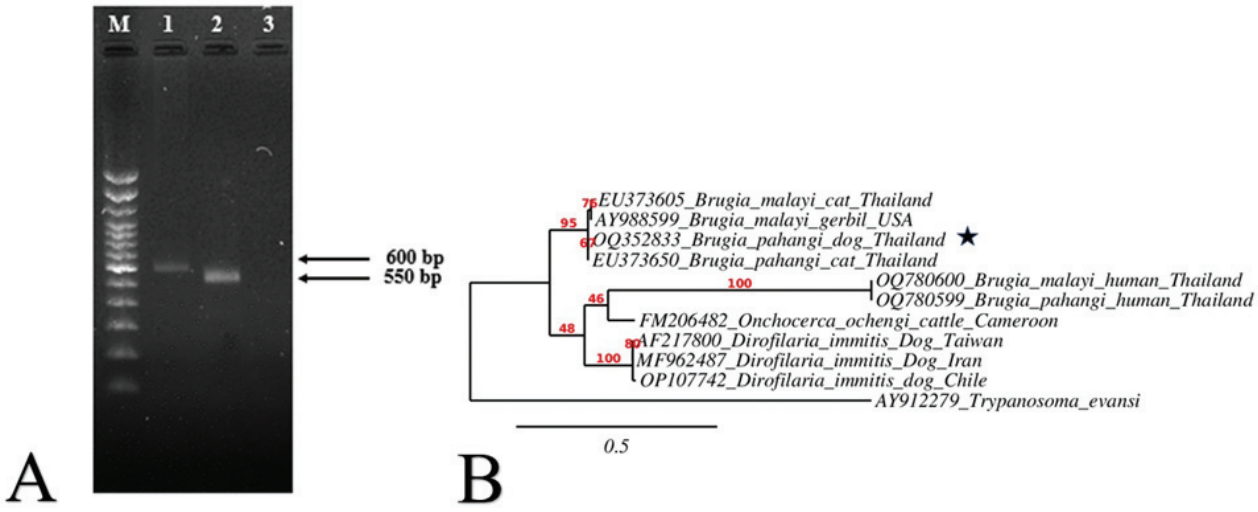

Figure 3. (A) Gel electrophoresis of PCR amplification targeting the 5.8S-ITS2-28S gene on a 2% agarose gel. Lane M represents the DNA ladder with a molecular weight of 100 bp. Lane 1 shows the PCR product from *B. pahangi* microfilaria (sample from this case report) with an expected size of approximately 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).

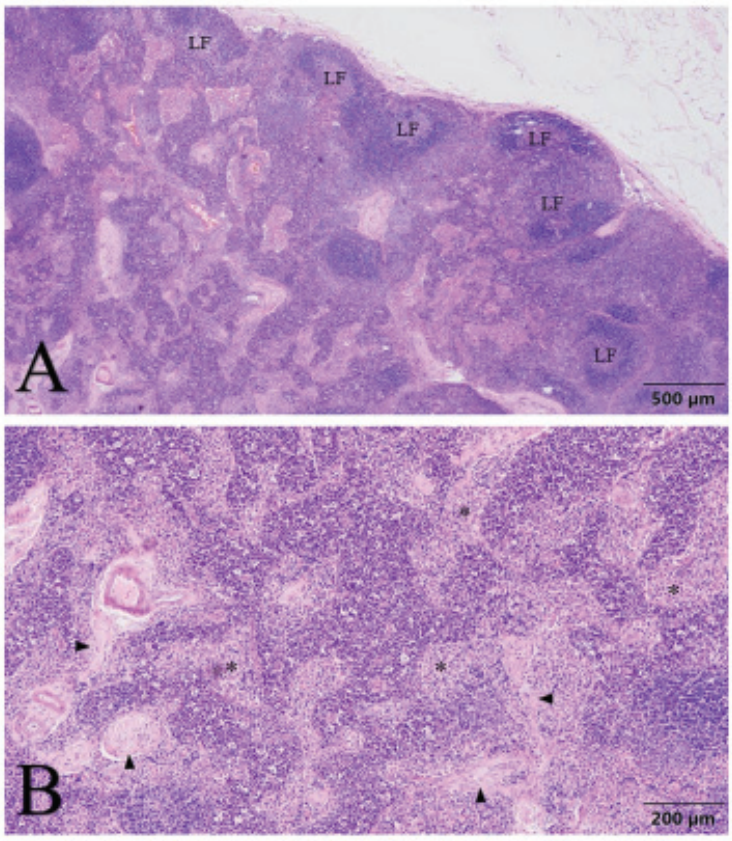

Figure 4. (A) Histological examination of lymph nodes revealing marked expansion of lymphoid follicles (LF). These follicles consist of central large lymphocytes surrounded by a distinct marginal zone, maintaining their polarity. Magnification: 100x (scale bar = 500 µm). (B) The medullary region shows 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.

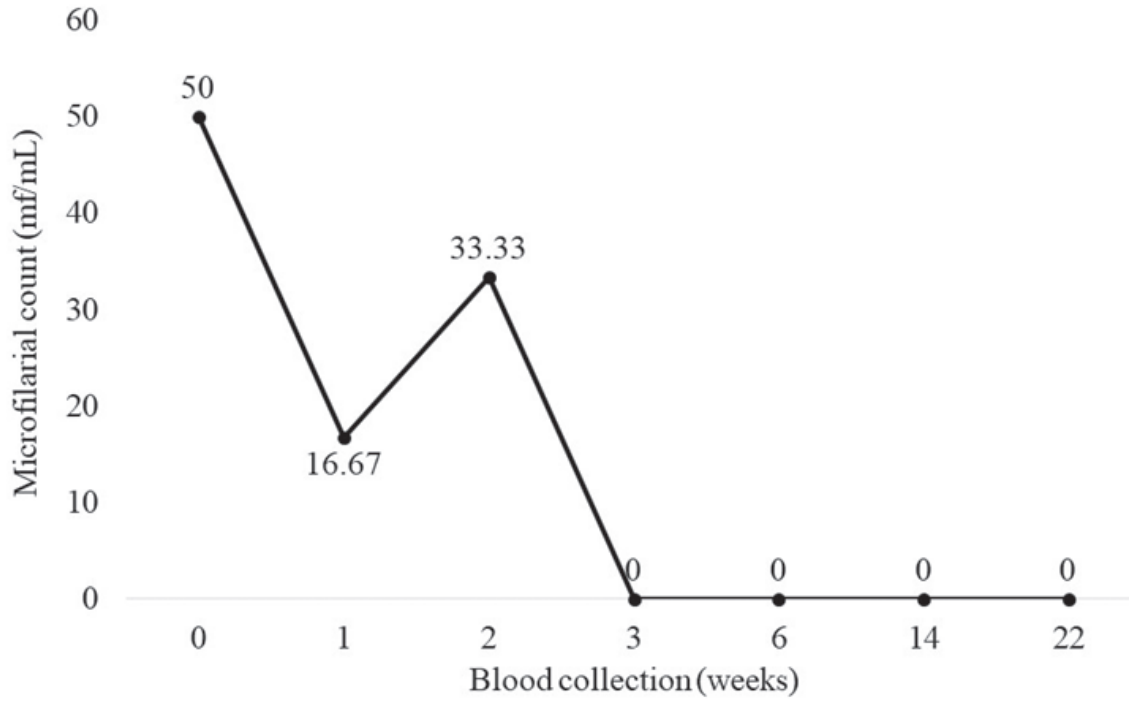

Figure 5. Graph depicting the microfilarial count (mf/mL) of *Brugia pahangi* from the buffy coat smear during the treatment period. The number of microfilariae per mL was estimated from a 60 μL blood sample.

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.

Acknowledgements

The author would like to express sincere gratitude to the owner for providing valuable information and granting permission for this case study. We would like to thank the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals for the molecular laboratory facility used in this study. Special appreciation is extended to all the staff at the Prasu Arthorn Veterinary Teaching Hospital for their assistance and support. Additionally, the author would like to acknowledge Asst. Prof. Rungrote Osathanon for providing valuable consultation on this case. This study was made possible with the support of the Faculty of Veterinary Science, Mahidol University.

References

Al-Abd NM, Nor ZM, Kassim M, Mansor M, Al-Adhroey AH, Ngui R, et al. Prevalence of filarial parasites in domestic and stray cats in Selangor State, Malaysia. Asian Pac J Trop Med. 2015;8(9):705-9.

Ambily VR, Pillai UN, Arun R, Pramod S, Jayakumar KM. Detection of human filarial parasite *Brugia malayi* in dogs by histochemical staining and molecular techniques. Vet Parasitol. 2011;181(2-4):210-4.

Bandi C, Anderson TJC, Genchi C, Blaxter ML. Phylogeny of *Wolbachia* in filarial worm. Mol Biochem Parasitol. 1998;265:2407-13.

Bandi C, McCall JW, Genchi C, Corona S, Venco L, Sacchi L. Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia*. Int J Parasitol. 1999;29(2):357-64.

Bowman DD, Mannella C. Macrocyclic lactones and *Dirofilaria immitis* microfilariae. Top Companion Anim Med. 2011;26:160-72.

CDC media centre. Lymphatic Filariasis [Internet]. CDC. 2018 [cited 2023 Mar 25] Available from: https://www.cdc.gov/parasites/lymphaticfilariasis/.

Chandrasakha P, Osathanon R, Suemanotham N. Case Report: Long-Term Management of Imidacloprid-Moxidectin in a Dog with Caval Syndrome. J Appl Anim Sci. 2022;14(2):33-44.

Chungpivat S, Taweethavonsawat P. The differentiation of microfilariae in dogs and cats using Giemsa's staining and the detection of acid phosphatase activity. J Thai Vet Pract. 2008;20:47-55.

Denham D.A, Rogers R. Structural and functional studies on the lymphatics of cats infected with *Brugia pahangi*. Trans R Soc Trop Med Hyg. 1975;69(2): 173-6.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008;36:465-9.

Edeson JFB, Wharton RH, Laing ABG. A preliminary account of the transmission, maintenance and laboratory vectors of *Brugia pahangi*. Trans R Soc Trop Med Hyg. 1960;54(5):439-49.

Ewert A, Singh M. Microfilarial levels in cats infected with *Brugia pahangi* by two alternative routes. Trans R Soc Trop Med Hyg. 1969;63(5):603-7.

Goldsmid JM. Studies on the laboratory diagnosis of human filariasis: preliminary communication. J Clin Pathol. 1970;23(7):632-5.

Jacobson LS, DiGangi BA. An Accessible Alternative to Melarsomine: 'Moxi-Doxy' for Treatment of Adult Heartworm Infection in Dogs. Front Vet Sci. 2021;8:702018.

Junhom W, Chungpivat S, Visseshakul N, The observation of microfilarial rate and density in cats inoculated with increasing numbers of *Brugia pahangi* infective larvae. Southeast Asian J Trop Med Public Health. 2006;37(suppl 3):40-2.

Kaikuntod M, Arjkumpa O, Kladkempetch D, Fukumoto S, Thongkorn K, Boonyapakorn C, et al. Geographic Spatial Distribution Patterns of *Dirofilaria immitis* and *Brugia pahangi* Infection in Community Dogs in Chiang Mai, Thailand. Animals. 2021;11(1):33.

Kaikuntod M, Thongkorn K, Tiwananthagorn S, Boonyapakorn C. Filarial worms in dogs in Southeast Asia. Vet Integr Sci. 2018;16(2):1-17.

Khowawisetsut L, Sarasombath PT, Thammapalo S, Loymek S, Korbarsa T, Nochote H, et al. Therapeutic trial of doxycyclin plus ivermectin for the treatment of *Brugia malayi* naturally infected cats. Vet Parasitol. 2017;245:42-7.

Laojun S, Hayitantu M, Janhyok J, Manklang N, Nammoontree J, Kaisaeng K, et al. Species Diversity of Mosquito Vectors in Kubae Salo Village, Kaluwo Sub-District, Mueang Narathiwat District, Narathiwat Province for Surveillance and Control. J Appl Anim Sci. 2022;15(2):25-38.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007; 23(21):2947-8.

Li BW, Rush AC, Weil GJ. High level expression of a glutamate-gated chloride channel gene in reproductive tissues of *Brugia malayi* may explain the sterilizing effect of ivermectin on filarial worms. Int J Parasitol Drugs Drug Resist. 2014;4(2):71-6.

Loymek S, Phuakrod A, Zaelai K, Sripumkhai W, Vongjaroensanti P, Wongkamchai S. Investigation on the Prevalence of Canine Microfilaremia in Thailand Using a Novel Microfluidic Device in Combination with Real-Time PCR. Vet Sci. 2021 ;8(3):39.

Marcos R, Pereira C, Santos M, Luzzago C, Lauzi S, Maia JP, et al. Buffy coat smear or Knott's test: Which to choose for canine microfilaria screening in field studies? Vet Clin Pathol. 2016;45(1):201-5.

Moreno Y, Nabhan JF, Solomon J, Mackenzie CD, Geary TG. Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. Proc Natl Acad Sci USA. 2010; 107(46):20120-5.

Mylonakis ME, Papadopoulos E, Koutinas AF, Paitaki C, Leontides L. Comparative methodology for the detection and differentiation of circulating microfilariae of *Dirofilaria immitis* in the dog. J Helminthol. 2004;78(2):137-40.

Omar MS. Distribution of acid phosphatase activity in the larval stages of *Wuchereria bancrofti*, *Brugia malayi*, *B. pahangi* and *Dirofilaria immitis* in the mosquito. Tropenmed Parasitol. 1977;28(1):100-8.

Orton S, Weinstock D, Hammerberg B. Association of elevated lymph node cell release of histamine and tumor necrosis factor with genetic predisposition to limb edema formation in dogs infected with *Brugia pahangi*. Am J Trop Med Hyg. 1998;58(6):695-704.

Paran RD, Svobodová V. Effect of therapy by using advocate spot-on combination (imidacloprid 10% and moxidectin 2.5%) on subcutaneous dirofilariosis in dogs. Vet Med Int. 2011;2011:482746.

Ravindran R, Varghese S, Nair SN, Balan VM, Lakshmanan B, Ashruf RM, et al. Canine filarial infections in a human *Brugia malayi* endemic area of India. Biomed Res Int. 2014;2014: 630160.

Regalbono A, Di Cesare A, Traversa D, Simonato G, Poser H, Danesi P, et al. Microfilaricidal efficacy of a single administration of Advocate® (Bayer Animal Health) in dogs naturally infected with *Dirofilaria immitis* or Dirofilaria repens. Vet Parasitol. 2016;226:30-4.

Rishniw M, Barr SC, Simpson KW, Frongillo MF, Franz M, Dominguez Alpizar J L. Discrimination between six species of canine microfilariae by a single polymerase chain reaction. Vet Parasitol. 2006; 135(3-4):303-14.

Sadarama PV, Chirayath D, Pillai UN, Lakshmanan B. Comparison of efficacy of ivermectin and diethylcarbamazine against naturally infected *Brugia malayi* microfilaria in dogs. J Parasit Dis. 2019;43(4):554-9.

Saeung A, Hempolchom C, Baimai V, Thongsahuan S, Taai K, Jariyapan N, et al. Susceptibility of eight species members of *Anopheles hyrcanus* group to nocturnally subperiodic *Brugia malayi*. Parasit Vectors. 2013;6:5.

Sarasombath PT, Thammapalo S, Loymek S, Angkanasinsiri A, Priyavoravong P, Wongkamchai S. First study of topical selamectin efficacy for treating cats naturally infected with *Brugia malayi* and *Brugia pahangi* under field conditions. Parasitol Res. 2019;118(4):1289-97.

Satjawongvanit H, Phumee A, Tiawsirisup S, Sungpradit S, Brownell N, Siriyasatien P, et al. Molecular Analysis of Canine Filaria and Its *Wolbachia* Endosymbionts in Domestic Dogs Collected from Two Animal University Hospitals in Bangkok Metropolitan Region, Thailand. Pathogens. 2019;8(3):114.

Schacher JF. Developmental stages of *Brugia pahangi* in the final host. J. Parasitol. 1962;48(5):693-706.

Snowden K, Hammerberg B, Smallwood JE. Clinical and xeroradiographic lymphangiography studies of acute and chronic *Brugia pahangi* infections in dogs. Ann Trop Med Parasitol. 1986;80(2):197-209.

Snowden KF, Hammerberg B. The lymphatic pathology of chronic *Brugia pahangi* infection in the dog. Trans R Soc Trop Med Hyg. 1989;83(5):670-8.

Taweethavonsawat P, Chungpivat S. Successful treatment of *Brugia pahangi* in naturally infected cats with ivermectin. Korean J Parasitol. 2013;51(6): 759-61.

Thongpiya J, Sa-nguanraksa D, Samarnthai N, Sarasombath PT. Filariasis of the breast caused by *Brugia pahangi*: A concomitant finding with invasive ductal carcinoma. Parasitol Int. 2021;80:102203.

TroCCAP. Guidelines for the diagnosis, treatment and control of canine endoparasites in the tropics, second edition [Internet]. TroCCAP. 2019 [cited 2023 Mar 25]. Available from https://www.troccap.com/2017press/wp-content/uploads/2019/05/TroCCAP_Canine_Endo_Guidelines_English_Ver2.pdf.

Research Articles

Effects of a Standardized Extract of *Centella asiatica* ECa 233 on Clinical Parameters and Radiography Using Computed Tomography in Dogs with Osteoarthritis

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Received: 23 May 2023; Revised: 16 July 2023; Accepted: 17 July 2023

Abstract

Osteoarthritis in dogs is a chronic disease that causes pain from inflammation of the joints. For treatment, medication is only used for the relief of the pain and inflammation of the joints. Side effects of long-term treatment affect multiple systems. This study aimed to evaluate the effects of a standardized extract of *Centella asiatica* (ECa 233) in dogs with osteoarthritis by examining the clinical score, C-reactive protein (CRP), radiographs, and computed tomography (CT) imaging. Eight dogs were diagnosed with osteoarthritis. The Eca 233 was given to the dogs orally at a dose of 10 mg/kg once a day for 28 days. Results showed that the clinical evaluation found that most parameters showed improvement compared to pretreatment. The mean clinical lameness score after treatment was found to have decreased significantly from 2.13±1.36 to 0.5±0.93 (p=0.01). Both the mean CRP values and radiographic score had decreased insignificantly. The average CT Hounsfield unit (HU) value was also decreased significantly, which indicated that ECa233 improved the soft tissue around the joint and the function of the osteoarthritis joint. These results showed that ECa233 reduced the pain and inflammatory reaction resulting in better walking ability and life quality in dogs with osteoarthritis.

Keywords: Computed tomography, dogs, ECa233, Osteoarthritis, Radiography

ผลของสารสกัดมาตรฐานบัวบกอีซีเอ 233 ต่อการประเมินทางคลินิกและภาพทางรังสีโดยใช้เครื่องเอกซเรย์คอมพิวเตอร์ในสุนัขที่มีภาวะข้อเสื่อม

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Received: 23 May 2023; Revised: 16 July 2023; Accepted: 17 July 2023

บทคัดย่อ

โรคข้อเสื่อมในสุนัขเป็นโรคเรื้อรังที่ทำให้เกิดความเจ็บปวดจากการอักเสบของข้อ การรักษาทำได้โดยการใช้ยาเพื่อลดอาการเจ็บปวดและการอักเสบของข้อ แต่เมื่อใช้ยาไปนานๆอาจเกิดผลข้างเคียงต่อระบบต่างๆตามมาได้ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของสารสกัดมาตรฐานบัวบกอีซีเอ 233 ต่อโรคข้อเสื่อมในสุนัขโดยประเมินค่าทางคลินิก ค่าโปรตีนซี-รีแอกทีฟ ภาพทางรังสีโดยใช้เครื่องเอกซเรย์คอมพิวเตอร์ สุนัข 8 ตัวที่มีภาวะข้อเสื่อมได้รับสารมาตรฐานอีซีเอ 233 ขนาด10 มิลลิกรัมต่อกิโลกรัมน้ำหนักตัวสุนัข โดยการกินวันละ 1 ครั้ง ติดต่อกันเป็นเวลา 28 วัน ผลการวิจัยพบว่าการประเมินทางคลินิกส่วนมากพบว่ามีอาการดีขึ้นหลังได้รับสารสกัดมาตรฐานบัวบกอีซีเอ 233 ค่าเฉลี่ยคะแนนความเจ็บปวดขณะเดินลดลงอย่างมีนัยสำคัญทางสถิติจาก 2.13±1.36 เหลือ 0.5±0.93 (p=0.01) ค่าโปรตีนซี-รีแอกทีฟและค่าเฉลี่ยคะแนนภาพรังสีลดลงแต่ไม่มีนัยสำคัญทางสถิติ ค่าเฉลี่ย HU ของเนื้อเยื่อรอบๆข้อสะโพกจากภาพเอกซเรย์คอมพิวเตอร์ลดลง อย่างมีนัยสำคัญทางสถิติ ซึ่งชี้แสดงให้เห็นว่าสารสกัดมาตรฐานบัวบกอีซีเอ 233 ทำให้เนื้อเยื่อรอบๆข้อมีการอักเสบลดลงช่วยให้ข้อทำงานดีขึ้น ผลการทดลองแสดงให้เห็นว่าสารสกัดมาตรฐานบัวบกอีซีเอ 233 ช่วยลดความเจ็บปวดและการอักเสบ ส่วนหนึ่งดูได้จากการเดินและคุณภาพชีวิตที่ดีขึ้นในสุนัขที่มีภาวะข้อเสื่อม

คำสำคัญ : เอกซเรย์คอมพิวเตอร์ สุนัข สารสกัดมาตรฐานบัวบกอีซีเอ 233 โรคข้อเสื่อม ภาพทางรังสี

Introduction

Osteoarthritis (OA) in dogs is a chronic disease that causes pain from inflammation of the joints resulting in poor life quality. The most common breeds are Labrador Retrievers and German Shepherds. In particular, German Shepherds that are overweight have a higher incidence (Anderson et al., 2018). No proper treatment for OA leads to long-term abnormality of the structure and function of the joints. Supportive treatment also only uses medication for the relief of the pain and inflammation of the joints. Furthermore, long-term use of anti-inflammatory drugs may cause the malfunctions of the liver and kidneys (Alhassani et al., 2021).

Diagnosis of OA by history taking, physical examination, radiography (X-rays), and computed tomography (CT-scan) determined the joint structure, bone formation, and subchondral bone sclerosis. Previous studies reported the same sensitivity and specificity for damage to an intervertebral disc diagnosis (Marino and Loughin 2010). However, it had been reported that a CT-scan could enable quantitative assessment of the soft tissues and materials using the Hounsfield unit (HU) (Barber and Dockery 2011). The HU is also referred to as CT numbers, which correspond to the average amount of radiation absorbed by the tissue (Ai et al., 2018). The absorption is measured for any given slice relative to water, which is one unit. In comparison, the bone is 700-3,000 units and air 0.001 units (Ai et al., 2018). Therefore, the HU value could be used to analyze the quality of the tissues around the joint. Serum markers, such as C-reactive protein (CRP) would indicate the severity, prognosis, and follow-up of treatment in dogs (de Bakker et al., 2017).

The goals of the OA treatment consist of improvement or preservation of the function of the joints, progressive prevention of the disease, pain control, and maintenance of the body weight and health conditions. There is differential management with individualized medication that includes a combination of non-medication (Ashford and Williard 2014). The recommended drugs used for treatment in OA are Non-Steroidal Anti-Inflammatory drugs (NSAIDs) such as Carprofen, Meroxicam, Deracoxib, Ketoprofen, etc. In patients with resistance to NSAIDs, adjunctive pain medication is used to control pain, such as Amantadine, Gabapentin, Tramadol, Codeine, Corticosteroid, etc. However, there is unclear information on their effectiveness in pain control in OA.

The herb *Centella asiatica* has anti-inflammatory effects and other properties (Somchit et al., 2004). The anti-inflammatory effect is the property of Eca233 extract, contributes to the wound healing process (Wannarat 2009; Anukunwithaya et al., 2017). Moreover, it enhances the healing effect on second-degree burns with the shrinkage of the wound, increasing of the blood flow to the skin, and increasing of the hair follicle, thus making the wound become dry and smooth, as well as reducing vascular inflammation and inflammatory cell migration. In addition, research has confirmed that ECa233 contributes to the healing of oral ulcers in humans by reducing inflammation, pain level and wound size (Ruengprasertkit et al., 2010). The anti-inflammatory effect of ECa233 is a good candidate for the prevention and treatment of inflammatory-related diseases (Sukketsiri et al., 2019). In rat and mouse experiments, it was found that up to 10 g/kg of ECa233 extract could be administered without acute toxicity (Chivapat et al., 2011).

However, no study has evaluated the ECa 233 for anti-inflammation purposes in OA dogs using the clinical score, CRP, radiography, and CT-scan imaging. The

purpose of this study was to investigate the effects of ECa233 on anti-inflammation by evaluating the clinical score, CRP, radiography, and CT-scan imaging in osteoarthritis dogs.

Materials and Methods

Animals

The experimental protocol was approved by the Faculty of Veterinary Science and the Ethics committee, Mahidol University, Thailand (MUVS-2021-05-20). Eight dogs were diagnosed with osteoarthritis, recruited from the Prasuarthorn Animal Hospital. All dogs fulfilled the inclusion criteria and were enrolled. All dogs did not receive any medication. All were prescribed with ECa233 for 28 days.

Inclusion/exclusion criteria

Dogs with clinical signs of chronic lameness (more than 1 month), 3-5 stiffness and joint pain, and radiological evidence of OA of the hip were eligible. Dogs were examined by veterinarian to confirm OA, previously entrance to this study. All osteoarthritis dogs were categorized with 1-4 lameness grades according to Table 1 (Nganvongpanit et al., 2014). Dog with the conditions of pregnancy or hepatic, cardiovascular, gastrointestinal, neurological disease were excluded. Dog with lameness due to lumbosacral instability, infection, immune disease, or fractures, including dogs which had previously received drug or dietary supplements for osteoarthritis treatment were also excluded.

The standardized extract ECa233

The standardized extract ECa233 containing madecassoside (51% w/w) and asiaticoside (38% w/w) was obtained from Siam Herbal Innovation Co. Ltd, Thailand). It is given to dogs orally at a dose of 10 mg/kg once a day after meal for 28 days. ECa233 10 mg/kg by conversion dose from the resulting reduce inflammation in mice (Nair and Jacob 2016).

Methods for assessing osteoarthritis

Clinical score

Efficacy of treatment for relieving inflammation in dogs with osteoarthritis can also be assessed using a clinical lameness scoring system which was examined by walking and trotted 12 meters (6 meters for evaluation) on a flat floor in the Prasuarthorn Animal Hospital. The test was conducted 3 times and evaluated by the veterinarian based on the criteria were modified from (Nganvongpanit et al., 2014) in (Table 1).

Table 1. Lameness scores for assessing dogs with osteoarthritis.

Grade	Clinical evaluation
1	Walks normally
2	Slightly lame when walking
3	Moderately lame when walking
4	Severely lame when walking
5	Reluctant to rise and will not walk more than five paces

Blood collection

Blood samples were collected from the vein of dogs (cephalic, saphenous or jugular vein) for 3 ml from each dog on before and after the treatment. Blood chemistry and serum CRP were performed at the laboratory of Prasuarthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University.

Radiography

Radiographs of the participating dogs were taken before and after 28 days of ECa233 administration by the same radiologist technician and standard computed radiography machine. Radiographs of dogs were performed in lateral and extended-ventrodorsal view of hip and stifle. Repositioning of the dog for subsequent radiographs was guided by the original film, and the same radiographic settings were used. Therefore, hip and stifle of x-rays were taken and evaluated by a veterinarian based on the criteria were modified from (Nganvongpanit et al., 2014) in Table 2

Table 2. Radiographic scoring system for assessing dogs with osteoarthritis.

Grade		Radiographic evaluation
0	Normal	Not affected
1	Mild	Doubtful narrowing of joint space and possible osteophytic lipping
2	Moderate	Definite osteophytes and possible narrowing of joint space
3	Severe	Moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
4	Very severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Computed tomography (CT) scan

General anesthesia

Feed and water are withheld for 12 and 6 hours respectively prior to being anesthetized. Moreover, a thorough physical examination, which includes body temperature, heart rate, respiratory rate, dehydration status, mucous membrane color, capillary refilling time, neuromuscular, integumentary and cardio-respiratory system, is performed by a veterinarian in order to evaluate risk of anesthetic complications.

After the patient is clinically evaluated that the anesthesia could be performed, the premedication and sedative agents are administered by a veterinarian. The satisfactory depth of anesthesia should be stage 3 planes 2 or 'surgical' anesthesia in order to perform a computed tomography. During anesthesia, the intravenous fluid is administered according to individual physical status. The veterinarian monitors animal's health status during the anesthesia which takes approximately 10-15 mins or throughout the CT-scan process. A veterinarian closely

monitors the patient until all the vital signs become stable within normal range and it is certain that the dog has no side effects of general anesthesia.

Computed tomography examination

CT-scan is to be performed on the area of hip joint with OA condition using Optimums 64 slice by positioning the joint with the caudal aspect of the limb on the scanning plane. Extend both hind limbs while the dog is in the ventral recumbency position. Then, place tibial plateau parallel to the scanning plane using scout view in order to take overlapping transverse pre-arthrography CT images of 1.25 mm from the distal third of the femur to the proximal third of the tibia/fibula, using a bone algorithm, 100 kVp, 140 mA, a field of view of 96 mm and a pitch of 0.75. A second scan was performed from the proximal aspect of the patella to the tibial plateau, with a slice thickness of 0.625 mm thickness. These images were acquired in a bone algorithm, 100 kVp, 180 mA, and a field of view of 96 mm and a pitch of 1. The contrast Iohexol (Omnipaque 350) was injected using a volume of 2 to 2.4 mL (median 2.2 mL) at a concentration 120 mg I/mL. The CT protocol was repeated for all stifles after intra-articular positive contrast injection (Van der Vekens et al., 2019). Every CT image is analyzed by veterinary diagnostic imaging specialist.

Statistical analysis

Clinical score, radiographs, CT-scan, CRP were calculated in the formulation of mean ± SD. The difference between day 0 and day 29 was compared using Non-parametric, Kruskal-Wallis Test. The data was analyzed with SPSS program version 21.0 and 95% level of confidence or < 0.05 statistical significance.

Results

Animals

Eight dogs were diagnosed with osteoarthritis, fulfilled the inclusion criteria and were enrolled. All dogs were 6-15 years old. The 8 dogs of experimental group consist of 5 males and 3 females. There were 2 Pomeranians, 2 mixed breeds, 2 Chihuahuas, 1 Beagle and 1 Poodle. Demographic data of OA dogs were summarized in Table 3. All dogs showed normal complete blood count and no blood parasites.

Table 3. Demographic data of osteoarthritis (OA) dogs.

Signalment	OA group (n=8)	*p*-value
Age (years old)	9.6 (6-15)	NA
Body weight (kg)	19.50 (8.20-38.56)	0.64
Sex	Male 5, Female 3	NA
Breed	Pomeranian (n=2), Mixed breed (2), Chihuahua (2), Beagle (1), Poodle (1)	NA

NA, Not applicable

CRP level

The experimental results showed decreasing of CRP value in 4 out of 8 dogs after receiving of ECa 233. The mean CRP before and after the administration of ECa 233 was 10.38±2.01 mg/L (range 9.00-14.63, n=8) and 9 mg/L (all dogs CRP value was 9 mg/L, n=8) respectively. The mean serum CRP was as shown in Table 4.

Clinical lameness score

Clinical evaluation in lameness showed significant improvement in the before the administration of ECa 233 compared with after the administration of ECa 233 (2.13±1.36, 0.5±0.93) respectively (p=0.01). The mean clinical lameness score was showed in Table 4.

Table 4. The C-reactive protein (CRP) value and clinical lameness score of osteoarthritis (OA) dogs before and after ECa233 administration.

Parameters	OA group (mean±SD)		*p*-value
	Before	After	
CRP value	10.38±2.01	9.00±0.00	0.06
Lameness score	2.13±1.36	0.5±0.93*	0.01*

Radiographic evaluation

Eight dogs from the OA group were diagnosed with OA of the hip and stifle joint and were classified as grades 1-3 according to the radiographic scoring system. The mean stifle and hip radiographic score as shown in Table 5. However, the results showed that there were no statistically significant differences (p>0.05) of the radiographic score between before and after the administration of ECa 233 in the stifle and hip joints. The radiographic score was 1, 2, and 3 of OA in the stifle joints and hip joints (Figure 1, 2), respectively.

Table 5. The radiographic score between before and after the administration of ECa233 in the stifle and hip joints of osteoarthritis (OA) dogs.

Radiographic score	OA group (mean±SD)		*p*-value
	Before	After	
Stifle joint	1.5±0.84	1.33±0.82	0.19
Hip joint	2.5±0.55	2.5±0.55	1.00

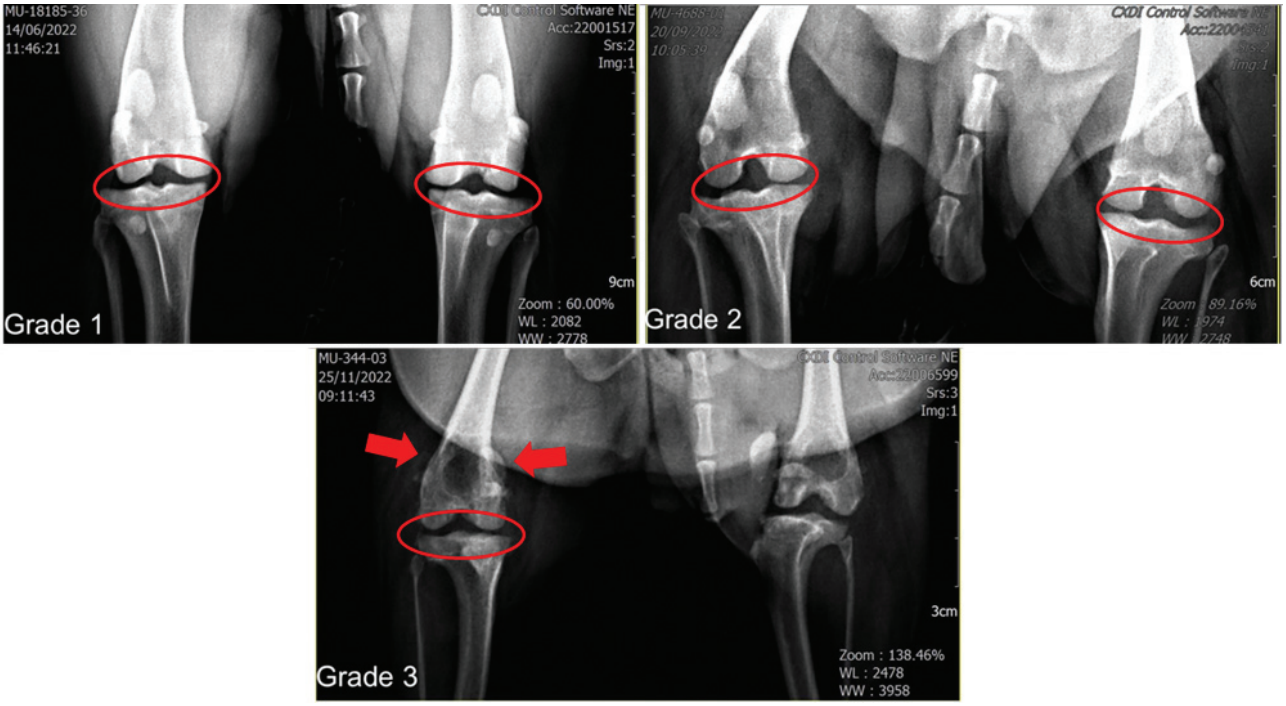

Figure 1. The radiographic score was 1, 2, and 3 of osteoarthritis in the stifle joints.

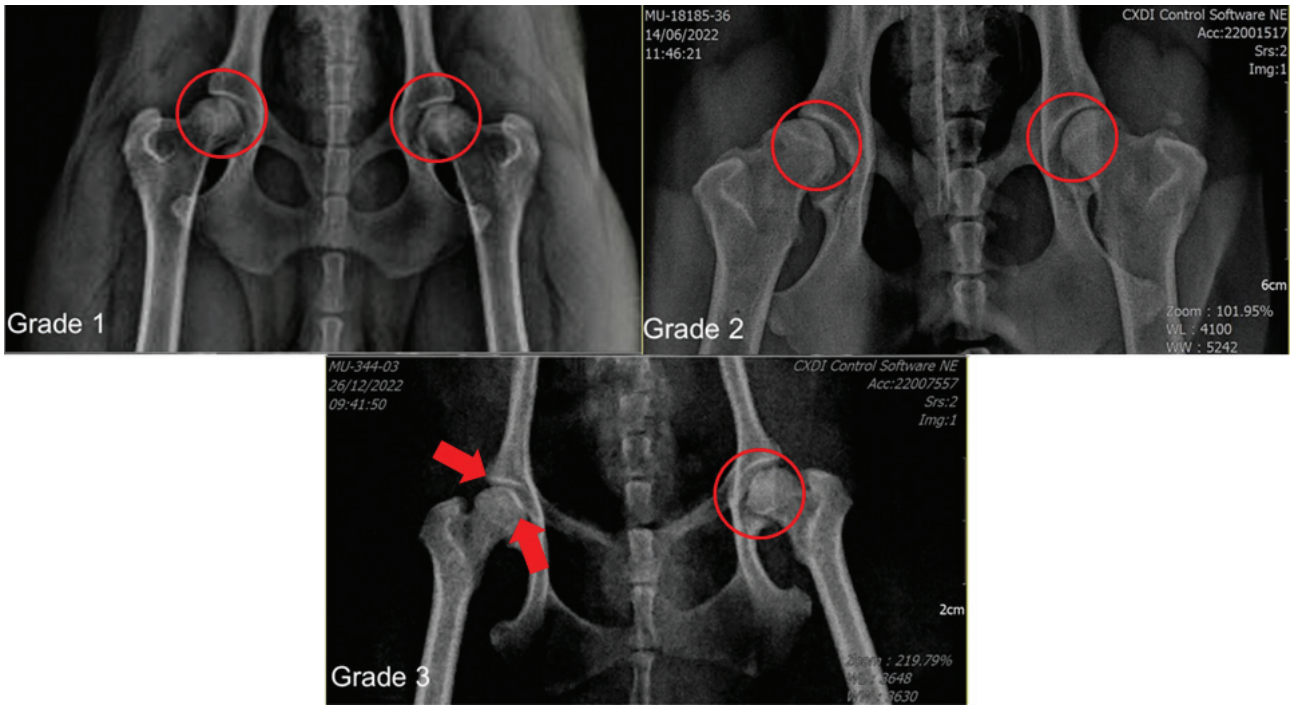

Figure 2. The radiographic score was 1, 2, and 3 of osteoarthritis in the hip joints.

Computed tomography evaluation

In this research study, a CT-scan was performed on six dogs before and after the administration of ECa 233 in order to evaluate the HU which would determine the radiographic density of the tissue of hip joint. The HU was measured from three random areas within three planes (sagittal, dorsal, and transverse plane). Two phases of the CT-scan were evaluated: pre-contrast and post-contrast phase.

From the two phases of the CT-scan result after the administration of ECa 233, the mean HU of the hip joint decreased from before ECa 233 administration and significantly different in post-contrast phase (p=0.037) as shown in Table 6. It means that ECa 233 improved tissue quality around the hip joint. CT images of hip joint of dog as shown in Figure 3.

Table 6. Hounsfield unit of pre-contrast and post-contrast of computed tomography imaging before and after the administration of ECa233.

Hip	Pre-contrast phase		Post-contrast phase	
	Before	After	Before	After
1	57.44	36.27	86.02	71.72
2	106.97	74.94	114.78	59.71
3	64.84	63.88	68.58	83.14
4	70.38	59.43	78.34	62.54
5	107.81	75.46	120.09	69.17
6	50.18	37.42	77.52	56.47
Mean±SD	76.27±25.05	57.9±17.46	90.89±21.36	67.12±9.71*
p-value	0.24		0.037*	

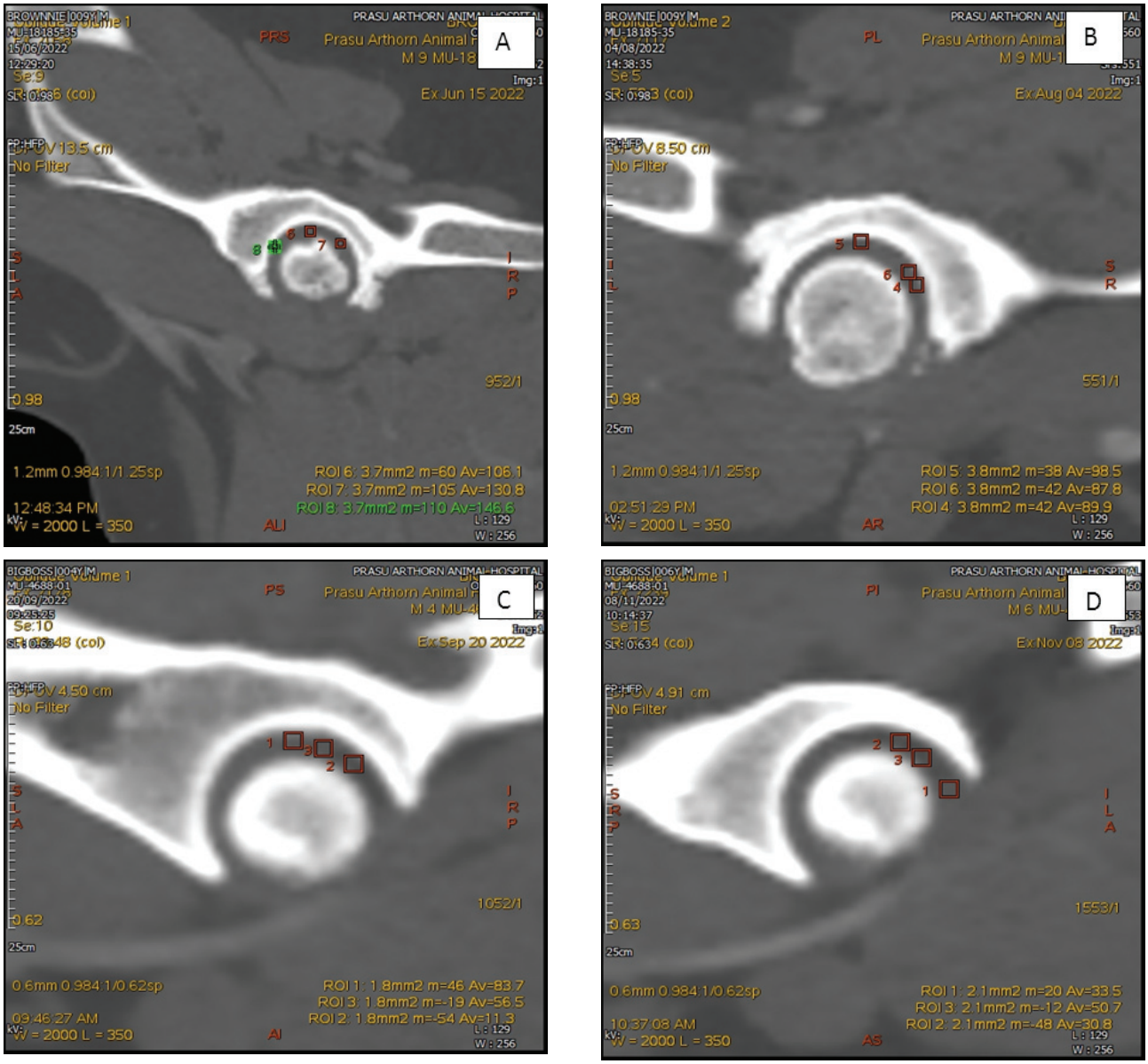

Figure 3. Computed tomography images of the hip joint of dog. Hounsfield unit was measured from 3 random areas of interest within the tissue around the right hip joint of dog (A, C) before and (B, D) after the administration of ECa 233.

Discussion

The research effects of ECa 233 in OA dogs were conducted by using the clinical lameness score, radiography, CT-scan, and CRP. The result of the clinical lameness score found that all dogs showed improvement in this parameter. Nevertheless, the radiographic score, CRP values, and CT-scan evaluation in some dogs did not improve.

Serum markers such as CRP indicate severity, prognosis, and follow-up plan of treatment in dogs (de Bakker et al., 2017). CRP values of the dogs with OA in this study were not high. No significant differences were found in the before and after treatment groups. The previous research has found that the CRP and IL-6 values are similarly correlated and used to differentiate between dogs with OA and suppurative arthritis. However, CRP values were not different in OA and normal joint dogs (Hillström et al., 2016). The increase in CRP values could not identify the inflammatory site (Christensen et al., 2014). In addition, synovial CRP was not correlated with serum CRP (Bennett et al., 2013).

An X-ray had the limited condition in the sense of the difficulty in holding the dog in the desired position. Additionally, the CT-scan required the animals to be anesthetized; consequently, the number of dogs participating in the project that went through the CT-scan was low. The radiographic findings between day 0 and 29 in the treatment group showed no significant change. Nevertheless, the radiographic images could not provide as much information about the tissues around the joint as MRI.

The diagnosis of OA by history taking, physical examination, X-rays, and CT-scan determined the joint structure, bone formation, and subchondral bone sclerosis. Previous studies reported the same sensitivity and specificity for damage to the intervertebral disc (Marino and Loughin 2010). However, it was reported that a CT-scan enabled quantitative assessment of the soft tissues and materials using the HU (Barber and Dockery 2011). The HU is also referred to as CT numbers, which correspond to the average amount of radiation absorbed by the tissue (Ai et al., 2018). Therefore, the HU value could be used to analyze the quality of tissues around joint.

Apart from CRP, there are other methods of indicating arthritis in order to follow-up the dog's condition. For example, other inflammatory markers such as IL, and TNF alpha, have been reported to be noninvasive markers of joint inflammation in dogs with idiopathic immune mediated polyarthropathy (Foster et al., 2014). Furthermore, the synovial fluid evaluation of the pH and glucose values was found to be significantly higher in degenerative joint disease dogs than in normal dogs (de Bakker et al., 2021). Therefore, further studies would be needed to determine the effect of ECa233 on specific inflammatory biomarkers like serum chondroitin sulfate and hyaluronan: biomarkers for osteoarthritis in canine hip dysplasia and other cytokines (Nganvongpanit et al., 2008). If possible, longer treatment of ECa 233 should be undertaken for three or six months to determine the bone structure and side effects.

The study design had limitation. Because this was a clinical study, the animals could not be controlled by using the same breed, sex, and/or age. Moreover, not all dogs in the study had the same OA grade. The total number of animals in this study was not large. Only 6/8 dogs were performed CT scan. Two dogs did not CT scan; one dog had an accident running into a door, causing a strained neck that required him to stay in the hospital for 2 weeks. In another dog, the owner had work commitments that made it inconvenient to appointment.

In conclusion, the result of the clinical lameness score found that all dogs showed improvement in this parameter. This could be a result that possibly the bone structure had not changed within 28 days. However, a s ignificant decrease in the lameness score of all dogs indicated that ECa 233 reduced the pain and inflammatory reaction, thus resulting in better walking ability and life quality. In addition, the decreased HU value from the CT imaging after treatment indicated that ECa 233 decreased the damage of tissues around the joint and the function of the osteoarthritis joint in dogs.

Acknowledgements

This work was supported by Faculty of Veterinary Science, Mahidol University. The authors would like to express their sincere gratitude to all the staffs of CT unit at Prasuarthorn Animal Hospital as well as the owners' permission and patients whose support and collaboration.

References

Ai HA, Meier JG, Wendt RE. HU deviation in lung and bone tissues: Characterization and a corrective strategy. Med Phys. 2018;45(5):2108-18.

Alhassani RY, Bagadood RM, Balubaid RN, Barno HI, Alahmadi MO, Ayoub NA. Drug therapies affecting renal function: an overview. Cureus. 2021;13(11):e19924.

Anderson KL, O'Neill DG, Brodbelt DC, Church DB, Meeson RL, Sargan D, et al. Prevalence, duration and risk factors for appendicular osteoarthritis in a UK dog population under primary veterinary care. Sci Rep. 2018;8(1):5641.

Anukunwithaya T, Tantisira MH, Tantisira B, Khemawoot P. Pharmacokinetics of a standardized extract of Centella asiatica ECa233 in rats. Planta Med. 2017;83(8):710-7.

Ashford S, Williard J. Osteoarthritis: A review. Nurse Pract. 2014;39(5):1-8.

Barber FA, Dockery WD. A computed tomography scan assessment of synthetic multiphase polymer scaffolds used for osteochondral defect repair. Arthroscopy. 2011;27(1):60-4.

Bennett D, Eckersall PD, Waterston M, Marchetti V, Rota A, McCulloch E, Sbrana S. The effect of robenacoxib on the concentration of C-reactive protein in synovial fluid from dogs with osteoarthritis. BMC Vet Res. 2013;9:42.

Christensen MB, Langhorn R, Goddard A, Andreasen EB, Moldal E, Tvarijonaviciute A, et al. Comparison of serum amyloid A and C- reactive protein as diagnostic markers of systemic inflammation in dogs. Can Vet J. 2014;55(2):161-8.

Chivapat S, Chavalittumrong P. Tantisira MH. Acute and sub-chronic toxicity studies of a standardized extract of Centella asiatica ECa233. Thai J Pharm Sci. 2011;35:55-64.

de Bakker E, Broeckx B, Demeyere K, Stroobants V, Van Ryssen B, Meyer E. Detection of osteoarthritis in dogs by metabolic, pro-inflammatory and degenerative synovial fluid biomarkers and traditional radiographic screening: A pilot study. Vet Immunol Immunopathol. 2021;237:110252.

de Bakker E, Stroobants V, VanDael F, Ryssen BV, Meyer E. Canine synovial fluid biomarkers for early detection and monitoring of osteoarthritis. Vet Rec. 2017;180(13):328-9.

Foster JD, Sample S, Kohler R, Watson K, Muir P, Trepanier LA. Serum biomarkers of clinical and cytologic response in dogs with idiopathic immune-mediated polyarthropathy. J Vet Intern . 2014;28(3):905-11.

Hillström A, Bylin J, Hagman R, Björhall K, Tvedten H, Königsson K, Fall T, Kjelgaard-Hansen M. Measure-ment of serum C-reactive protein concentration for discriminating between suppurative arthritis and osteoarthritis in dogs. BMC Vet Res. 2016;12(1):240.

Marino DJ, Loughin CA. Diagnostic imaging of the canine stifle: a review. Vet Surg. 2010;39(3): 284-95.

Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27-31.

Nganvongpanit K, Itthiarbha A, Ong-Chai S, Kongtawelert P. Evaluation of serum chondroitin sulfate and hyaluronan: biomarkers for osteoarthritis in canine hip dysplasia. J Vet Sci. 2008;9(3):317-25.

Nganvongpanit K, Tanvisut S, Yano T, Kongtawelert P. Effect of swimming on clinical functional parameters and serum biomarkers in healthy and osteoarthritic dogs. ISRN Vet Sci. 2014;2014:459809.

Ruengprasertkit C, Hongprasong N, Tantisira B, Tantisira MH. Preliminary study of effects of a standardized extract of Centella asiatica ECa233 on minor aphthous ulcers. CU Dent J. 2010;33:131-42.

Somchit MN, Sulaiman MR, Zuraini A, Samsuddin L, Somchit N, Israf DA, et al. Antinociceptive and antiinflammatory effects of Centella asiatica. Indian J Pharmacol. 2004;36:377-80.

Sukketsiri W, Tanasawet S, Moolsap F, Tantisira MH, Hutamekalin P, Tipmanee V. ECa233 suppresses LPS-induced proinflammatory responses in macrophages via suppressing ERK1/2, p38 MAPK and Akt pathways. Biol Pharm Bull. 2019;42(8): 1358-65.

Van der Vekens E, de Bakker E, Bogaerts E, Broeckx BJG, Ducatelle R, Kromhout K, et al. High-frequency ultrasound, computed tomography and computed tomography arthrography of the cranial cruciate ligament, menisci and cranial meniscotibial ligaments in 10 radiographically normal canine cadaver stifles. BMC Vet Res. 2019;15(1):146.

Wannarat K. Effect of Centella asiatica standardized extract ECa233 on healing of second-degree burns in normal and diabetic induced rats [Thesis in Pharmacology]. Bangkok: Chulalongkorn University; 2009.

Case Report

Partial Cystectomy and Ureteroneocystostomy in a Dog with Urinary Bladder Squamous Cell Carcinoma

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Received: 28 June 2023; Revised: 30 July 2023; Accepted: 31 July 2023

Abstract

Squamous cell carcinoma (SCC) is a rare type of urinary bladder carcinoma in dogs. A 10-year-old spayed bitch was presented with pollakiuria. Ultrasonography revealed right obstructive hydronephrosis and proximal hydroureter. Urinary bladder wall thickening and an irregular mucosal surface were presented. Partial cystectomy and ureteroneocystostomy of the right ureter were performed for urinary bladder mass removal in the area close to the right ureteral opening. The histopathological characteristics of urinary bladder SCC were confirmed by negative immunohistochemical staining of uroplakin III, cytokeratin 7, and cytokeratin 20. Two months after surgery, multiple nodules in all hepatic lobules were presented from ultrasonography. The distant metastasis was suspected. This report demonstrates the diagnostic approach and management of urinary bladder SCC, a rare type of canine urinary bladder cancer.

Keywords: Bladder cancer, Dog, Partial cystectomy, Squamous cell carcinoma

การตัดกระเพาะปัสสาวะออกบางส่วนร่วมกับเปลี่ยนย้ายรูเปิดท่อไตบริเวณกระเพาะปัสสาวะในสุนัขที่เป็นมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์

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Received: 28 June 2023; Revised: 30 July 2023; Accepted: 31 July 2023

บทคัดย่อ

สุนัขเพศเมียที่ทำหมันแล้ว อายุ 10 ปี เข้ารับการรักษาที่โรงพยาบาลสัตว์ด้วยอาการปัสสาวะบ่อย จากการวินิจฉัยด้วยการทำอัลตร้าซาวด์พบว่า ไตข้างขวาและท่อไตส่วนต้นด้านขวาบวมร่วมกับผนังกระเพาะปัสสาวะมีการหนาตัวและลักษณะเยื่อบุด้านในไม่เรียบ จากการผ่าตัดพบว่ามีก้อนเนื้องอกที่กระเพาะปัสสาวะจึงได้มีการตัดก้อนเนื้องอกและกระเพาะปัสสาวะที่ใกล้กับตำแหน่งรูเปิดท่อไตด้านขวาออกบางส่วนร่วมกับเปลี่ยนย้ายรูเปิดท่อไตด้านขวา ผลการตรวจทางจุลพยาธิวิทยาพบว่าเป็นมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์ และมีการยืนยันผลการวินิจฉัยนี้ด้วยการทดสอบทางอิมมูโนฮิสโตเคมีซึ่งให้ผลการย้อมแอนติเจนยูโรไพคิน 3 (uroplakin III) ไซโตเคราติน7 (cytokeratin7) และไซโตเคราติน20 (cytokeratin20) เป็นลบ อย่างไรก็ตามภายหลังการผ่าตัดเป็นเวลา 2 เดือนได้มีการอัลตร้าซาวด์พบก้อนที่ตับ จึงสันนิษฐานว่าเป็นการแพร่กระจายไปยังอวัยวะอื่น ข้อมูลรายงานสัตว์ป่วยนี้จะนำเสนอการผ่าตัดกรณีของมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์ซึ่งเป็นมะเร็งชนิดที่พบได้ยากในสุนัข

คำสำคัญ : มะเร็งกระเพาะปัสสาวะ สุนัข การผ่าตัดกระเพาะปัสสาวะออกบางส่วน มะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์

Introduction

Canine urinary bladder neoplasia, which accounts for 2% of all cancers, can result in partial or complete obstruction of the urinary tract. The most frequent type of bladder cancer is transitional cell carcinoma (TCC) (Fulkerson and Knapp 2019), while squamous cell carcinoma (SCC) is less common in dogs with a 1.5 - 2% incidence of bladder cancers (Ramos-Vara et al., 2003; de Brot et al., 2018). To present, in Thailand, only urinary bladder TCC (Rungsipipat et al., 2003; Suwankanit and Manee-in 2018) and urinary bladder leiomyoma have been reported (Ploypetch et al., 2021). Secondary cancer on the urinary bladder has rarely been observed. Studies in humans have found that bladder SCC usually occurs in the trigone area as well as the urethra or ureters. Moreover, common sites of metastasis of SCC of the urinary bladder are the lung, bone, and liver (Manley et al., 2017). Surgery, radiation, chemotherapy, medicinal therapy or a combination of these are used to treat bladder cancer in humans and dogs (Zahoor et al., 2018; Burgess and DeRegis 2019; Fulkerson and Knapp 2019). Radical cystectomy is a conventional treatment for human bladder SCC, as it provides better disease control and recurrence-free survival time than other treatments, when combined with radiotherapy (Richie et al., 1976; Martin et al., 2016).

This case study illustrates the surgical management of urinary obstruction due to urinary cancers, and the SCC process to confirm the diagnosis.

Case Description

A 10-year-old spayed bitch American Cocker Spaniel with myxomatous mitral valve disease stage B2 was referred to Prasu-Arthorn Animal Hospital, Mahidol University for evaluation of anorexia and pollakiuria. The vital signs were normal, except for a grade 4/6 murmur heart sound. Blood profiles were within the reference interval, as shown in Table 1. Serum biochemical analysis showed hyperproteinemia (8 g/dl, reference 6 - 7.5 g/dl), slightly increased alanine aminotransferase (265 IU/l, reference 10 - 100 IU/l), and high blood urea nitrogen (55 mg/dl, reference 7-27 mg/dl). The cystocentesis urinalysis results revealed a slightly transparent, yellow, inappropriate concentration of urine (urine specific gravity 1.010) and pH 7. Proteinuria was also present, which was detected by urine strip testing. Due to some bacteriuria, a urine sample was taken for bacterial identification and antibiotic susceptibility testing. *Proteus mirabilis* sensitive to cephalosporins was interpreted as the bacterial culture and antimicrobial sensitivity result. Thoracic and abdominal radiography did not present any visible abnormalities. Abdominal ultrasonography showed right hydronephrosis (Figure 1A). The right renal pelvis dimension is 1.6 x 2.1 cm with a transverse view (Figure 1B). The right proximal ureter was obstructed and dilated (Figure 1C) with a hyperechoic structure. The urinary bladder had wall thickening, with an irregular mucosal surface pronounced at the right apex part (Figure 1D). Therefore, the patient was diagnosed with right outflow obstructive hydronephrosis due to early ureteral obstruction. Surgical treatment to correct the right ureteral obstruction was planned.

Table 1. Hematology report (absolute) from the first visit and 7 days after surgery.

Parameters*	First visit	Seven days after surgery	Normal value
WBC (µl)	11,160	9,500	6,000 - 17,000
Monocyte	669.6	475	150 - 1,350
Neutrophil	8,370	6,745	300 - 11,500
Band	0	0	0 - 300
Lymphocyte	2,120.4	2,185	1,000 - 4,800
Eosinophil	0	95	100 - 1,250
Basophil	0	0	<100
RBC (10^6 cell/µl)	6.65	7.05	5 - 9
Hb (g/dl)	16	17.0	10 - 18
Hct (%)	47.9	50.7	35 - 55
MCV (fL)	72	71.9	60 - 77
MCH (pg)	24.1	24.1	20 - 25
MCHC (g/dl)	33.4	33.5	32 - 36
PLT (10^3/µl)	197	213	200 - 500
Platelet smear	Adequate	Adequate	
Plasma Protein	8	8	6.0 - 7.5
ALT (U/L)	265	255	10 - 100
ALP (U/L)	107	118	23 - 212
BUN (mg/dL)	55	50	7 - 27
Creatinine (mg/dL)	1.36	1.44	0.5 - 1.8

*WBC: White blood cells; RBC: Red blood cells; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; BUN: Blood urea nitrogen.

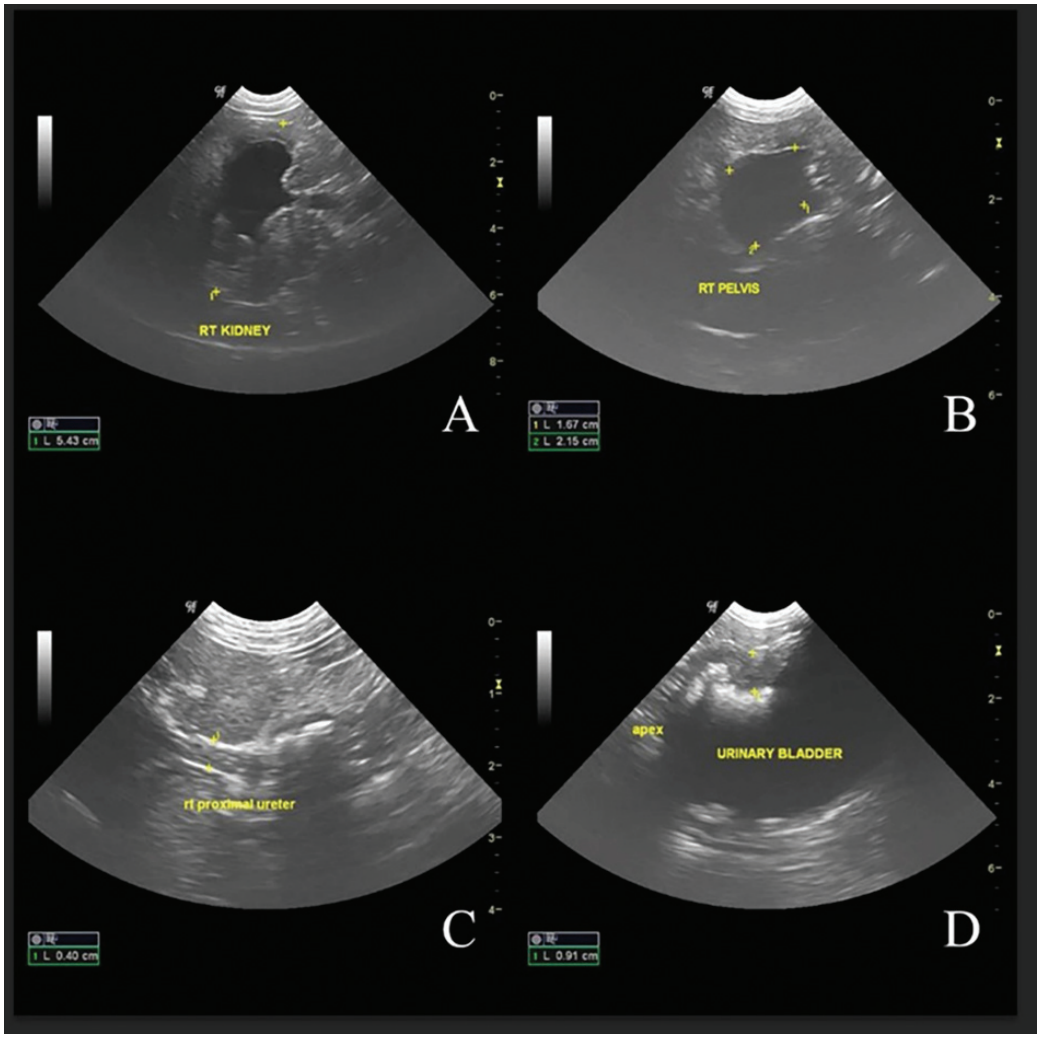

Figure 1. Abdominosonographic findings. (A) Right hydronephrosis. (B) The dilatation of the right renal pelvis. (C) Dilated right proximal ureter. (D) The urinary bladder has wall thickening with an irregular mucosal surface pronounced at the apex part.

The patient underwent a partial cystectomy at the right ureter orifice, as well as a right ureteroneocystostomy. Anaesthetic drugs were selected to minimize the cardiac and renal side effects of general anaesthesia. In this case, fentanyl (3 μg/kg fentanyl-Hameln; Siam Bioscience Co. Ltd., Thailand) and midazolam (0.3 mg/kg midazolam; F. Hoffmann-La Roche Ltd., Switzerland) were intravenously administered as a premedication, then inducted with alfaxalone (2 mg/kg Alfaxan®; Jurox Pty. Ltd., Australia). An injectable drug with morphine (0.3 mg/kg morphine sulphate injection; M&H Manufacturing Co. Ltd., Thailand) was injected intramuscularly for analgesia and amoxicillin/clavulanic acid (20 mg/kg Cavumox®; Siam Bheasach Co. Ltd., Thailand) was intravenously administered for anti-microbial prophylaxis. The patient was intubated and maintained at an anaesthetic level using isoflurane (AERRANE; Baxter Healthcare of Puerto Rico, United States) inhalation with oxygen. The patient was positioned in dorsal recumbency. A ventral midline celiotomy was performed through the caudal aspect of the abdominal wall with a ventral midline cystotomy. Intraoperation, a mass that involved the right

ureteral opening and bladder apex was presented, causing right ureteral dilatation (Figure 2A). Partial cystectomy was then performed, respecting a margin of 20 mm on the sides. Resection comprised about 40 to 50% of all bladder tissue (Figure 2B). The right ureter was removed approximately 5 mm. A ureteroneocystostomy was done with an interrupted pattern using absorbable 4-0 polydioxanone suture material (PDS™ II 4-0; Johnson & Johnson Medical N.V., Belgium) (Figure 2C) to reimplant the right ureter into the bladder. The bladder wall was closed with a simple continuous pattern using absorbable 3-0 poliglecaprone 25 suture material (Monocryl™ 4-0; Johnson & Johnson Medical N.V., Belgium). Finally, the abdomen wall was closed utilizing the routine technique. A postoperative urinary catheter was placed to monitor urine output for 7 days. The bladder mass (Figure 2D), measuring 18 mm (length) x 15 mm (width) x 15 mm (height), was examined histopathologically and stained with haematoxylin & eosin (H&E) and immunohistochemistry (IHC) performed.

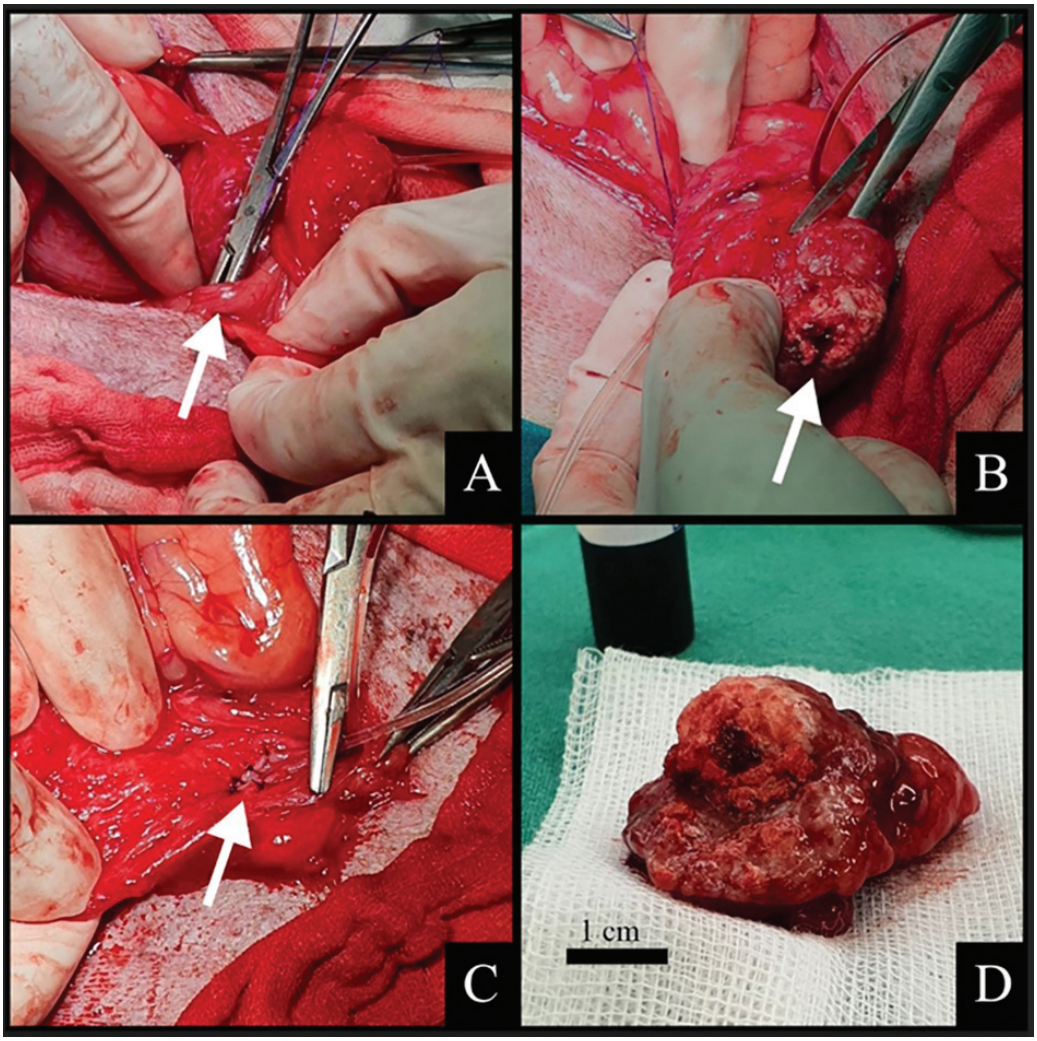

Figure 2. Ureteroneocystotomy and partial cystectomy procedure. (A) A laparotomy found right ureteral dilatation (arrow). (B) Partial cystectomy for removal of the entire mass (arrow). (C) Ureteroneocystotomy for reimplantation of the ureter on the surface of the bladder (arrow). (D) Excised mass with rough shape and irregular surface.

Microscopically, the mass revealed multifocally non-encapsulated, well-differentiated, strongly proliferative, pleomorphic squamous cells in the submucosal layer and invading through the transitional epithelial layer. Pleomorphic cells were arranged in cords to islets of proliferative squamous cells. Squamous cells were hyperplastic with anisocytosis, round, of cuboidal to polyhedral shape with abundant amphophilic cytoplasm with occasional intercellular bridges. Nuclei had anisokaryosis, were vesiculated oval to round with prominent nucleoli. Mitotic figures were occasionally noticed. Eosinophilic keratin precipitation was occasionally detected in cords. Submucosal layers were occupied by hyperplastic fibrous tissues with focally extensive submucosal layers. There was no evidence of metastasis. Keratin pearl was well differentiated for SCC diagnosis (Figure 3A). The streptavidin-biotin peroxidase technique with commercial kits was used for immunohistochemistry. The primary antibodies including Uroplakin III (UR III) (1: 450 Clone AU-1; Cell Marque, USA), cytokeratin 7 (CK7) (1: 1,000 Clone OV-TL 12/30; Cell Marque, USA(, and cytokeratin 20 (CK 20) (1: 3,000 Clone Ks20.8; Cell Marque, USA) were used to determine SCC of the urinary bladder. Only the apical border of the cytoplasmic membrane of transitional cells showed strongly intense positivity for three markers. The intermediate and superficial cells were also cytoplasmically stained. The lower cells and basal area were negative. The dysplasia of proliferative squamous cells had a lack of immunoreactivity. All negative immunohistochemistry (Figures 3B, 3C, and 3D) results confirmed the presence of SCC.

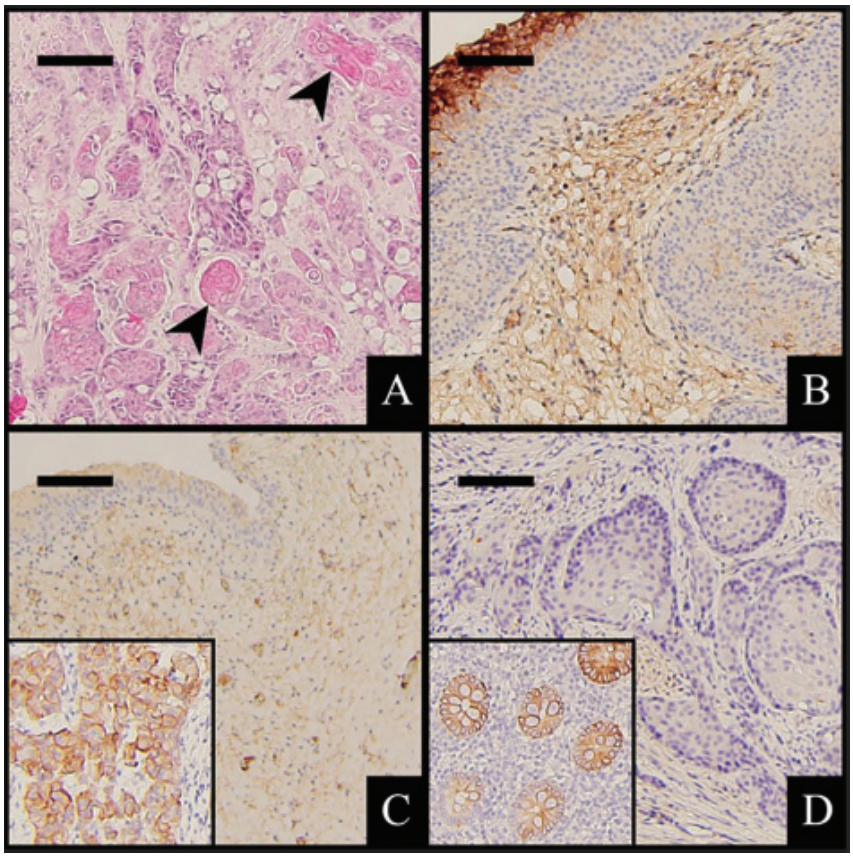

Figure 3. Immunohistopathologic findings. (A) The rounded-looped shapes are diagnosed as keratin pearl (arrowheads) from H&E staining. Immunohistochemical staining of UR III (B), CK 7 (C), and CK 20 (D). The positive controls of CK 7 and CK 20 are shown as an inset in the lower left corner of (C) and (D), respectively. Bar = 200 μm.

Afterwards, the patient was admitted to the hospital for 7 days for post-operative care. Vital signs, especially blood pressure and urine output, were closely measured. Moreover, the post-operative practices including fluid therapy, antimicrobials, analgesics, wound dressing and recheck blood profiles were performed in the critical care unit. For pain management, an injectable drug with morphine (0.3 mg/kg morphine sulphate injection; M&H Manufacturing Co. Ltd., Thailand) was injected subcutaneously every 8 hours, depending on the pain score. No clinical signs of the urinary system were observed 7 days after surgery. Blood profiles were rechecked before discharge from the hospital. Hematologic values were showed in Table 1.

Ultrasonography was used to check for renal hydronephrosis and ureteral dilation, as well as the recurrence of cancer one month after surgery. The dilatation of the right renal pelvis and right proximal ureter was reduced when compared with before surgery. The urinary bladder's wall thickness was normal. The right ureteral opening of the bladder had a mucosal surface that was slightly irregular and assumed fibrosis following ureteroneocystostomy. Although the dog showed no clinical signs, ultrasonography in two months after surgery revealed multiple nodules in all hepatic lobules and left medial iliac lymphadenopathy. Metastasis was suspected. Metronomic chemotherapy with a combination of chlorambucil (4 mg/m2 LeukeranTM; Aspen Pharmacare Australia Pty Ltd., Australia) and firocoxib (2 mg/kg Previcox®; Boehringer Ingelheim, Germany) was used to improve the quality of life. No side effects from this low-dose chemotherapy were observed in this case. Moreover, the owner described that no clinical signs of haematuria or trouble urinating had appeared at least 10 months after metronomic administration.

Discussion

According to the universal consensus in humans, radical cystectomy is required for the surgical treatment of urinary bladder SCC. Recurrence-free survival rates for the entire patient population after radical cystectomy at 5 and 10 years were 68 and 66%, respectively (Stein et al., 2001). On the contrary, previous studies in dogs that received this technique had many complications, including pyelonephritis, peritonitis, oliguria, azotaemia and ureteral obstruction (Ricardo Huppes et al., 2017). A partial cystectomy with extensive resection, which was analogous to traditional surgery for removing urinary bladder carcinoma, was undertaken alternatively. Except for tumour metastasis, no complications were discovered during or after surgery. However, the presence of residual tumour at the site of resection through neoplastic transformation of the remaining bladder mucosa and implantation of tumour cells into the surgical wound, resulting in local tumour recurrence, might be a common consequence after bladder SCC resection (Zahoor et al., 2018). The study in humans undergoing partial cystectomy for bladder cancer reported that bleeding, infection, reduced bladder capacity, and urinary extravasation are all common consequences after surgery (Kates et al., 2014).

Once distinguishing characteristics such as keratin pearls and intercellular bridges have been recognized, SCC can be definitively confirmed histologically. Moreover, these features can only be seen when the SCC is still a well-differentiated neoplasm (Lopez-Beltran et al., 2007). Despite the presence of keratin pearls and intercellular bridges, this case was also diagnosed using the IHC technique. For the detection of canine urothelial cancers, UP III is more specific than CK 7 and CK 20. Between SCC and TCC, the immunohistochemistry staining

patterns of CK 20, CK 7 and UP III are significantly different. All these markers are negative in bladder SCC as in this case (Ramos-Vara et al., 2003). Therefore, this case was definitively diagnosed as canine urinary bladder SCC, confirmed by histopathology and the IHC technique.

Regarding the treatment of bladder SCC, combining preoperative radiation with radical cystectomy resulted in a significantly greater 5-year survival rate for humans than any other treatment (Martin et al., 2016; Manley et al., 2017). A recent study in dogs has suggested that radiotherapy, with or without adjunctive chemotherapy and nonsteroidal anti-inflammatory drugs, presumably achieves locoregional tumour control and prolonged survival in genitourinary carcinoma, including bladder carcinoma (Clerc-Renaud et al., 2021). However, information about a radiation protocol for the treatment of canine urinary bladder SCC is still limited. Treatment with chemotherapy in bladder carcinoma should be used in dogs with metastases. Due to the presence of metastasis in liver and abdominal lymph node, low-dose metronomic chemotherapy was prescribed in this case postoperatively. Although there is a lack of evidence for chemotherapy in dogs with urinary bladder SCC, the metronomic administration of chlorambucil in canine TCC has been shown to be well tolerated, with 70% of cases achieving partial remission or stable condition (Schrempp et al., 2013).

Conclusions

This report has described a dog that had a urinary obstruction caused by urinary bladder SCC, which occurs rarely. Partial cystectomy and ureteroneocystostomy are optional surgical techniques for SCC in the dog's ureteral orifice. Interestingly, the biomarker approach for diagnosing urinary bladder SCC in dogs should be investigated in further studies.

Acknowledgements

The authors are most grateful to the owner for the information and permission. The authors would like to give a special thank you to Asst. Prof. Dr. Somkiat Huaijantug for his guidance on diagnostic imaging. The authors would like to thank Dr. Piyanoot Fonghem and all staff from the Prasu-Arthon Animal Hospital, Faculty of Veterinary Science, Mahidol University for providing patient information.

References

Burgess KE, DeRegis CJ. Urologic Oncology. Vet Clin North Am Small Anim Pract. 2019;49(2):311-23.

Clerc-Renaud B, Gieger TL, LaRue SM, Nolan MW. Treatment of genitourinary carcinoma in dogs using nonsteroidal anti-inflammatory drugs, mitoxantrone, and radiation therapy: A retrospective study. J Vet Intern Med. 2021;35(2):1052-61.

de Brot S, Robinson BD, Scase T, Grau-Roma L, Wilkinson E, Boorjian SA, et al. The dog as an animal model for bladder and urethral urothelial carcinoma: Comparative epidemiology and histology. Oncol Lett. 2018;16(2):1641-9.

Fulkerson CM, Knapp DW. Tumors of the urinary system. In: Vail DM, Thamm DH, Liptak JM, editors. Withrow and MacEwen's Small Animal Clinical Oncology. Tumors of the Urinary System. St. Louis (MO): W.B. Saunders; 2019. p. 645-56.

Kates M, Gorin MA, Deibert CM, Pierorazio PM, Schoenberg MP, McKiernan JM, et al. In-hospital death and hospital-acquired complications among patients undergoing partial cystectomy for bladder cancer in the United States. Urol Oncol. 2014;32(1): 53 e9-14.

Lopez-Beltran A, Requena MJ, Alvarez-Kindelan J, Quintero A, Blanca A, Montironi R. Squamous differentiation in primary urothelial carcinoma of the urinary tract as seen by MAC387 immunohistochemistry. J Clin Pathol. 2007;60(3):332-5.

Manley KV, Hubbard R, Swallow D, Finch W, Wood SJ, Biers SM. Risk factors for development of primary bladder squamous cell carcinoma. Ann R Coll Surg Engl. 2017;99(2):155-60.

Martin JW, Carballido EM, Ahmed A, Farhan B, Dutta R, Smith C, et al. Squamous cell carcinoma of the urinary bladder: Systematic review of clinical characteristics and therapeutic approaches. Arab J Urol. 2016;14(3):183-91.

Ploypetch S, Maneein S, Phantharangsi S. Computed tomographic appearance of bladder and vaginal leiomyoma in dog: case report. Thai J Vet Med. 2021;51(4):809-14.

Ramos-Vara JA, Miller MA, Boucher M, Roudabush A, Johnson GC. Immunohistochemical detection of uroplakin III, cytokeratin 7, and cytokeratin 20 in canine urothelial tumors. Vet Pathol. 2003;40(1): 55-62.

Ricardo Huppes R, Crivellenti LZ, Barboza De Nardi A, Roque Lima B, Alves Cintra C, Luiz Costa Castro J, et al. Radical cystectomy and cutaneous ureterostomy in 4 dogs with trigonal transitional cell carcinoma: description of technique and case series. Vet Surg. 2017;46(1):111-9.

Richie JP, Waisman J, Skinner DG, Dretler SP. Squamous carcinoma of the bladder: treatment by radical cystectomy. J Urol. 1976;115(6):670-2.

Rungsipipat A, Sunyasootcharee B, Ousawaphlangchai L, Sailasuta A, Thanawongnuwech R, Teankum K. Neoplasms of dogs in Bangkok. Thai J Vet Med. 2003;33(1):59-66.

Schrempp DR, Childress MO, Stewart JC, Leach TN, Tan KM, Abbo AH, et al. Metronomic administration of chlorambucil for treatment of dogs with urinary bladder transitional cell carcinoma. J Am Vet Med Assoc. 2013;242(11):1534-8.

Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol. 2001;19(3):666-75.

Suwankanit K, Manee-in S. Treatment of transitional cell carcinoma of urinary bladder using meloxicam in a dog: a case report. J Appl Anim Sci. 2018;11(2): 45-56.

Zahoor H, Elson P, Stephenson A, Haber GP, Kaouk J, Fergany A, et al. Patient characteristics, treatment patterns and prognostic factors in squamous cell bladder cancer. Clin Genitourin Cancer. 2018; 16(2):e437-e42.

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