JOURNAL OF
APPLIED ANIMAL SCIENCE

ISSN 1906-2257 VOL.14 NO.2 JULY-DECEMBER 2021

"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

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

999 ถนนพุทธมณฑล สาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

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

จัดพิมพ์โดย :

ห้างหุ้นส่วนสามัญนิติบุคคล ปอยท์ กราฟิค

177/2 ซอยจรัญสนิทวงศ์ 11 ถนนจรัญสนิทวงศ์ แขวงวัดท่าพระ เขตบางกอกใหญ่ กรุงเทพฯ 10600

โทร. 08-1826-5455 E-mail address: pointswift@yahoo.com

"Journal of Applied Animal Science" (JAAS)

สารจากคณบดี

แม้ในสถานการณ์ที่ยากลำบากที่ต้องปรับวิถีการใช้ชีวิตตามรูปแบบ new normal จากภาวะการระบาดโควิด-19 ที่มีผลกระทบเป็นวงกว้างไปทั่วโลก แต่ดูเหมือนว่าการปรับตัวของประชาคมในโลกเป็นไปตามแนวทางที่ตนถนัดและพัฒนางานต่าง ๆ ออกมาเรื่อย ๆ ไม่หยุดยั้ง เฉกเช่นเดียวกับบุคลากรสัตวแพทย์ที่มีส่วนร่วมในการขับเคลื่อนผลงานออกมาอย่างต่อเนื่อง ดังปรากฏในบทความรูปแบบ research article และ case report ของ Journal of Applied Animal Science (JAAS) Vol 14 No.2 Jul-Dec 2021 ที่ครอบคลุมมิติการทำงานของสัตวแพทย์ในด้านต่าง ๆ รวมทั้งการทำงานข้ามศาสตร์ตามแนวทางสุขภาพหนึ่งเดียว ซึ่งเป็นไปตามเจตนารมณ์ของทีมบรรณาธิการที่นำทีมโดยบรรณาธิการวารสาร รศ.ดร.น.สพ.ธนศักดิ์ ช่างบรรจง เป็นฉบับที่ 4 แล้ว

และตามที่ได้เกริ่นไว้ในฉบับที่แล้ว ทางบรรณาธิการวารสารได้เริ่มต้นที่จะยกระดับมาตรฐานวารสารให้ตรงตามเกณฑ์คุณภาพวารสารของศูนย์ดัชนีการอ้างอิงวารสารไทย (Thai-Journal Citation Index Centre, TCI) และวางแผนที่จะนำวารสารเข้าสู่ระบบฐานข้อมูลวารสารอิเล็กทรอนิกส์กลางของประเทศไทย (Thai Journals Online (ThaiJO)) ที่พัฒนาอยู่บนระบบเดียวกันกับระบบ Online Journal System (OJS) ซึ่งพัฒนาโดย Public Knowledge Project (PKP) ที่เป็นหน่วยงานระดับนานาชาติที่พัฒนาจากมหาวิทยาลัยของประเทศต่าง ๆ และสำหรับวารสารฉบับหน้าจะเปิดช่องทางให้สามารถส่งบทความออนไลน์ผ่านทางระบบ ThaiJO ได้แล้ว

ขอขอบคุณบรรณาธิการวารสารตลอดจนผู้รับผิดชอบงานวารสารทุกท่าน ที่ได้สละเวลาอันมีค่าจัดทำเล่มวารสาร ประสานงานและอำนวยความสะดวกกับผู้เขียนบทความ ตั้งแต่เริ่มต้นจนแล้วเสร็จ และขอเป็นกำลังใจให้ผู้รับผิดชอบ Journal of Applied Animal Science สามารถผลิตผลงานและบรรลุผลตามวัตถุประสงค์ทุกประการ

รองศาสตราจารย์ ดร.สัตวแพทย์หญิงวลาสินี ศักดิ์คำดวง
คณบดีคณะสัตวแพทยศาสตร์
มหาวิทยาลัยมหิดล

"Journal of Applied Animal Science" (JAAS)

ปีที่ 14 ฉบับที่ 2 กรกฎาคม-ธันวาคม 2564 Vol. 14 No. 2 July-December 2021

สารบัญ

คำแนะนำสำหรับผู้แต่ง

"Journal of Applied Animal Science" (JAAS)

วารสารสัตวศาสตร์ประยุกต์เป็นวารสารวิชาการราย 6 เดือน (2 ฉบับต่อปี เดือนมกราคม-มิถุนายน และเดือนกรกฎาคม-ธันวาคม) ของคณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล เผยแพร่ผลงานวิจัยครอบคลุมสหสาขาวิชาทั้งสัตวแพทยศาสตร์ และสัตวศาสตร์ ตั้งแต่พื้นฐานถึงระดับโมเลกุล รวมถึงรายงานทางคลินิก บทความที่ได้รับการตีพิมพ์ในวารสารต้องผ่านการประเมินโดยผู้ทรงคุณวุฒิอย่างน้อย 2 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 http://www.vs.mahidol.ac.th/jaas/

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 2 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, http://www.vs.mahidol.ac.th/jaas.

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

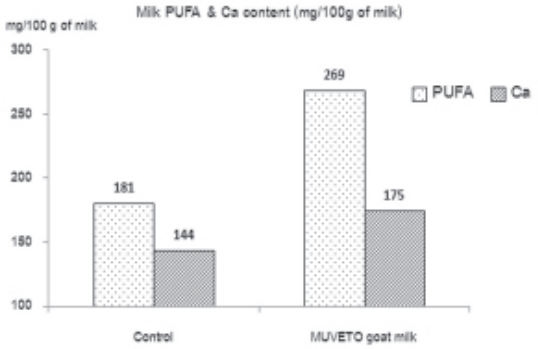
ในปี พ.ศ. 2564 วารสาร Journal of Applied Animal Science (JAAS) ยังคงได้รับการตอบรับอย่างดียิ่งจากอาจารย์ นักวิจัย และสัตวแพทย์จากหลากหลายหน่วยงาน โดยการร่วมส่งผลงานมาตีพิมพ์ทั้งในรูปแบบของบทความปริทัศน์ บทความวิจัย และรายงานสัตว์ป่วย สำหรับวารสาร JAAS ฉบับถัดไปซึ่งเป็นฉบับแรกของปี พ.ศ. 2565 ทางวารสารจะเริ่มเปิดใช้งานผ่านทางระบบฐานข้อมูลวารสารอิเล็กทรอนิกส์กลางของประเทศไทย (Thai Journals Online: ThaiJO) เพื่อเป็นการพัฒนาระบบการจัดการของวารสารให้มีประสิทธิภาพมากยิ่งขึ้นและเป็นไปตามมาตรฐานสากล โดยท่านผู้สนใจสามารถดูรายละเอียดของวารสารได้ที่เว็บไซต์ https://he02.tci-thaijo.org/index.php/jaas_muvs

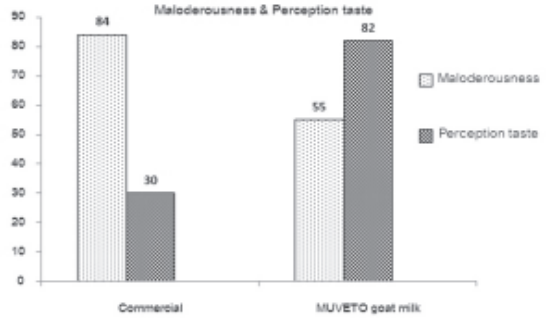
สำหรับวารสารฉบับที่ 2 (เดือนกรกฎาคมถึงเดือนธันวาคม) ของปีที่ 14 นี้ ประกอบด้วยบทความที่น่าสนใจหลายเรื่อง ได้แก่ บทความวิจัย 2 เรื่อง **“การประเมินชุดตรวจ N-terminal pro-B-type natriuretic peptide ในการแยกระดับความรุนแรงของโรคลิ้นหัวใจเสื่อมในสุนัข”** และ **“การประเมินประชากรจระเข้ในบึงบอระเพ็ด ประเทศไทย”** รายงานสัตว์ป่วย 2 เรื่อง **“การรักษาพยาธิหนอนหัวใจระยะที่ 4 (Caval Syndrome) ในสุนัขด้วย อิมิดาโคลพริด ม็อกซี่เด็กติน แบบระยะยาว”** และ **“การผ่าตัดแก้ไขโรคหลอดเลือดหัวใจคงค้างในสุนัขปอมเมอราเนียนเต็มวัย”**

สุดท้ายนี้ ในนามหัวหน้ากองบรรณาธิการ ขอขอบพระคุณกองบรรณาธิการวารสาร ผู้ทรงคุณวุฒิพิจารณาบทความ และผู้นิพนธ์บทความทุกท่าน ที่มีส่วนร่วมทำให้วารสาร JAAS ของปีนี้สำเร็จลุล่วงไปด้วยดี

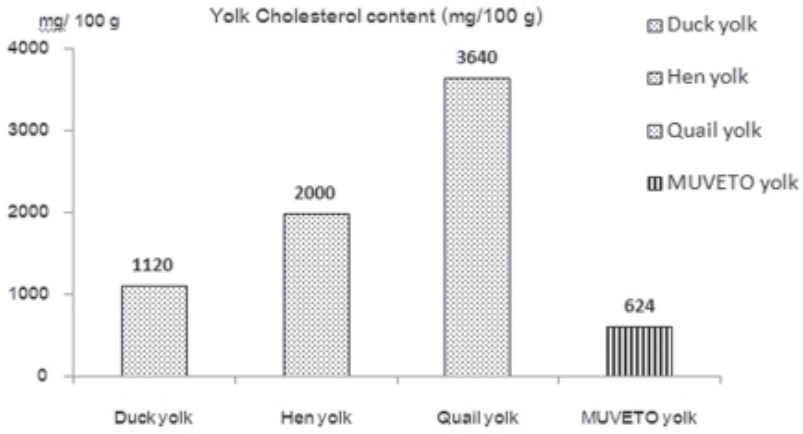
รองศาสตราจารย์ ดร.นายสัตวแพทย์ธนศักดิ์ ช่างบรรจง
บรรณาธิการ (Editor-in-Chief)

MUVETO





Functional goat milk: Naturally high PUFA,Ca and maloderousness



Functional egg: Low cholesterol

Distributed by : Faculty of Veterinary Science, Mahidol University
999 Phutthamonthon Sai 4 Road, Salaya, Phutthamonthon,
Nakhonpathom, 73170, Thailand. Tel. 02-4410933.

Research Articles

Assessment of Two New Commercial Rapid Tests for Canine N-terminal Pro-B-type Natriuretic Peptide to Distinguish the Severity of Mitral Valve Disease in Dogs

Nattapon Riengvirodkij[1] Walasinee Sakcamduang[2*]

[1]Prasu-Arthorn Veterinary Teaching Hospital, Faculty of Veterinary Science, Mahidol University, Salaya, Phutthamonthon, Nakhon Pathom, Thailand

[2]Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Salaya, Phutthamonthon, Nakhon Pathom, Thailand

[*]Corresponding author, E-mail address: walasinee.sak@mahidol.ac.th

Received: 8 September 2021; Revised: 10 November 2021; Accepted: 15 November 2021

Abstract

N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) is a potential cardiac biomarker released primarily from ventricular myocytes. Previous studies in dogs suggest that the NT-proBNP assay can be used to distinguish dogs with respiratory signs between congestive heart failure and primary respiratory illnesses. The objective of this study is to determine and assess the clinical sensitivity and specificity of the two new NT-pro-BNP immunoassay tests for distinguishing dogs affected by myxomatous mitral valve disease (MMVD) between with and without congestive heart failure (CHF), as well as between dogs with MMVD and normal dogs. Thirty-seven dogs were enrolled in this study. Thirty-two dogs were measured by the NT-proBNP immunoassay test (Bionote®), including 11 healthy dogs, 11 dogs with MMVD stage B2 (absence of CHF) and 10 dogs with MMVD stage C (presence of CHF). Twenty-eight dogs were measured by the NT-proBNP rapid detection kit (Dianotech®), including 6 healthy dogs, 7 dogs with MMVD stage B2 and 15 dogs with MMVD stage C.

The median NT-proBNP concentration in dogs with MMVD stage C was significantly higher than the median NT-proBNP concentration in dogs with MMVD stage B2 and normal healthy dogs (p-value <0.001). The Receiver operating characteristic (ROC) curve analysis showed that serum NT-proBNP concentration could differentiate dogs with CHF signs (MMVD stage C) from dogs without CHF signs (normal healthy dogs and MMVD stage B2. The area under the curve (AUC) was 0.932 and 0.928 for NT-proBNP Bionote® and Dianotech® test, respectively. It also could discriminate dogs affected by MMVD with CHF (MMVD stage C2) from dogs affected by MMVD without CHF (MMVD stage B2) with AUC of 0.818 and 0.867 for NT-proBNP Bionote® and Dianotech® tests, respectively. In conclusion, the results suggested that serum NT-proBNP concentrations tests from both companies could discriminate between dogs affected by CHF from those without CHF and normal healthy dogs with reasonable accuracy.

Keywords: N-terminal pro-B-type natriuretic peptide, NT-pro-BNP, mitral valve disease, dogs

การประเมินชุดตรวจ N-terminal pro-B-type natriuretic peptide ในการแยกระดับความรุนแรงของโรคลิ้นหัวใจเสื่อมในสุนัข

ณัฐพล เรียงวิโรจน์กิจ[1] วลาสินี ศักดิ์คำดวง[2*]

[1]โรงพยาบาลสัตว์ประศุอาทร คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ถนนพุทธมณฑล สาย 4
ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170
[2]ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ถนนพุทธมณฑล สาย 4
ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

[*]ผู้รับผิดชอบบทความ E-mail address: walasinee.sak@mahidol.ac.th

Received: 8 September 2021; Revised: 10 November 2021; Accepted: 15 November 2021

บทคัดย่อ

N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) เป็นตัวชี้วัดทางชีวภาพที่มีศักยภาพในการวินิจฉัยโรคหัวใจ NT-pro-BNP จะถูกหลั่งออกมาจากเซลล์กล้ามเนื้อหัวใจห้องล่างเป็นหลัก มีรายงานจากการศึกษาก่อนหน้าระบุว่าสามารถใช้ชุดตรวจ NT-pro-BNP ในการวินิจฉัยสุนัขที่มีปัญหาโรคลิ้นหัวใจเสื่อม และใช้ตรวจแยกสุนัขที่มีอาการหายใจลำบากที่มีสาเหตุมาจากโรคหัวใจออกจากรายที่มีสาเหตุมาจากโรคระบบทางเดินหายใจได้ การศึกษานี้มีเป้าหมายในการศึกษาประสิทธิภาพของชุดตรวจ NT-pro-BNP รุ่นใหม่จาก 2 บริษัท ในการใช้ตรวจวินิจฉัยโรคลิ้นหัวใจเสื่อมในสุนัข และ เพื่อประเมินความไวและความจำเพาะในการใช้ตรวจแยกสุนัขโรคลิ้นหัวใจเสื่อมที่เกิดภาวะหัวใจล้มเหลวออกจากรายที่ยังไม่เกิดภาวะหัวใจล้มเหลว รวมไปถึงการใช้ตรวจแยกสุนัขที่มีโรคลิ้นหัวใจเสื่อมออกจากสุนัขปกติ

การศึกษาทำโดยเก็บตัวอย่างจากสุนัขทั้งหมด 37 ตัว โดยตัวอย่างจากสุนัข 32 ตัวจะทำการตรวจวัดระดับ NT-pro-BNP ด้วยชุดตรวจจากบริษัท Bionote® โดยแบ่งเป็นสุนัขปกติ 11 ตัว สุนัขลิ้นหัวใจเสื่อมระยะ B2 (ยังไม่มีภาวะหัวใจล้มเหลว) 11 ตัว และสุนัขลิ้นหัวใจเสื่อมระยะ C (มีภาวะหัวใจล้มเหลว) 10 ตัว และตัวอย่างจากสุนัข 28 ตัวจะทำการตรวจวัดระดับ NT-pro-BNP ด้วยชุดตรวจจากบริษัท Dianotech® แบ่งเป็นสุนัขปกติ 6 ตัว สุนัขลิ้นหัวใจเสื่อมระยะ B2 7 ตัว และสุนัขลิ้นหัวใจเสื่อมระยะ C 15 ตัว

ผลการศึกษาพบว่าระดับ NT-pro-BNP ในสุนัขลิ้นหัวใจเสื่อมระยะ C มีค่าสูงกว่าระดับ NT-pro-BNP ในสุนัขลิ้นหัวใจเสื่อมระยะ B2 และสุนัขปกติอย่างมีนัยสำคัญ (p-value < 0.001) การวิเคราะห์ด้วยเส้นโค้ง Receiver operating characteristic (ROC) พบว่าระดับ NT-pro-BNP สามารถใช้ตรวจแยกสุนัขลิ้นหัวใจเสื่อมที่มีภาวะหัวใจล้มเหลว (ระยะ C ออกจากสุนัขที่ยังไม่มีภาวะหัวใจล้มเหลว (ระยะ B2 และสุนัขปกติ) ได้ โดยมีค่าพื้นที่ใต้เส้นโค้ง (area under the curve; AUC) ที่ 0.932 และ 0.928 สำหรับชุดตรวจจากบริษัท Bionote® และ Dianotech® ตามลำดับ การวิเคราะห์ด้วยเส้นโค้ง ROC ยังสามารถใช้ตรวจแยกสุนัขลิ้นหัวใจเสื่อมที่มีภาวะหัวใจล้มเหลว (ระยะ C) ออกจากสุนัขลิ้นหัวใจเสื่อมที่ยังไม่มีภาวะหัวใจล้มเหลว (ระยะ B2) ได้อีกด้วย โดยมีค่าพื้นที่ใต้เส้นโค้งอยู่ที่ 0.818 และ 0.867 สำหรับชุดตรวจจากบริษัท Bionote® และ Dianotech® ตามลำดับ โดยสรุปพบว่าชุดตรวจ NT-pro-BNP จากทั้ง 2 บริษัทมีประสิทธิภาพในระดับที่ยอมรับได้ในการใช้เป็นตัวช่วยในการตรวจวินิจฉัยโรคลิ้นหัวใจเสื่อมในสุนัข และใช้ตรวจแยกสุนัขโรคลิ้นหัวใจเสื่อมระยะที่เกิดภาวะหัวใจล้มเหลวออกจากรายที่ยังไม่เกิดภาวะหัวใจล้มเหลว

คำสำคัญ : เปปไทด์ เอ็นเทอร์มินอลโปรบีเอ็นพี โรคลิ้นหัวใจเสื่อม สุนัข

Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs (Borgarelli et al., 2012; Buchanan 1977). It often leads to congestive heart failure (CHF) and cardiac-related death, eventually (Borgarelli et al., 2012). Differentiation between dogs affected by MMVD with and without CHF is quite challenging. Diagnosis of mitral valve disease (MMVD) with different stages in dogs has conventionally been made on the basis of physical examination, cardiorespiratory auscultation, thoracic radiography and echocardiography. Unfortunately, clinical signs of MMVD are usually non-specific and could be similar to some respiratory tract diseases making it difficult to distinguish between those two diseases just from the physical examination. Detection of lung edema in the perihilar area from thoracic radiography could also be used for identifying dogs with CHF. However, in patients with severe respiratory distress, this technique is limited. For this reason, a biomarker that could be used to differentiate disease severity would be helpful in the diagnosis of dogs with MMVD clinically.

N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) is a cardiac peptide released primarily from ventricular myocytes when stretching (Hosoda et al., 1991). It is synthesized as a prohormone and cleaved into 2 peptide fragments, NT-pro BNP and C-terminal BNP (C-BNP). They are increased in the blood circulation of dogs with heart disease (Boswood et al., 2008; Oyama et al., 2008). However, C-BNP is rapidly degraded and unstable in blood circulation (Thomas and Woods 2003), making it difficult to be used as a clinical biomarker. In contrast, NT-pro-BNP is more stable with a longer half-life than C-BNP (Mueller et al., 2004). In human medicine, the NT-pro-BNP test has been used to differentiate between patients with respiratory distress cause from cardiac and non-cardiac causes (Alibay et al., 2005; Januzzi et al., 2005). Evaluation of serum NT-pro-BNP concentration is currently also recommended as a screening test for patients suspected of heart failure (Swedberg et al., 2005). Previous studies in dogs indicate the use of NT-proBNP assays in differentiating the presence of congestive heart failure from primary respiratory diseases (Boswood et al., 2008; Fine et al., 2008; Fox et al., 2015; Oyama et al., 2008). Recently, two new NT-pro-BNP tests from Bionote® and Dianotech® companies have been introduced for clinical use in dogs with heart disease. Both tests are fluorescent immunoassay which can quantitatively measure the NT-pro BNP concentrations in dogs within minutes, allowing clinicians to immediately decide the appropriate treatment. A serum sample of 100 μl is recommended for the Bionote® test. This test can detect NT-pro BNP levels ranging from 500-10,000 pmol/L with a storage temperature of 2-8 oC. For the Dianotech® test, the detection range is 150-6,000 pmol/L. Either serum or plasma sample is acceptable for this test. Moreover, its storage temperature is broader than the Bionote® test, with a temperature range of 4-30 °C.

This study aims to determine and evaluate the clinical utility of these two new NT-pro-BNP tests from the Bionote® and Dianotech® companies for differentiating dogs affected by MMVD between with and without CHF and between dogs with MMVD versus normal dogs. We hypothesized that these two new NT-pro-BNP assays could be used as diagnostic tests for identifying dogs affected by MMVD with CHF from those without CHF and dogs with MMVD from normal dogs.

Meterials and Methods

Thirty-seven dogs visited at Prasu-Arthorn Small Animal Teaching Hospital, Faculty of Veterinary Science, Mahidol University, were enrolled in the study after their owners have signed consent forms. The protocol used in this study was approved by Mahidol University-Institute Animal Care and Use Committee of the Faculty of Veterinary Science, number MUVS-2017-12-57. All enrolled dogs underwent complete physical examination, thoracic radiography, echocardiography, and electrocardiography. Diagnosis of mitral valve disease (MMVD) was made based on echocardiographic findings of thickened or prolapsed mitral leaflets with evidence of color-flow mitral regurgitation. American College of Veterinary Internal Medicine (ACVIM) staging system was used to classify the severity of dogs with MMVD. Stage B2 was defined by detection of echocardiographic evidence of cardiomegaly, including the left atrial to the aorta (LA: Ao) ratio >1.6 and the normalized left ventricular internal diameter in diastole >1.7, but without clinical signs of CHF. Stage C was defined by the presence of CHF signs with evidence of cardiac enlargement as mentioned above in stage B2 (Keene et al., 2019). Animals were divided into three groups: 1. Normal healthy dogs as a control group, 2. MMVD stage B2 group and 3. MMVD stage C group. Three milliliters (ml) of blood were obtained from each patient. Two ml of blood were placed in the EDTA tube and Heparinized tube to measure complete blood count (CBC) and routine biochemistry. Dogs with abnormalities of hematological and/or biochemical parameters were excluded from the study. The remaining 1 ml was placed in a plain tube, and serum was separated by centrifugation and was kept at -80^{o}C until analyses. Canine NT-proBNP immunoassay test (Bionote®) and canine NT-proBNP rapid detection kit (Dianotech®) were used to determine serum NT-proBNP concentrations.

Measurements of both assays were according to the product instrustions (https://www.bionote.com/vcheck-canine-nt-probnp, and https://irp-cdn.multiscreensite.com/20fad1a2/files/uploaded/Dianotech%20Fluorescence%20Quatitative%Analyer.pdf for products of the Bionote® and Dianotech respectively).

The attending laboratory was blinded to the clinical classification of the cases to avoid bias in the analysis.

Statistical analysis

Normal distribution was tested by the Shapiro Wilk test. Data were present as mean and standard deviation (SD) if data were normally distributed or median and interquartile range if data were not normally distributed. ANOVA or Kruskal-Wallis test was used to determine the difference of age, body weight and NT-proBNP concentration among the groups. The chi-square test was used for the proportion between females and males among the groups. Receiver Operating Characteristic (ROC) curve was plotted to identify the sensitivity, specificity and area under the curve (AUC) and suggested cut-off values of NT-proBNP concentrations. P-value <0.05 was considered significant.

Results

Thirty-seven dogs were included in this study, 32 dogs were analyzed for the canine NT-proBNP immunoassay test (Bionote®) and 28 dogs were analyzed for the canine NT-proBNP rapid detection kit (Dianotech®). Of the 32 dogs with Bionote® test, 11 were considered normal healthy, 11 were considered MMVD stage B2 and 10 were considered MMVD stage C. Six from 11 dogs of the normal healthy group were Chihuahuas. Three were Poodles, 1 was Pug and 1 was Miniature Pincher. Dogs with MMVD stage B2 consisted of 4 Chihuahuas, 2

Pomeranian and 1 of each following breed: Poodle, Yorkshire Terrier, Shih Tzu, Dachshund and mixed-breed dog. The MMVD stage C group included 3 Chihuahuas, 3 Shih Tzus, 3 mixed-breed dogs and 1 Poodles. Of the 28 dogs with Dianotech® test, 6 were classified in the normal healthy group, which consisted of 2 Chihuahuas, 2 Poodles, 1 Pug and 1 Miniature Pincher. Seven were classified in MMVD stage B2 group, which comprised 3 Chihuahuas and 1 of each following breed: Pomeranian, Yorkshire Terrier, Poodle and mixed-breeding. Fifteen were considered MMVD stage C, which included 5 Chihuahuas, 4 mixed-breeding, 3 Poodles, and 3 Shih Tzus. All dogs enrolled in stage C were in the chronic stage of CHF. Demographic data and NT-proBNP concentrations of the 3 groups measured by the canine NT-proBNP immunoassay test (Bionote®) and the canine NT-proBNP rapid detection kit (Dianotech®) were present in Table 1 and Table 2, respectively. Data were reported as a median and interquartile range because the data were not normally distributed. The median age, the median body weight and the proportion of females versus males were not significantly different among the groups. From the 32 samples of the Bionote® test, there were two outliers of body weight in the MMVD stage B2 group (14.2 and 13 kg) and one outlier in the MMVD C group. (16.4 kg). From the 28 samples of the Dianotech® test, there were one outlier of body weight in the MMVD stage B2 group (14.2 kg) and two outliers in the MMVD C group (16.4 and 16 kg). For both NT-proBNP tests, the median NT-proBNP concentration in dogs with MMVD stage C was significantly higher than the median NT-proBNP concentration in dogs with MMVD stage B2 and normal healthy dogs (p-value <0.001) (Table 1, 2 and Figure 1). The NT-proBNP levels measured by the Bionote® test in the normal dogs and dogs with MMVD stage B2 were equal at 500 pmoL/L because of the testís detection limit.

Table 1. Demographic data and NT-proBNP concentrations of 32 dogs measured by the canine NT-proBNP immunoassay test (Bionote®). Data was present as median and interquartile range.

Variables	Normal dogs (n=11)	MMVD stage B2 (n=11)	MMVD stage C (n=10)	p-vaule
Age (years)	7 (5-10.5)	11 (9.25-13)	10.05 (8.98-13.03)	0.065
Body weight (kg)	4.5 (3.7-7.2)	5.4 (4.5-8.2)	5 (4.28-7.08)	0.606
Female (percent)	7/11 (63.6)	2/11 (18.2)	6/10 (60%)	0.062
NT-proBNP (pmoL/L)	500 (500-762)	500 (500-4817.5)	2189.9* (646.7-5198.7)	<0.001

*Significantly different (p<0.01) from the other groups.

Table 2. Demographic data and NT-proBNP concentrations of 28 dogs measured by the canine NT-proBNP rapid detection kit (Dianotech®). Data was present as median and interquartile range.

Variables	Normal dogs (n=6)	MMVD stage B2 (n=7)	MMVD stage C (n=15)	p-vaule
Age (years)	7.25 (4.8-10.88)	11 (6-13)	10.1 (8.9-13)	0.232
Body weight (kg)	5 (3.6-7.63)	5.1 (4.5-6.1)	4.8 (4.3-7.5)	0.86
Female (percent)	4/6 (66.7%)	2/7 (28.6%)	7/15 (46.7%)	0.389
NT-proBNP (pmoL/L)	172 (150-445)	353 (266-2,295)	1,341* (471-6,785)	<0.001

*Significantly different (p<0.01) from the other groups.

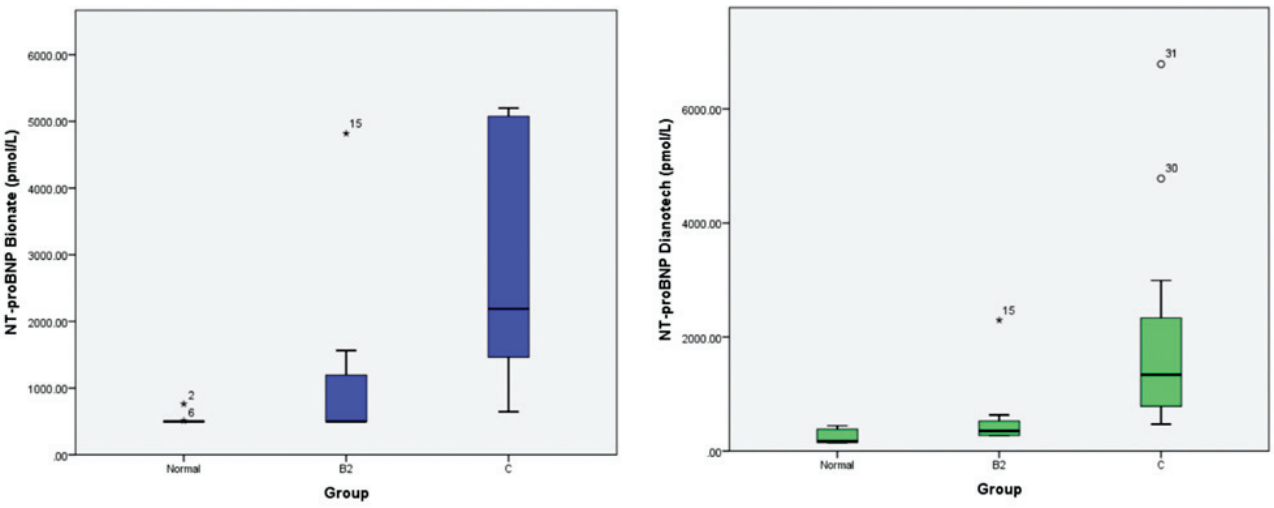


Figure 1. (A) A box plot demonstrates NT-proBNP concentrations measuring by canine NT-proBNP immunoassay test (Bionote®) (blue box) in 11 normal healthy dogs, 11 dogs with MMVD stage B2 and 10 dogs with MMVD stage C. (B) A box plot demonstrates NT-proBNP concentrations measuring by canine NT-proBNP rapid detection kit (Dianotech®) (green box) in 6 normal healthy dogs, 7 dogs with MMVD stage B2 and 15 dogs with MMVD stage C. The whiskers represent the range of values, the box represents the 25th and 75th percentiles and the line within the box represents the median of data. Outliers are shown by individual points.

ROC curve analysis showed that serum NT-proBNP concentration could differentiate dogs with CHF signs (MMVD stage C) from dogs without CHF signs (normal healthy dogs and MMVD stage B2). The AUC were 0.932 and 0.928 for NT-proBNP Bionote® and Dianotech® tests, respectively (Figure 2). Suggested cut-off values for differentiation of dogs with CHF signs from those without CHF signs were established in Table 2.

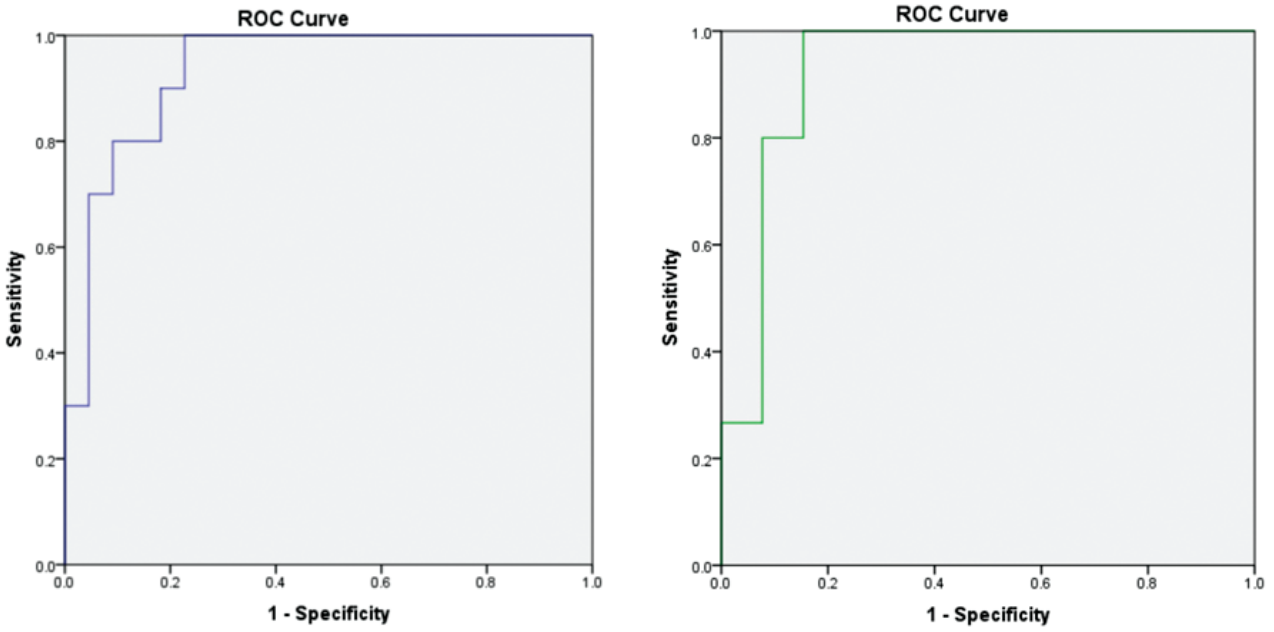


Figure 2. ROC curves discriminate dogs with CHF signs from dogs with no CHF signs. (A) AUC were 0.938 for NT-proBNP Bionote® test and (B) 0.923 for NT-proBNP Dianotech® test.

Table 3. Suggested cut-off values of NT-proBNP test with sensitivity and specificity for the differentiation of dogs with CHF and no CHF.

Assay	Suggested cut-off value	Sensitivity (%)	Specificity (%)
Canine NT-proBNP	633.7 pmol/L	100	77.3
immunoassay test	772 pmol/L	90	81.8
(Bionote®)	1,440 pmol/L	80	90.9
	1,646 pmol/L	70	95.5
Canine NT-proBNP rapid	458 pmol/L	100	84.6
detection kit (Dianotech®)	705 pmol/L	80	92.3
	2,378 pmol/L	26.7	100

ROC curve analysis also displayed that serum NT-proBNP concentration could discriminate dogs affected by MMVD with CHF (MMVD stage C2) from dogs affected MMVD without CHF (MMVD stage B2). The AUC were 0.818 and 0.867 for NT-proBNP Bionote® and Dianotech® tests, respectively (Figure 3). Suggested cut-off values for differentiation between these 2 stage of MMVD were established in Table 3.

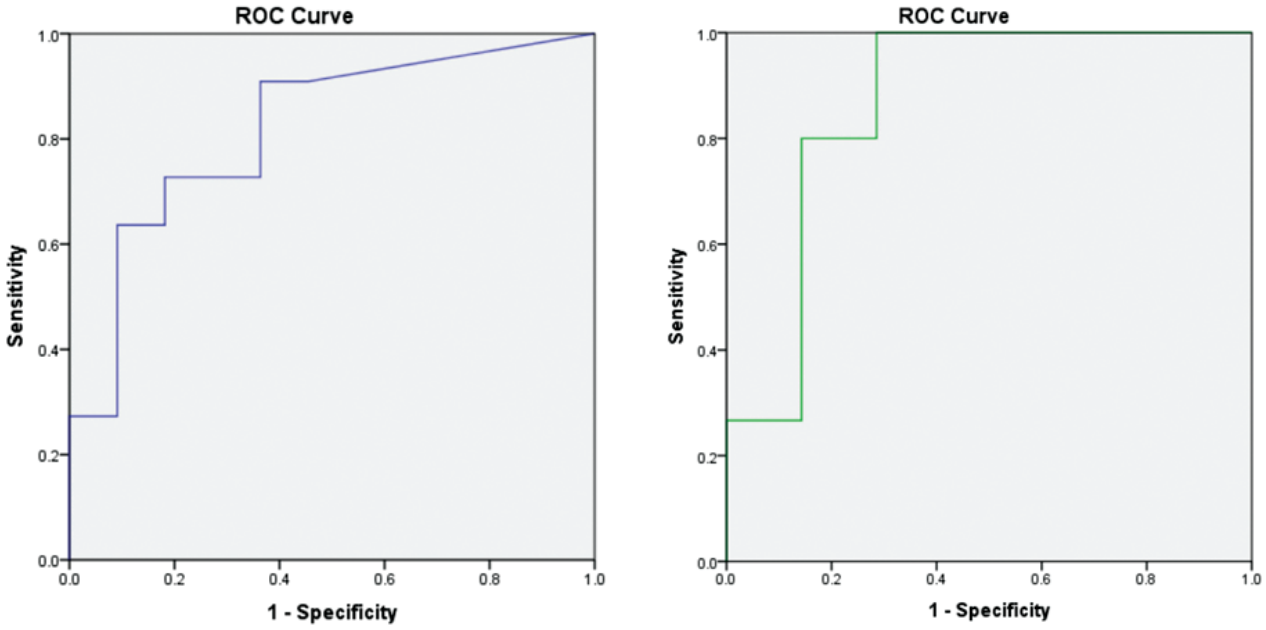


Figure 3. ROC curves discriminate dogs with MMVD stage C from dogs with MMVD stage B2. (A) AUC were 0.818 for NT-proBNP Bionote® test and (B) 0.867 for NT-proBNP Dianotech® test.

Table 4. Suggested cut-off values of NT-proBNP test with sensitivity and specificity for the differentiation of dogs with MMVD stage C and stage B2.

Assay	Suggested cut-off value	Sensitivity (%)	Specificity (%)
Canine NT-proBNP	633.7 pmol/L	90.9	63.6
immunoassay test	1,440.7 pmol/L	72.7	81.8
(Bionote®)	1,646.15 pmol/L	63.6	90.9
Canine NT-proBNP	444.5 pmol/L	100	71.4
detection kit (Dianotech®)	546 pmol/L	80	85.7
	2,250 pmol/L	26.7	100

Serum NT-proBNP concentration could also be used to discriminate dogs with MMVD (MMVD stage B2 and C) from normal healthy dogs (normal control). The AUC were 0.789 and 0.932 for NT-proBNP Bionote® and Dianotech® tests, respectively (Figure 4). Suggested cut-off value for differentiation of dogs with MMVD from those without MMVD signs was established in Table 4.

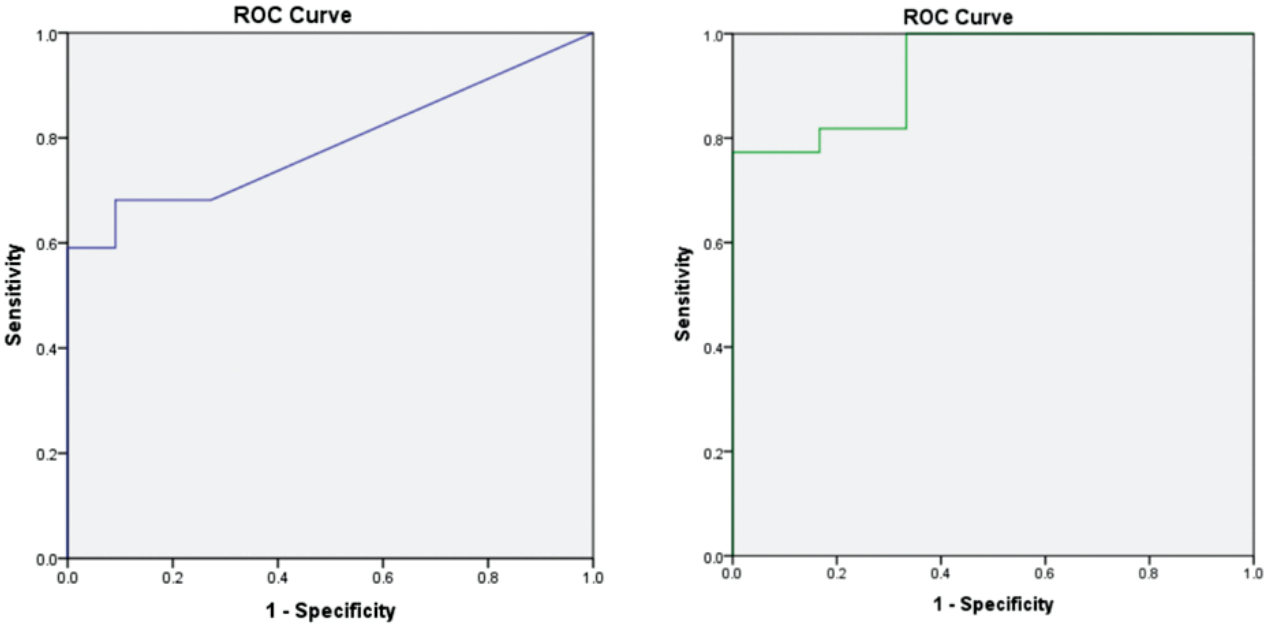


Figure 4. ROC curves discriminate dogs with MMVD stage B2 and C from normal healthy dogs. (A) AUC were 0.789 for NT-proBNP Bionote® test and (B) 0.932 for NT-proBNP Dianotech® test.

Table 5. Suggested cut-off value of NT-proBNP test with sensitivity and specificity for discriminating dogs with MMVD stage B2 and C from normal healthy dogs.

Assay	Suggested cut-off value	Sensitivity (%)	Specificity (%)
NT-proBNP V-check test (Bionote®)	565.8 pmol/L	68.2	90.9
Canine NT-proBNP detection kit (Dianotech®)	224.5 pmol/L	100	66.7
	400 pmol/L	81.8	83.3
	458 pmol/L	77.3	100

Discussion

The results of this study showed that serum NT-proBNP concentrations when measured by canine test from Bionote® and Dianotech® can discriminate between dogs with CHF signs from dogs without CHF signs, and between dogs with MMVD stage C from dogs with MMVD stage B2.

In the present study, serum NT-proBNP concentrations from both companies (Bionote® and Dianotech®) were significantly higher in dogs with CHF (MMVD stage C) than in dogs with evidence of MMVD and cardiomegaly but without CHF (MMVD stage B2) and normal healthy dogs. The ROC curve analysis suggested that serum NT-proBNP concentrations could be used to discriminate these groups. For the canine NT-proBNP immunoassay test (Bionote®), the authors recommended using the cut-off value of 772 pmol/L, which yielded a sensitivity of 90% and specificity of 81.2%. The high sensitivity (90%) indicated that most dogs with CHF have serum NT-proBNP concentrations higher than 772 pmol/L. Additionally, the high specificity (81.2%) indicated that most dogs with NT-proBNP concentrations higher than this cut-off would truly have CHF. Another suggestive cut-off value could be 633.7 pmol/L, which yielded a sensitivity of 100% and specificity of 77.3%. A 100% sensitivity indicated that all dogs with CHF have serum NT-proBNP concentrations higher than this cut-off value which meant dogs with serum NT-proBNP concentration lower than this cut-off value could truthfully rule out CHF. However, its specificity was lower than the first suggestive cut-off value. The moderate specificity (77.3%) indicated that some dogs with serum NT-proBNP concentrations higher than this value might falsely interpret for developing CHF, and other confirmative procedures such as thoracic radiography and echocardiography, are further suggested to identify CHF. For the canine NT-proBNP rapid detection kit (Dianotech®), only one suggested cut-off value of 458 pmol/L was recommended. This cut-off value had 100% sensitivity indicated that all dogs with serum NT-proBNP concentration lower than this cut-off can truly rule out CHF. It also had good specificity at 84.6%, indicating that only a small amount of dogs without CHF had serum NT-proBNP concentration higher than this level. Other suggested cut-off values were not recommended due to their inappropriate sensitivity and specificity.

In dogs with MMVD, ROC curve analysis revealed that serum NT-proBNP concentration could differentiate dogs with CHF (MMVD stage C2) from dogs without CHF (MMVD stage B2). For the canine NT-proBNP immunoassay test (Bionote®), the authors recommended using the cut-off value of 633.7 pmol/L, which yielded a sensitivity of 90.9% and specificity of

63.6%. The high sensitivity (90.9%) indicated that most dogs with MMVD stage C have serum NT-proBNP concentrations higher than 633.7 pmol/L. However, its specificity was too low (63.6%) to be used as a basis for clinical diagnosis. For the canine NT-proBNP rapid detection kit (Dianotech®), the authors recommended using the cut-off value of 444.5 pmol/L, which yielded a sensitivity of 100% and specificity of 71.4%. A 100% sensitivity indicated that all dogs with serum NT-proBNP concentration lower than this cut-off could truly rule out MMVD stage C. The moderate specificity at 71.4 % indicated that some dogs with MMVD stage B2 might have serum NT-proBNP concentration greater than this level. Echocardiography and thoracic radiography are further recommended to confirm MMVD stage C. Previous study from Oyama et al. (2008) has reported the high levels of NT-proBNP concentrations in dogs with heart diseases and its usefulness in distinguishing dogs affected by MMVD between with and without CHF. They reported that serum NT-proBNP was significantly higher in dogs with CHF than dogs with heart disease without CHF. The ROC curve with AUC 0.83 and a suggested cut-off value of serum NT-proBNP concentration >1,725 pmol/L demonstrated that serum NT-proBNP concentration could be used to discriminate dogs affected by MMVD with CHF from those without CHF with a sensitivity of 88.2% and specificity of 76.7% (Oyama et al., 2008). All suggestive cut-off values obtained from this study were lower than the value in the previous study. The difference of these cut-off values could be due to the difference of serum NT-proBNP tests (Canine Cardiocare NT-proBNP, Irvine, Calif vs. Canine NT-proBNP immunoassay test (Bionote®) and canine NT-proBNP rapid detection kit (Dianotech®)). The manufacturers of NT-proBNP tests from Bionote® and Dianotech® suggest that dogs with heart failure (MMVD stage C) will have serum NT-proBNP levels > 1,800 pmol/L and >800 pmol/L, respectively. These cut-offs are higher than the results in the present study. As a result, more research is needed to optimize the cut-off values of these two companies for clinical usage.

When using these 2 NT-proBNP tests to distinguish dogs affected by MMVD (MMVD stage B2 and C) from normal healthy dogs, the ROC curve analysis demonstrated several cut-off values to differentiate between these groups. For the canine NT-proBNP immunoassay test (Bionote®), the authors did not suggest any cut-off value due to its inappropriate sensitivity or specificity. At a cut-off value of 565.8 pmol/L, serum NT-proBNP level had a sensitivity of 68.2% and specificity of 90.9% for discriminating dogs with MMVD from normal dogs. The clinical utility of this cut-off value in this setting was limited by its low sensitivity. This indicated that some dogs with MMVD stage B2 and C might have serum NT-proBNP concentration lower than this cut-off (false negative). For the canine NT-proBNP rapid detection kit (Dianotech®), a suggested cut-off value of 224.5 pmol/L had 100% sensitivity indicated that all dogs with serum NT-proBNP concentration were lower than this cut-off could truly rule out MMVD stage B2 and C. However, the specificity was quite low (66.7%), indicating that several normal dogs would have serum NT-proBNP concentration higher than this cut-off (false positive). A suggested cut-off value of 458 pmol/L had 100% specificity indicated that all dogs with serum NT-proBNP concentration higher than this cut-off truly had MMVD stage B2 or C. The moderate sensitivity at 77.3 % indicated that some dogs with MMVD stage B2 or C might have serum NT-proBNP concentration lower than this level. These sensitivity and specificity were quite close to the previous

study by Oyama et al. (2008), which investigated serum NT-proBNP concentration in dogs with mitral valve disease (MMVD) and dilated cardiomyopathy (DCM) compared with healthy dogs. They reported that serum NT-proBNP was significantly higher in dogs with heart diseases than in normal control dogs. The ROC curve with AUC 0.92 and suggested cut-off value of serum NT-proBNP concentration > 445 pmol/L demonstrated that serum NT-proBNP concentration could be used to discriminate dogs with heart diseases (MMVD or DCM) from healthy normal dogs with a sensitivity of 83.2% and specificity of 90% (Oyama et al., 2008).

The suggestive cut-off values for differentiating dogs with CHF from those with no CHF of the Bionote® assay are generally higher than the Dianotech® assay. The sensitivity and specificity of the Dianotech® assay were slightly higher than the Bionote® assay. There are some advantages of the Dianotech® assay when compared to the Bionote® assay, including less sample volume for analysis (50 μL vs. 100 μL), less detection time (10 min vs. 15 min), broader storage temperature (4-30oc vs. 2-8oc). In addition, both serum and plasma samples can be used in the Dianotech® assay. This is contrast to the measurement of the Bionote® assay, which requires only a serum sample. However, significant differences do not exist between these two assays. According to this result, the authors recommended that these two assays can be used alternatively with different cut-off values as screening tests for identifying dogs affected by MMVD with CHF.

There were some limitations in this study. First, the relatively small sample size in each group might result in less significant diagnostic power for differentiation dogs between the groups. Another limitation was that the sample size of each group in Bionote® and Dianotech® NT-proBNP tests was not equal Lastly, the study was lack of positive and negative control to validate the tests. These limited a comparison of diagnostic accuracy in the differentiation of dogs with MMVD between these two tests. Despite these limitations of the sample size, these two tests remained satisfactory results in discriminating the CHF. This study was considered a preliminary study, and further investigations with more sample sizes in each group are expected to support our results.

In conclusion, the results of this study suggested that serum NT-proBNP concentrations from the new tests of both companies could be used to discriminate between dogs with CHF from those without CHF and normal healthy dogs with acceptable accuracy, but at different cut-off values. It has shown that the measurement of serum NT-proBNP concentrations by these tests would be helpful as an adjunctive clinical assessment for identifying dogs with CHF and evaluating the severity of MMVD in dogs.

Acknowledgements

The authors would like to thank all staff at the laboratory unit, Prasu-Arthorn Veterinary teaching Hospital, Faculty of Veterinary Science, Mahidol University, for sample storage and NT-proBNP measurement. We would like to thank BestAgro Companion Co, Ltd., and VetAnyMall Co, Ltd. for their support of the NT-proBNP tests in this study.

Conflict of Interest

This study was performed independently, with both BestAgro Companion Co, Ltd., and VetAnyMall Co, Ltd. having no influence over study design, data acquisition, analyses, results, manuscript preparation or scientific publication. None of the authors has any other financial or personal relationship that could inappropriate influence or bias the content of the manuscript.

References

Alibay Y, Beauchet A, El Mahmoud R, Schmitt C, Brun-Ney D, Benoit MO, et al. Plasma N-terminal pro-brain natriuretic peptide and brain natriuretic peptide in assessment of acute dyspnea. Biomed Pharmacother. 2005;59(1-2):20-4.

Borgarelli M, Crosara S, Lamb K, Savarino P, La Rosa G, Tarducci A, et al. Survival characteristics and prognostic variables of dogs with preclinical chronic degenerative mitral valve disease attributable to myxomatous degeneration. J Vet Intern Med. 2012; 26(1):69-75.

Boswood A, Dukes-McEwan J, Loureiro J, James RA, Martin M, Stafford-Johnson M, et al. The diagnostic accuracy of different natriuretic peptides in the investigation of canine cardiac disease. J Small Anim Pract. 2008;49(1):26-32.

Buchanan JW. Chronic valvular disease (endocardiosis) in dogs. Adv Vet Sci Comp Med. 1977; 21:75-106.

Fine DM, DeClue AE, Reinero CR. Evaluation of circulating amino terminal-pro-B-type natriuretic peptide concentration in dogs with respiratory distress attributable to congestive heart failure or primary pulmonary disease. J Am Vet Med Assoc. 2008; 232(11):1674-9.

Fox PR, Oyama MA, Hezzell MJ, Rush JE, Nguyenba TP, DeFrancesco TC, et al. Relationship of plasma N-terminal pro-brain natriuretic peptide concentrations to heart failure classification and cause of respiratory distress in dogs using a 2nd generation ELISA assay. J Vet Intern Med. 2015;29(1):171-9.

Hosoda K, Nakao K, Mukoyama M, Saito Y, Jougasaki M, Shirakami G, et al. Expression of brain natriuretic peptide gene in human heart. Production in the ventricle. Hypertension. 1991;17(6 Pt 2):1152-5.

Januzzi JL, Jr., Camargo CA, Anwaruddin S, Baggish AL, Chen AA, Krauser DG, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol. 2005;95(8):948-54.

Keene BW, Atkins CE, Bonagura JD, Fox PR, Haggstrom J, Fuentes VL, et al. ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. J Vet Intern Med. 2019;33(3):1127-40.

Mueller T, Gegenhuber A, Poelz W, Haltmayer M. Head-to-head comparison of the diagnostic utility of BNP and NT-proBNP in symptomatic and asymptomatic structural heart disease. Clin Chim Acta. 2004;341(1-2):41-8.

Oyama MA, Fox PR, Rush JE, Rozanski EA, Lesser M. Clinical utility of serum N-terminal pro-B-type natriuretic peptide concentration for identifying cardiac disease in dogs and assessing disease severity. J Am Vet Med Assoc. 2008;232(10): 1496-503.

Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. Eur Heart J. 2005;26(11): 1115-40.

Thomas CJ, Woods RL. Haemodynamic action of B-type natriuretic peptide substantially outlasts its plasma half-life in conscious dogs. Clin Exp Pharmacol Physiol. 2003;30(5-6):369-75.

Research Articles

Population Assessment of Crocodiles in Bueng Boraphet, Thailand

Parntep Ratanakorn[1] Tatiyanuch Chamsai[2] Poonyapat Sedwisai[2] Tat Sujittosakul[2] Thanaphum Lapjatuporn[2] Peerawat Wongluechai[2] Ekasit Tiyanun[3] Akalak Kunsorn[3] Somsook Puangdee[3] Thaweesak Chooma[3] Kadsirin Mattayasap[3] Phimchanok Srongmonkol[4] Jiradej Boonmak[4] Nareerat Sangkachai[2*]

[1]Faculty of Veterinary Medicine and Applied Zoology, Chulabhorn Royal Academy, 906 Kamphaeng Phet 6 Road, Talat Bang Khen, Lak Si, Bangkok 10210, Thailand

[2]Faculty of Veterinary Science, Mahidol University, 999 Phutthamonthon Sai 4 Road, Salaya, Phutthamonthon District, Nakhon Pathom 73170, Thailand

[3]Mahidol University Nakhonsawan Campus, 402 Khao Thong, Phayuha Khiri District, Nakhon Sawan 60130, Thailand

[4]Department of National Parks, Wildlife and Plant Conservation, 61 Pholyothin Road, Ladyao, Chatuchak, Bangkok 10900, Thailand

[*]Corresponding author, E-mail address: nareerat.san@mahidol.edu

Received: 15 November 2021; Revised: 8 January 2022; Accepted: 12 January 2022

Abstract

This study aimed to estimate the number and distribution of crocodiles in Bueng Boraphet, Nakhon Sawan Province, Thailand. The surveys were conducted between November 2017 and August 2018. The data was obtained from nighttime spotlight and daylight visual surveys using motorboat along two line transects totaling 38 kilometers and covered an area about 7.6 square kilometers of Bueng Boraphet coastal area. Our results revealed that the number of crocodiles was at least 17 individuals with density of 2.24 individuals per square kilometers. The calculated population of crocodiles in the Bueng Boraphet was estimated to be about 17-37 individuals. In this study, most crocodiles were found in the first transect line located in the aquatic sanctuary area where all fishery is strictly prohibited. It shows that human activities directly affect crocodile encounters and these small numbers of crocodiles in Bueng Boraphet remain critically endangered. Therefore, the implementation of protection action is a high-priority project that needs to be done first before further restocking can be undertaken. Bueng Boraphet can be developed into national natural crocodile conservation, while eco-tourism activities can be expanded in such area. Furthermore, population surveys should be carried out continuously to determine population dynamics and develop a crocodile surveillance system. At the same time community engagement should be initiated to support the system to sustainable conservation.

Keywords: Population, Crocodiles, Bueng Boraphet, Thailand

การประเมินประชากรจระเข้ในบึงบอระเพ็ด ประเทศไทย

ปานเทพ รัตนากร[1] ตติยนุช แช่มใส[2] ปุญญพัฒน์ เศษวิสัย[2] ธรรศ สุจิตโตสกุล[2] ธนภูมิ ลาภจตุพร[2]
พีรวัส วงษ์ลือชัย[2] เอกสิทธิ์ ติยานันต์[3] เอกลักษณ์ คันศร[3] สมสุข พวงดี[3] ทวีศักดิ์ ชูมา[3]
เกศศิรินทร์ มัธยทรัพย์[3] พิมพ์ชนก สรงมงคล[4] จิระเดช บุญมาก[4] นรีรัตน์ สังขะไชย[2*]

[1]คณะสัตวแพทยศาสตร์และสัตววิทยาประยุกต์ วิทยาลัยวิทยาศาสตร์การแพทย์เจ้าฟ้าจุฬาภรณ์ 906
ถ.กำแพงเพชร 6 แขวงตลาดบางเขน เขตหลักสี่ กรุงเทพมหานครฯ 10210
[2]คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล 999 ถ.พุทธมณฑลสาย 4 ต.ศาลายา อ.พุทธมณฑล จ.นครปฐม 73170
[3]โครงการจัดตั้งวิทยาเขตนครสวรรค์ มหาวิทยาลัยมหิดล 402 หมู่ 5 ต.เขาทอง อ.พยุหะคีรี จ.นครสวรรค์ 60130
[4]กรมอุทยานแห่งชาติสัตว์ป่าและพันธุ์พืช 61 ถ.พหลโยธิน แขวงลาดยาว เขตจตุจักร กรุงเทพมหานครฯ 10900

[*]ผู้รับผิดชอบบทความ E-mail address: nareerat.san@mahidol.edu

Received: 15 November 2021; Revised: 8 January 2022; Accepted: 12 January 2022

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อประเมินจำนวนและการกระจายของจระเข้ในบึงบอระเพ็ด จังหวัดนครสวรรค์ ประเทศไทย โดยทำการสำรวจระหว่างเดือนพฤศจิกายน พ.ศ. 2560 ถึงเดือนสิงหาคม พ.ศ. 2561 ด้วยวิธีส่องไฟในเวลากลางคืนและสำรวจในเวลากลางวันด้วยเรือยนต์ตามเส้นสำรวจ 2 เส้นทาง ระยะทางรวม 38 กิโลเมตร และครอบคลุมพื้นที่บริเวณชายฝั่งของบึงบอระเพ็ดประมาณ 7.6 ตารางกิโลเมตร ผลการสำรวจพบจระเข้ไม่น้อยกว่า 17 ตัว มีความหนาแน่นของจระเข้ไม่น้อยกว่า 2.24 ตัว/ตารางกิโลเมตร คำนวณจำนวนประชากรจระเข้ในบึงบอระเพ็ดได้ประมาณ 17-37 ตัว ในการศึกษาครั้งนี้พบว่าจระเข้ส่วนมากพบในเส้นสำรวจที่หนึ่งซึ่งอยู่ในเขตพื้นที่รักษาพันธุ์สัตว์น้ำที่ห้ามทำการประมงโดยเด็ดขาด แสดงให้เห็นว่ากิจกรรมของมนุษย์ส่งผลกระทบโดยตรงต่อการพบจระเข้และจำนวนจระเข้ที่เหลืออยู่ไม่มากในบึงบอระเพ็ดนี้ยังมีความเสี่ยงใกล้สูญพันธุ์อย่างยิ่ง ดังนั้นการป้องกันเชิงพื้นที่จึงมีความสำคัญที่ต้องดำเนินการเป็นอันดับแรกจึงจะสามารถฟื้นฟูประชากรจระเข้ต่อไปในอนาคตได้ บึงบอระเพ็ดสามารถพัฒนาสู่การเป็นพื้นที่อนุรักษ์จระเข้ธรรมชาติของประเทศและส่งเสริมกิจกรรมการท่องเที่ยวเชิงนิเวศ นอกจากนี้ควรดำเนินการสำรวจประชากรอย่างต่อเนื่องเพื่อศึกษาพลวัตของประชากรซึ่งจะนำไปสู่การพัฒนาระบบเฝ้าระวังจระเข้ร่วมกับการสร้างการมีส่วนร่วมของชุมชนเพื่อการอนุรักษ์อย่างยั่งยืนต่อไป

คำสำคัญ : ประชากร จระเข้ บึงบอระเพ็ด ประเทศไทย

Introduction

In Thailand, three species of crocodilians are historically abundant throughout the country consisting of the Siamese crocodile (*Crocodylus siamensis*), saltwater crocodile (*Crocodylus porosus*), and false gharial (*Tomistoma schlegelii*). However, the Siamese crocodile is the species normally found in natural freshwater ecosystems such as rivers, swamps, wetlands in lowland central and eastern Thailand, particularly in the Yom, Ping, Chao Phraya, and Pasak River in the past (Smith 1919; Taylor 1969). Recently, their wild population is dramatically depleting due to habitat destruction, illegal fishing, and crocodile poaching (Bezuijen et al., 2012). In addition, many people believe that crocodiles are dangerous animals that can harm humans, pets, and livestock (Aust et al., 2009; Gopi and Pandav 2009). The population of Siamese crocodiles remaining in the wild is scattered in different areas and each area has very small individuals. (Ratanakorn et al., 1994; Platt et al., 2002). Currently, several small remnant populations of wild Siamese crocodiles are found scattered in protected areas including Thung Salaeng Luang National Park, Pang Sida National Park, Kaeng Krachan National Park, Yod Dome Wildlife Sanctuary, Khao Yai National Park, Khao Ang Rue Nai Wildlife Sanctuary, and Bueng Boraphet Non-Hunting Area (Manolis 2017). Hence, there is a great risk of the extinction of Siamese crocodiles in the near future.

In the past, Bueng Boraphet was a natural swamp. Later, a weir was built to store water and become wetlands permanently. Therefore, Bueng Boraphet is the largest man-made freshwater reservoir located in central Thailand surrounded by local communities (Sriwongsitanon et al., 2007). This is an internationally important wetland proposed as the Ramsar site due to its high biodiversity (Office of Environmental Policy Planning 2002). Many rivers carry silts and nutrients flow into the swamp which makes this place suitable for the growth of various aquatic planktons, plants, and is also a proper to be habitat for numerous important wildlife, including aquatic species, birds, and the Siamese crocodile (Office of Environmental Policy Planning 2003; Chaichana and Choowaew 2013). There are records of crocodile sightings from the past to present but the precise status of crocodiles over time in Bueng Boraphet has never been scientifically evaluated. Moreover, people use the area for a variety of purposes, including utilizing natural resources for household consumption or as a source of income, water reservoirs for agriculture activities, and tourist attractions. The beautiful scenery of nature in Bueng Boraphet could be the model and develop sustainable conservation work and promote eco-tourism (Khundiloknattawasa 2019). Therefore, it is necessary to understand populations and habitats to obtain basic information of crocodiles in Bueng Boraphet because no population surveys have been conducted in the area before, and it is necessary for resources management.

The Siamese crocodile is now listed as a critically endangered species by the IUCN and Office of Natural Resources and Environmental Policy and Planning, Thailand. Since 1992, the IUCN/SSC Crocodile Specialist Group has established the Crocodile Action Plan and Siamese crocodile has been listed as the highest priority for action. The basic survey and identification of habitat and population should be done immediately to prevent extinction and to build a basis of specified management and conservation action (Ross 1998). Thailand has well-organized protected areas so implementation of protection of habitat and restocking of the population is the high priority action that should be done (Manolis and Stevenson 2019). However, there is still inadequate policy

and financial support, and a lack of proper studies of population, habitat, and factors affecting the survival which is necessary for habitat management planning in the country. Consequently, the crocodile conservation projects in Thailand are urgently needed. The umbrella project of crocodile conservation in Thailand consists of (1) studying the population of crocodiles in the natural habitat, (2) social studies to initiate a participatory conservation approach, and (3) restoring the population of crocodiles in protective areas. Thus, this study was designed to estimate the number and distribution of crocodiles in Bueng Boraphet which is a fundamental first step towards developing a sustainable management protocol. The data obtained from the surveys was used to assess factors that influence crocodile habitat use which will be used to determine the suitability of the area as a crocodile habitat and develop sustainable management plans for this vulnerable species.

Materials and Methods

Study area

This study was conducted in Bueng Boraphet located in Nakhon Sawan Province, central Thailand. The geographical location is between latitude of 15°40'N and 15°45'N and longitude of 100°10'E and 100°23'E. It is a semi-natural swamp covering an area of 212 square kilometers. The area is divided the area into 2 zones. Zone 1 is an aquatic sanctuary area where all fisheries are strictly prohibited, and zone 2 is an area where people are allowed to do the fishery by using permitted equipment only. Therefore, we designed two transect lines for the crocodile survey according to the mentioned zoning. The first line transect was 20 kilometers in zone 1 and the second line transect was 18 kilometers in zone 2 (Figure 1).

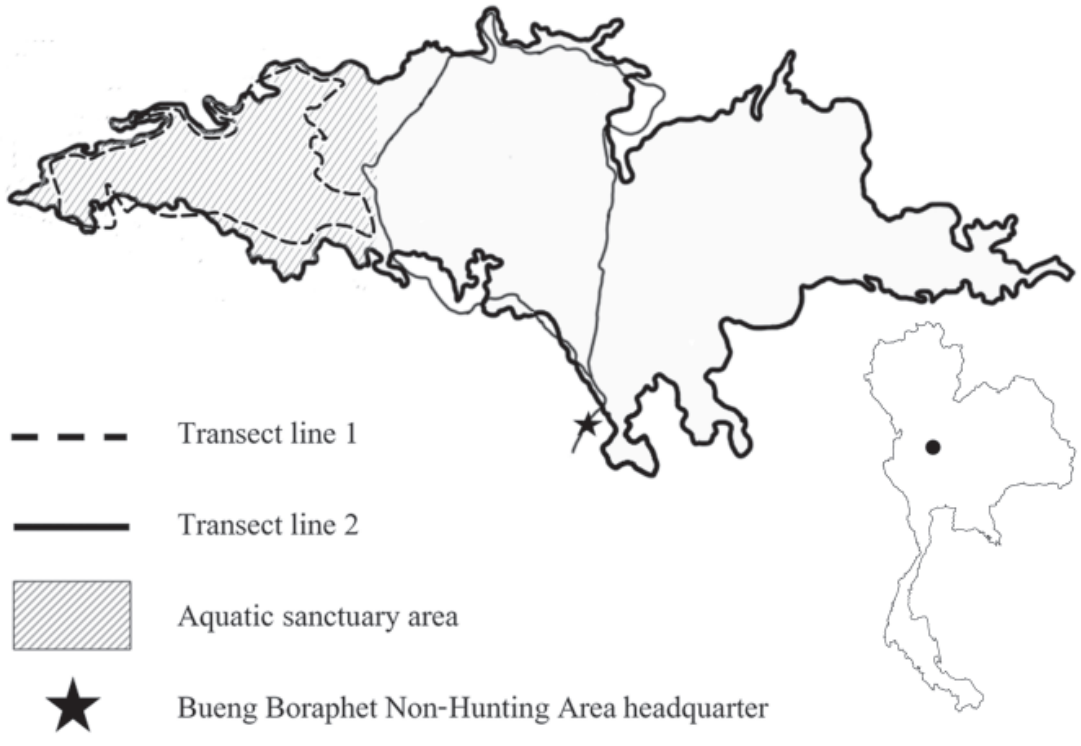


Figure 1. Location of the study area and transect lines for spotlight survey.

Survey Methods

The surveys were done between November 2017 and August 2018. We conducted 36 nocturnal spotlight surveys in the first transect line and 28 in the second transect line. Moreover, we did 33 daytime surveys in the first transect line and 27 in the second transect line.

A motorboat was used to explore the transect lines. On the night, spotlight surveys (Bayliss 1987; Fukuda et al., 2013) were used for collecting data. Crocodiles were counted along the study routes by observing "Eye-shine" because their eyes have tapetum lucidum that will reflect light when exposed to light from our light sources. A hand-held LED spotlight of at least 100W was used as the light source. During the survey, spotters will hold light sources in a horizontal plane at an angle of 180 degrees to the surface of the water, which makes it possible to observe eye-shine well. The average speed of the boat was about 23-26 km/h and this reduced upon each sighting to approach the crocodile as close as possible and estimate its size. A crocodile was recorded as a hatchery if their size was less than 1 meter, a juvenile if their size was between 1 to 1.8 meters, an adult if body length was more than 1.8 meters, unknown when it cannot be identified, and pod if we found a group of hatcheries. The geographic coordinates of each sighting spot were logged using GPS. We recorded time, air and water temperatures and the relative humidity for all surveys. Moreover, we conducted walking surveys on a day mainly in the location where the crocodile had been spotted during previous nocturnal spotlight surveys for observed footprints, tail marks.

Data analyses

The relative abundance was estimated from collected data and the population observed in the area during per transect during the study period was analyzed. The relative abundance was calculated as the number of crocodiles observed per kilometer (Bayliss 1987). The sighting area was calculated by total line transect distance multiplied by the estimated sighting distance from the observer and shore (200 meters). The encounter rate of the total survey was calculated. In addition, the sighting fraction can be calculated from the formula (King et al., 1990).

$$N = \frac{\bar{x}}{p} \pm \frac{[1.96(SD)]^{1/1}}{p}$$

where p is a percentage of the population observed, is the average number of crocodiles spotted, and SD is the standard deviation. Then, crocodile population size with 95 percent cent confidence limits was calculated from the following equation (Messel et al., 1981).

Results

From November 2017 to August 2018, the maximum air temperature was 31.5 Celsius and the minimum was 18.3 Celsius. Whereas, the maximum air temperature was 32.1 Celsius and the minimum was 19.2 Celsius. The water temperature was warmer than the air temperature. The average temperature difference was 1.6 Celsius. There was a 10.8 Celsius difference on a windy day. The maximum the relative humidity was 91.2 and the minimum was 60.5. A total of 45 crocodiles were observed at night time. One crocodile was found in the daytime (Figure 3). In 38 kilometers of survey distance, the encounter rate was 1.211 individuals per kilometer overall range. When comparing the coordinates where the crocodile was found, time, and characteristics of each individual from overall surveys, we estimated observed crocodiles at least 17 individuals in the overall transect lines which 14 crocodiles were spotted in the first transect line and three crocodiles were found in the

second transect line. In addition, two more crocodiles were found outside the study tracks (Figure 2). From all the observed data, they were classified to 4 hatchlings (body length less than 1 meter), 8 juveniles (body length 1 to 1.8 meters), 3 adults (body length over 1.8 meters), and 4 unknowns. Crocodiles are often found near shore. 99.39% of the habitat where crocodiles were found had floating wetland vegetation (e.g., water hyacinth, sedges, reeds, lotus, cattail), and 69.48% of the areas had dense grasses, shrubs, and trees at the coastline.

The relative abundance was 2.24 crocodiles per square kilometer. Furthermore, the calculated the population of crocodiles on the survey line is 7.6 square kilometers were 5.919±4.343 individuals and a density of 0.779±0.571 individuals per square kilometer. Bueng Boraphet covers a total area of 212 square kilometers. The calculated population of crocodiles in the Bueng Boraphet area was estimated to be about 5-37. Thus, it was concluded that the crocodile population in the study area was 17-37 when comparing the calculated population with observed coordinates.

Figure 2. Distribution of crocodiles from spotlight surveys.

Figure 3. Photographs of the crocodiles found during the surveys.

Discussion

According to historical records, crocodiles have been discovered all around Thailand. However, their population is dramatically declined even Thailand has more than forty important lowlands and wetlands. There are a few places where the Siamese crocodiles can survive in their natural environment. Bueng Boraphet is one of the sites where still have reports of crocodiles inhabited since the past to present (Taylor 1969; Smith 1919; Ratanakorn et al., 1994; Platt et al., 2002). Formerly, up to a hundred individuals were most found in Bueng Boraphet. However, there was a recent report found 8 adult Siamese crocodiles during the spotlight survey (Manolis 2017). We found a similar trend from our results which indicated that the population of crocodiles in Bueng Boraphet is 17-37 individuals with at least three adults and three unknowns. However, we could not confirm their population structure because the crocodiles were attentive to changing stimulus. Most crocodiles dove into the water or hid behind floating plants before the observer could estimate body length. In our study, most crocodiles were found in the first transect line located in the aquatic sanctuary area where strict regulations are implemented to protect fish breeding sites. It shows that human activities directly affect crocodile encounters. Fishing regulations in the protected zone can prevent the killing and unintentional catching of crocodiles in fishing nets (van Weerd and van der Ploeg 2003). Crocodiles will have a low incidence of being harmed by fishing gear. Furthermore, this protected zone has higher biological resources such as macrophytes and fish compared with a fishing zone (Chaichana and Choowaew 2013). We mostly

found crocodiles in dense wetland vegetation environment. Similarly, Crocodylus siamensis preferably abundant in still water in the Sre Ambel River where their habitat is dominated by sedges, extensive mats of water hyacinth, floating grasses, and scattered tree cover along the river. The vegetation is important for a hiding place and material for nesting (Platt et al., 2006).

There are some limitations of this study. Although, spotlight surveys are the most common method of crocodile research because crocodiles are difficult to see especially during the daytime but they are easily visible at eye-shine when the light is reflected. The obtained data can provide precise indices of abundance. However, there is visibility bias that can lead to inaccurate observations associated with various factors including animal behavior, degree of wariness, vegetation covering, and weather (Bayliss 1987; Fukuda et al., 2013). Since crocodiles can hardly be seen directly. Therefore, it is necessary to assess the population using obtained data and calculated using the sighting fraction (Messel et al. 1981; King et al., 1990). In Bueng Boraphet, there are many canals with high sinuosity or densely covered with vegetation which can be visibility bias of this study. On rainy days, the reflections from the crocodile's eyes are obscured by reflections from water droplets or dew. Hence, our study started after the rainy season to minimize that error and to avoid an accident that might occur from strong winds during traveling by boat in a large open water area. The water depth of the swamp seasonally changes because it served as a flood control area in the rainy season and water resource in the dry season for local communities to rely on (Haq et al., 2018). There were slight changes in the survey route due to lower water levels affecting areas accessible by boat. Therefore, there are challenges in designing the survey route to cover the entire area. Interestingly, the most common area where crocodiles are found is in a small canal called Khlong Boraphet which has Chao Mae Mon Thong Shrine. This shrine has a myth related to crocodiles that are revered by the local people. Therefore, the belief of the villagers may be another influencing factor that helps the crocodile survive. This requires further social studies to understand the social context of wildlife conservation.

Currently, Bueng Boraphet is the largest freshwater wetland on the Chao Phraya River Basin. Around this area, there are 31 villages, with approximately 5,000 households. Bueng Boraphet provides significant values in various aspects. It has high socio-economic and ecological values. It is used as a water reservoir for domestic and agricultural water supply and flood control. Moreover, it is also an important fishery site for local communities to harvest natural resources for food and income. It is facing threats majority from human activities. Large numbers of aquatic plants, phytoplankton, zooplankton, fish, and birds were reported in the swamp. Even Bueng Boraphet has ecologically important for a wide variety of flora and fauna but dramatic changes in hydrology and landscapes over the past decade have had a great impact on wildlife (Haq et al., 2018). Especially for crocodiles, if an area was established to conserve waterbirds may not be suitable for large reptiles such as crocodiles that need appropriate and careful management of suitable wetland habitat (Webb and Jenkins 1991). The sediment dredging and environmental modification for water storage to solve flooding problems are also significant threats that affect the suitability of crocodile habitat and nesting site in the future. Although fishing is prohibited, illegal fishery still exists. Destructive fishing methods such as electro-fishing and anesthetic chemical substances are still found, even

though the penalties are high. The use of illegal fishing gear will also directly affect the crocodile population (van der Ploeg and van Weerd 2006).

Crocodilians play an important role in the structure and function of the ecosystem. These large-bodied predators are keystone species that contribute to nutrient and energy translocation across ecosystems (Ashton 2010). Therefore, they have the potential to be ecological indicators considering their ecological values (Somaweera et al., 2020). Conservation of crocodiles is the great benefits for ecosystem. This estimate of the population and distribution of crocodiles in Bueng Boraphet is very important that gives the basic information for planning crocodile population management. Our results indicate that these small numbers of crocodiles in Bueng Boraphet remain critically endangered. However, the area with highly strict regulations has a huge impact on helping crocodiles survive in the wild. Therefore, the implementation of protection action is a high-priority project that needs to be done first before further restocking can be undertaken. The crocodile population can recover if their habitats were intact (Fukuda et al., 2011).

Bueng Boraphet can be developed as a national natural crocodile conservation site to conserve the genetic diversity of Siamese crocodiles because it has enough area and ecological integrity. The advantage of being a Ramsar Site will have a positive effect on the conservation of crocodiles in the area. Moreover, the conservation of crocodiles will have a positive effect on the development of eco-tourism site because it can attract wildlife enthusiasts, researchers, and zoologists. Eco-tourism focuses on protecting the resource base and it is also used as a tourism tool that can strengthen conservation with local community participation. They can have additional income from tourism activities (Ryan and Harvey 2000; Rafsanjani and Karami 2011; van der Ploeg et al., 2011). Bueng Boraphet has potential to be a model of sustainable co-habitation of crocodiles and local people. This place has a natural potential and beautiful nature. It is located in the central region. Travelers can visit without having to travel too far. It does not cost too much and no additional equipment is required. The boat ride can accommodate tourists of various ages in a diverse society. This place is already famous for tourist destination. There are a variety of activities suitable for various people who want to learn about nature or the local community lifestyle. At present, tourists visit this scenic place for the purpose of surfing nature because they can take a boat trip to bird watching, see beautiful lotus fields and enjoy traditional food. Therefore, further population surveys should be carried out continuously to determine population dynamics and develop a crocodile surveillance system together with built community engagement for sustainable conservation.

Acknowledgements

This work was successfully accomplished because of the support of many individuals and organizations. This study was supported by grants from the Agricultural Research Development Agency (Public Organization). We are grateful to Yossapong Temsiripong for providing expertise that greatly assisted the work. We are thankful to Crocodile Cooperative of Thailand, Thailand for supports. We are also thankful to staffs from Bueng Boraphet Non-Hunting Area (Department of National Park, Wildlife, and Plant Conservation) and staffs from Bueng Boraphet Inland Fisheries Control Unit (Nakhon Sawan) (Department of Fishery) for field assistance. Nevertheless, we would like to thank Nakhon Sawan Provincial Administrative Organization for supports. Therefore, we would like to express our sincere gratitude to all of them.

References

Aust P, Boyle B, Fergusson R, Coulson T. The impact of Nile crocodiles on rural livelihoods in northeast Namibia. South African. J wildl Res. 2009;39:57-69.

Ashton PJ. The demise of the Nile Crocodile (*Crocodylus niloticus*) as a keystone species for aquatic ecosystem conservation in South Africa: The case of the Olifants River. Aquat Conserv Mar Freshw Ecosyst. 2010;20(5):489-93.

Bayliss P. Survey methods and monitoring within crocodile management programmes. In: Webb GJ, Manolis SC, Whitehead PJ, editors. Wildlife Management: Crocodiles and Alligators. Sydney: Surrey Beatty and Sons. 1987. p. 157-75.

Bezuijen M, Simpson B, Behler N, Daltry J, Tempsiripong Y. Crocodylus siamensis. [Internet]. The IUCN Red List of Threatened Species 2012. 2012 [cited 2021 September 27]. Available from: https://dx.doi.org/10.2305/IUCN.UK.2012.RLTS.T5671A3048087.en.

Chaichana R, Choowaew S. Ecological importance and biological resource conservation of Boraphet marsh, Thailand. Sci Asia. 2013;39(2):105-10.

Fukuda Y, Saalfeld W, Webb G, Manolis C, Risk R. Standardised method of spotlight surveys for crocodiles in the tidal rivers of the Northern Territory, Australia. North Territ Nat. 2013; 24: 14-32.

Fukuda, Y, Webb G, Manolis C, Delaney R, Letnic M, Lindner G, et al. Recovery of saltwater crocodiles following unregulated hunting in tidal rivers of the Northern Territory, Australia. J Wildl Manag. 2011;75:1253-66.

Gopi GV, Pandav B. Humans sharing space with Crocodylus porosus in Bhitarkanika Wildlife Sanctuary: conflicts and options. Curr Sci. 2009;96:459-60.

Haq RU, Eiam-Ampai K, Ngoprasert D, Sasaki N, Shrestha RP. Changing landscapes and declining populations of resident waterbirds: A 12-year study in bung Boraphet Wetland, Thailand. Trop Conserv Sci. 2018;11:1-17.

Khundiloknattawasa, S. Community participation in development of ecotourism Bung boraped, Nakhon Sawan Province. Humanities and Social Sciences Journal of Pibulsongkram Rajabhat University. 2019;14(1):194-211.

King FW, Espinal M, Cerrato LCA. Distribution and status of the crocodilians of Honduras. Proceedings of the 10th Working Meeting of the Crocodile Specialist Group, IUCN-The World Conservation Union, Gland, Switzerland; 1990. p. 313-54.

Platt SG, Lynam AJ, Temsiripong Y, Kampanakngarn M. Occurrence of the Siamese crocodile (Crocodylus siamensis) in Kaeng Krachan National Park, Thailand. Nat Hist Bull Siam Soc. 2002;50:7-14.

Platt SG, Sovannara H, Kheng L, Stuart BL, Walston J. Crocodylus siamensis along the Sre Ambel River, southern Cambodia: habitat, nesting, and conservation. Herpetol Nat Hist. 2006;9(2):183-8.

Manolis, C. "2nd Siamese Crocodile Meeting on Husbandry and Conservation" and "Siamese Crocodile Task Force Meeting" (1-2 June 2017). Crocodile Specialist Group Newsletter. 2017;36(2):5-9.

Manolis C, Stevenson C. Crocodiles. Status Survey and Conservation Action Plan. Darwin: Crocodile Specialist Group; 2019.

Messel H, Vorlicek GC, Wells AG, Green WJ. Surveys of tidal river systems in the Northern Territory of Australia and their crocodile populations. Sydney: Monograph, Pergamon Press; 1981.

Office of Environmental Policy Planning. An inventory of wetlands of international and national importance in Thailand. Bangkok: Ministry of Science, Technology, and Environment. 2002.

Office of Environmental Policy Planning. Biodiversity in Bueng Boraphet. Bangkok: Ministry of Science, Technology, and Environment. 2003.

Rafsanjani AK, Karami M. Eco-tourism necessity to preserve and maintain endangered species: A study of mugger crocodile. J Geogr Reg Plann. 2011;4(14):708-14.

Ratanakorn P, Amget B, Ottley B. Preliminary Surveys of crocodiles in Thailand. Proceedings of the 12th Working Meeting of the Crocodile Specialist Group. Gland, Switzerland: IUCN- The World Conservation Union, Thailand; 1994. p. 35-49.

Ross JP. Crocodiles. Status Survey and Conservation Action Plan. 2nd ed. IUCN/SSC Crocodile Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK; 1998.

Ryan C, Harvey K. Who likes saltwater crocodiles? Analysing socio-demographics of those viewing tourist wildlife attractions based on saltwater crocodiles. J Sustain Tour. 2000; 8(5):426-33.

Somaweera R, Nifong J, Rosenblatt A, Brien ML, Combrink X, Elsey RM, et al. The ecological importance of crocodylians: towards evidence-based justification for their conservation. Biol Rev. 2020;95(4):936-59.

Smith MA. Crocodylus siamensis. J Nat Hist Soc Siam. 1919;3:217-22.

Sriwongsitanon, N, Surakit K, Hawhins PR, Chandrasena N. Decision support tools for water resource management: a case study of Bung Boraphet wetland, Thailand. J Dev Sustainability Agric. 2007;2:17-26.

Taylor EH. The turtles and crocodiles of Thailand and adjacent waters. Univ Kansas Sci Bull. 1969;49(3):87-179.

Van der Ploeg J, Van Weerd M. A paradigm shift in Philippine crocodile conservation. Proceedings of the 18th working meeting of the Crocodile Specialist Group. IUCN-the World Conservation Union, Gland, Switzerland and Cambridge, UK; 2006. p. 164-86.

Van der Ploeg J, Cauillan-Cureg M, van Weerd M, Persoon G. 'Why must we protect crocodiles?' Explaining the value of the Philippine crocodile to rural communities. J Integr Environ Sci. 2011;8:1-12

Van Weerd M, Van der Ploeg J. A new future for the Philippine crocodile Crocodylus mindorensis. Sylvatrop. 2003;13(1&2):31-50.

Webb G, Jenkins R. Management of Crocodilians in Thailand: A Review with Recommendations. Canberra: Australian National Parks and Wildlife Service; 1991.

Case Report

Case Report: Long-Term Management of Imidacloprid-Moxidectin in a Dog with Caval Syndrome

Panyakamol Chandrasakha Rungrote Osathanon Namphung Suemanotham*

Faculty of Veterinary Science, Mahidol University, Phuttamonthon 4 Rd., Salaya, Nakhon Pathom 73170, Thailand

***Corresponding author, E-mail address: namphung.sue@mahidol.edu**

Received: 14 July 2021; Revised: 11 September 2021; Accepted: 15 September 2021

Abstract

Caval syndrome is a condition from heavy heartworm infestations, causing intracardiac obstruction, potentiating in multiple fatal complications, and poor prognosis. Early diagnosis is imperative for prompt treatment and echocardiography has the benefit of aiding in exploring the cardiac structure and the worm burden. According to The American Heartworm Society guideline (2020), it has established several treatment regimens both medical and surgical. The purpose of this case report is to determine the clinical efficacy of long-term macrocyclic lactone in the treatment of heartworm in a dog with caval syndrome. An 8-year-old female French bulldog was presented with ascites. Numerous heartworms were detected intracardiac from an echocardiography. A monthly application of 10% imidacloprid and 2.5% moxidectin with 4 weeks of 10 mg/kg doxycycline had shown the efficacy in controlling heartworms of a dog with caval syndrome thus far, along with an exercise restriction to prevent complication from pulmonary thromboembolism. Reassessment of this patient was planned for a monthly health screening and a 3-month echocardiography for worms migration, thrombi, and antigen testing. After 3 months, the worms were unable to detect from echocardiography and the dog was no longer required abdominocentesis. The dog is currently living well and planned on giving a regular heartworm prevention program after 12 months.

Keywords: caval syndrome, canine heartworm, long term macrocyclic lactone

รายงานสัตว์ป่วย: การรักษาพยาธิหนอนหัวใจระยะที่ 4 (Caval Syndrome) ในสุนัขด้วย อิมิดาโคลพริด ม็อกซี่เด็กติน แบบระยะยาว

ปัญญกมล จันทรสาขา รุ่งโรจน์ โอสถานนท์ น้ำผึ้ง สื่อมโนธรรม*

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล พุทธมณฑลสาย 4 ศาลายา นครปฐม 73170

***ผู้รับผิดชอบบทความ E-mail address: namphung.sue@mahidol.edu**

Received: 14 July 2021; Revised: 11 September 2021; Accepted: 15 September 2021

บทคัดย่อ

การติดพยาธิหนอนหัวใจระยะที่ 4 (Caval syndrome) เกิดจากการติดเชื้อหนอนพยาธิในปริมาณมากจนเกิดการอุดตันในหัวใจ ส่งผลให้เกิดภาวะแทรกซ้อนที่หลากหลายซึ่งเป็นอันตรายต่อชีวิต และการพยากรณ์โรคไม่ดี ความรวดเร็วในการวินิจฉัยเป็นสิ่งจำเป็นเพื่อทำการรักษาได้อย่างทันท่วงที การตรวจหัวใจด้วยคลื่นเสียงความถี่สูงถือเป็นเครื่องมือที่ช่วยให้เห็นโครงสร้างของหัวใจและตัวหนอนพยาธิที่อยู่ในหัวใจ โดยในปี ค.ศ. 2020 American Heartworm Society ได้ออกแนวปฏิบัติในการรักษาทั้งการรักษาทางยาและผ่าตัด รายงานสัตว์ป่วยนี้มีวัตถุประสงค์เพื่ออภิปรายผลการรักษาพยาธิหนอนหัวใจระยะที่ 4 ด้วยอิมิดาโคลพริด ม็อกซี่เด็กติน แบบระยะยาว สุนัขพันธุ์เฟรนช์บูลด็อก เพศเมีย อายุ 8 ปี มาด้วยปัญหามีน้ำคั่งในช่องท้อง เมื่อตรวจด้วยคลื่นเสียงความถี่สูงพบหนอนพยาธิหัวใจจำนวนมากในหัวใจ รักษาโดยให้ยา 10% อิมิดาโคลพริด และ 2.5% ม็อกซี่เด็กติน ร่วมกับด็อกซีไซคลิน 10 มก./กก. เป็นเวลา 4 สัปดาห์ ผลการรักษาพบว่ายาดังกล่าวมีประสิทธิภาพในการควบคุมหนอนพยาธิในสุนัขป่วยด้วยโรคพยาธิหนอนหัวใจระยะที่ 4 มีการจำกัดกิจกรรมของสุนัขร่วมด้วยเพื่อป้องกันภาวะลิ่มเลือดอุดกั้นในปอด ทำการการติดตามผลการรักษาทุกเดือนและตรวจด้วยคลื่นเสียงความถี่สูงเมื่อครบ 3 เดือน เพื่อประเมินการเคลื่อนย้ายของหนอนพยาธิ ลิ่มเลือด และตรวจหาแอนติเจนต่อพยาธิ โดยไม่พบหนอนพยาธิเมื่อผ่านไป 3 เดือน จากการตรวจด้วยคลื่นเสียงความถี่สูง และไม่ต้องเจาะระบายน้ำในช่องท้องสุนัข สุนัขยังคงมีชีวิตและวางแผนป้องกันพยาธิหนอนหัวใจต่อไปหลังรักษาครบ 12 เดือน

คำสำคัญ : Caval syndrome พยาธิหนอนหัวใจ แม็คโครไซคลิกแลคโตนแบบระยะยาว

Introduction

Caval syndrome is known as dirofilarial hemoglobinuria (AHS 2020). The condition develops in dogs which severely infested with heartworms. The presence of worms in right atrium, ventricle, pulmonary artery, and often vena cava causes blood flow obstruction (Jones 2016). The dogs constantly present signs of acute anorexia, respiratory distress, weakness, right-sided cardiac murmur, anemia, hemoglobinuria, hepatic and renal dysfunction, signs of forward and backward heart failure, and possibly, disseminated intravascular coagulation (DIC) (Strickland 1998).

Dirofilaria spp., a juvenile form called microfilaria, is the main cause of heartworm disease. It could be encountered through a modified Knott or a filtration test (Kamyingkird et al., 2017). Another screening test using antigen detection can be done through a rapid test kit. Nevertheless, it takes up to 6 months post-infection, as well as the detection of microfilariae, to be able to reveal the antigen from the active adult female (Atkins 2003; Taylor et al., 2016). Thus, the acceptable gold standard for heartworm testing is the presence of adult worms in the pulmonary arteries and/or heart on necropsy examination. The visualization of heartworms within the tricuspid orifice and posterior vena cava from echocardiography can be performed (Courteney and Zeng 2001; Atkins 2003). Considering necropsy is the terminal decision and gives no benefit in the individual treatment. Early diagnosis can help to reduce the inevitable dilemma and favoring the treatment plan.

The treatment goal of heartworm disease is to eliminate all stages of this nematode and to improve the quality of life of the patient (AHS 2020). Multimodal treatment and slow-killing of the heartworm have been proposed after fatal complications from mechanical removing or using an adulticide of melarsomine dihydrochloride. The adult worms are less susceptible and taking longer to die. However, within 2 years of treatment, it is expected that 95% of the worms should be killed (Bowman 2012). The anecdotal reports a supplementation of doxycycline has been widely used to eliminate intracellular bacteria and it works against gram negative bacteria called Wolbachia spp. in all stages of the worms (Bazzocchi et al., 2008). Bacteria are living with the worm as symbiosis microorganisms and are essential to the development of larvae into adult worms (McCall et al., 2008). By reducing the bacteria, it is subsequently reducing the number of adult worms as well as microfilaria (Frank and Heald 2010). Several case reports have been attempted to find a safe approach for physical removal of heartworms. In Yoon et al., the study had demonstrated success in using a nitinol basket with catheter-guided to remove the worm (Yoon et al., 2010). When killing heartworm by using any medicine, it should be taken with precautions from wormís debris inducing thrombi. As from Virchowís triad, it has been explained that such case as heartworm is predisposed to thrombi formation, Hypercoagulable state, vascular stasis, and damage to the vascular endothelium, which often encountered in dirofilariasis (Yun et al., 2010). The thrombi prevention was performed with clopidogrel (Sanofi-Aventis, France) 2-4 mg/kg, q24h.

Accordingly, the case report aimed to use a long-term macrocyclic lactone as the alternative method, with adjunctive therapy for heart failure and inflammation from the wormís antigen in dogs with the severe manifestation of heartworms and expressed the signs of caval syndrome.

Figure 1. The dog had abdominal distention and was difficult to stand on the first visit. General body condition was poor and given 2/9 on her body condition score

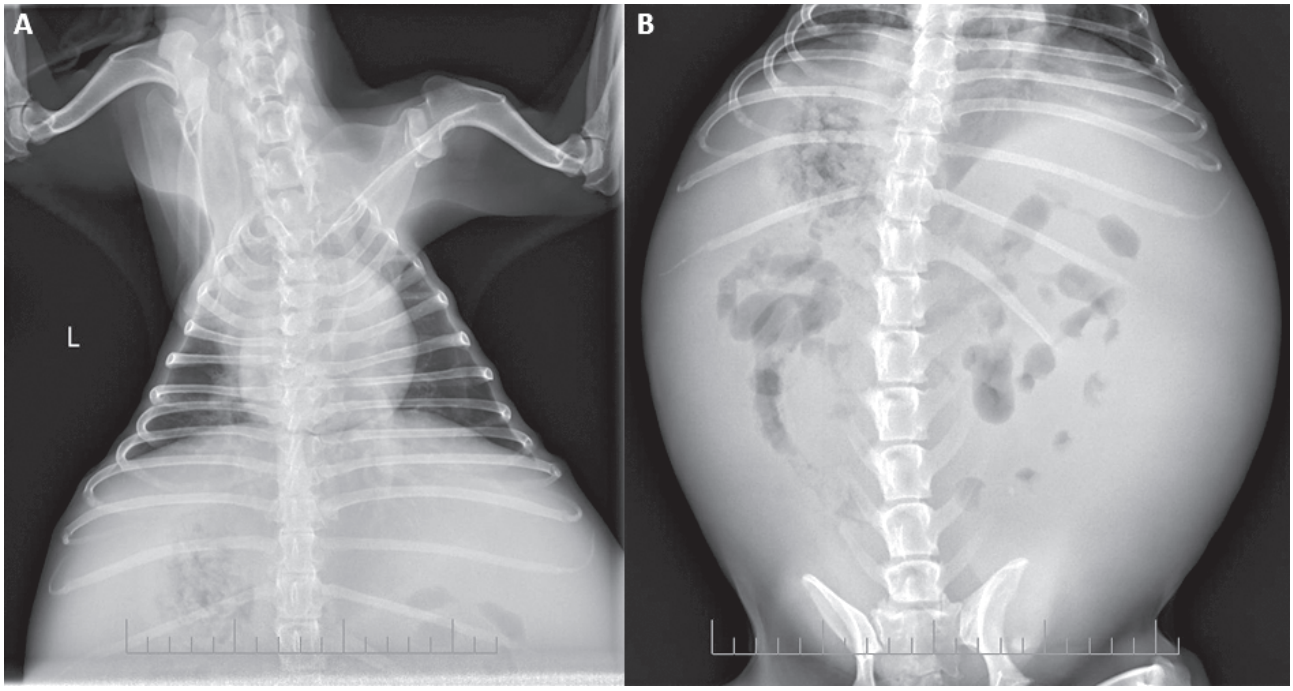


Figure 2. Radiography of thoracic and abdominal cavity ventrodorsal view. Inverted D-shape heart with pulmonary knob was seen with tortuous pulmonary artery and pulmonary artery enlargement (A). Ground-glass soft tissue opacity in the abdominal cavity was marked (B).

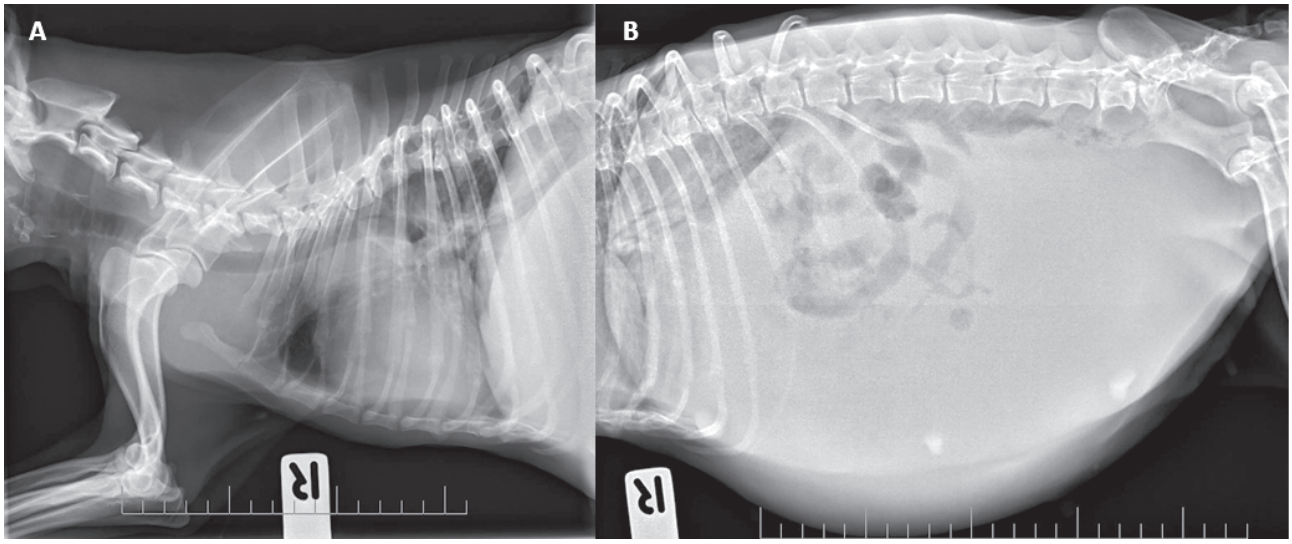


Figure 3. Radiography of thoracic and abdominal cavity lateral view. Sternal contact of the heart, elevated trachea, thickening vena cava (comparing to the 9th rib), vascular pattern of lung and unstructured interstitial pattern at perivascular region, especially at large pulmonary artery of caudodorsal lobe in thoracic cavity(A). Liver enlargement and ground glass soft tissue opacity in the abdominal cavity (B).

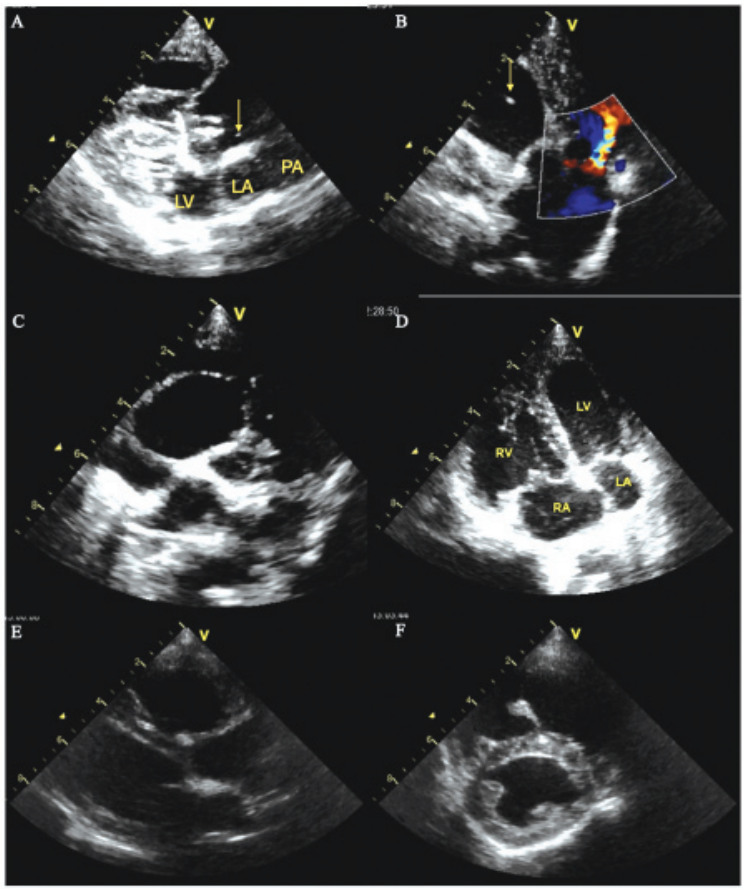


Figure 4. Echocardiogram images of the heart from right parasternal long-axis and short-axis with intracardiac heartworm, appearing as parallel hyperechoic line (A and B). Severe right atrial enlargement can be observed from right parasternal short axis (B and C). Marked thickening of tricuspid valve can be seen from left apical four chamber view (D and F). No worm detected after 3-6 months of treatment (E and F).

Figure 5. A serosanguineous fluid acquired from an abdominocentesis

Table 1. Hematology report (absolute) from the first visit.

Parameters	First visit	Normal value
WBC (/ul)	12,510	6,000 - 17,000
Monocyte	500.4	150 - 1,350
Neutrophil	10,633.5	3,000 - 11,500
Band	0	0 - 300
Lymphocyte	1251	1,000 - 4,800
Eosinophil	125.1	100 - 1,250
Basophil	0	< 100
RBC (10^6/ul)	5.41	5.0 - 9.0
Hb (g/dl)	12.2	10.0 - 18.0
Hct (%)	35.4	35 - 55
MCV (fL)	65.5	60 - 77
MCH (pg)	22.6	20 - 25
MCHC (g/dl)	34.4	32.0 - 36.0
PLT (10^3/ul)	317	200 - 500
Platelet smear	Adequate	
Plasma Protein	6.0	6.0-7.5
ALT (U/L)	42	10 - 100
ALP (U/L)	38	23 - 212
BUN (mg/dL)	25	7 - 27
Creatinine (mg/dL)	1.76	0.50 - 1.80
Albumin	2.8	2.7-3.8

Table 2. Echocardiogram variables from the first and recent visit.

Variables	First visit	At 6 months
HR (BPM)	132.37	130
LVIDd (mm)	15.23	28.0
LVIDs (mm)	8.88	16.0
LA diameter (mm)	15.77	21.0
Ao diameter (mm)	11.55	14.0
LA:Ao short axis	1.37	1.53
TR Max PG (mmHg)	75.83	55.40
PR Max PG (mmHg)	72.52	38.83
RA Max PG (mmHg)	65.5	-
PV Max PG (mmHg)	14.27	17.58
AV Max PG (mmHg)	1.82	2.69
MV Max PG (mmHg)	34.4	-
MR Max PG (mmHg)	88.68	57.29
MV E:A ratio	0.948	0.83

Case description

An 8-year-old intact female French bulldog was presented with 3-month abdominal distension. The dog has never undergone any vaccination program or heartworm prevention and had no previous medical history. She was kept outdoor with 3 other dogs in the house. Cough, fatigue, and difficulty breathing were occasionally observed.

During the first visit, the dog was moderately depressed and weak. She was emaciated with a body condition score of 2/9. From the physical examination, murmur heart sound was graded 5/6 and prominent at the pulmonic region. The mucous membrane was pale pink with prolonging capillary refill time (CRT). Jugular pulsation was markedly seen. The dog had mild tachypnea with mild increase lung sound at the right caudal lobe with an enlarged abdomen. Abdominal ballottement was presented without cramp (Figure 1).

Thoracic and abdominal radiographs were taken in ventrodorsal and lateral views. Liver enlargement and ground-glass soft tissue opacity were seen in the abdominal cavity. The internal organs were difficult to distinguish from both views (Figure 2 and 3). The thoracic radiography showed an elevated trachea, thickening vena cava, vascular pattern of the lungs and an unstructured interstitial pattern at the perivascular region, especially at the large pulmonary artery of the caudodorsal lobes from lateral view (Figure 3). From the ventrodorsal view, an inverted D-shape heart with pulmonary knob was seen with tortuous pulmonary arteries, and pulmonary artery enlargement (Figure 2A and B).

Hematology values were within the reference interval. No parasite was found from the blood smear

during the first visit (Table 1). An echocardiography revealed parallel hyperechoic lines of numerous heartworms in the right atrium (RA), right ventricle (RV), and pulmonary artery (PA) causing severe tricuspid regurgitation (TR) and marked pulmonary regurgitation. Severe right cardiomegaly and pulmonary hypertension with right-side heart failure signs were also presented (Figure 4). Echocardiography and clinical signs suggested a caval syndrome.

The initial plan was mainly targeting on improving the quality of life for this patient as the prognosis was poor to grave if pulmonary thromboembolism occurred. Thus, abdominocentesis was performed and the 2,000 milliliters of serosanguineous fluid (Figure 5) were obtained for the first time and gradually decrease with diuretic drug (furosemide 1 mg/kg). The fluid was identified as modified transudate. Medical treatment was prescribed, using a long-term macrocyclic lactone with right side heart failure management. A monthly spot on imidacloprid 250 mg and moxidectin 62.5mg (Advocate® spot-on, Bayer Animal Health GmbH, Leverkusen, Germany) was used to kill microfilaria and a 10 mg/kg doxycycline to cut off heartworm life cycle by reducing *Wolbachia* spp. in the adult worms. Prednisolone 0.25 mg/kg was started and planned to taper down each week. Ramipril 0.125 mg/kg and spironolactone 1.25 mg/kg were used for heart failure treatment. The owner was instructed to give the dog an absolute exercise restriction, adequate food and water intake, as well as a recommendation of heart worm prevention for other dogs in the house.

The dog was scheduled for a follow-up and initial reassessment weekly. Her general condition was improved. A reaccumulation of ascites was less and took longer to return. After 3 and 6 months, the patient was reevaluated with an echocardiography and no heartworm was detected in the right atrium, pulmonary artery, and ventricles. Moreover, the parameters from echocardiographic findings and clinical signs are improved (Table 2). Abdominal ultrasound aided in determines the current ascites. A rapid test for heartworm antigen (The SNAP® 4DX® Plus Test, IDEXX Laboratories, Inc., Westbrook, ME) was used at 6 months after treatment and no heartworm antigen was detected. During the 4th month visit, small fluid was found in cystocolic site and difficult to obtain from abdominocentesis. By adjusting diuretic drugs, furosemide 0.5 mg/kg q12h was prescribed along with an adjunctive of spironolactone 1.25 mg/kg q12h. Despite the owner had missed the 3rd and the 5th visit, the dog had a well respond. By the 7th month, abdominocentesis was no longer required. The dog had been living well and was planned on a monthly reassessment with a regular heartworm prevention program (AHS 2020).

Discussion

Caval syndrome is the final stage of a heartworm disease. The major characteristic signs are intracardiac worms obstruction of blood flow, interference of tricuspid valve closure, causing tricuspid valve regurgitation, heart murmur, and jugular pulsations as a consequence from liver congestion. Other than these clinical signs, caval syndrome is often diagnosed and confirmed by the present of the intracardiac worms from an echocardiography or necropsy. Screening test such as a rapid test kit is useful in early diagnosis and for following up the treatment. However, it requires the antigen from a female heartworm to develop at least 6 months after the dog has exposed to the worm (AHS 2020; TroCCAP 2017). The SNAP 4Dx Plus? Test has a 99.0% sensitivity and a 99.3% specificity, comparing to the gold standard, necropsy (Barr et al. 2011;

IDEXX 2016; Liu et al. 2018; Lee et al. 2011). Atkins and Genchi et al. had demonstrated up to 100% sensitivity (95% confidence limit) with 3 worms infestation (Atkins 2003; Genchi et al., 2018). Echocardiography aids in visualizing the structures of the heart with parameters measurement (Romano et al., 2020) and worms migration (Courteney and Zeng 2001; Atkins 2003) despite a lower sensitivity, comparing to antigen detection (DeFrancesco 2001; Esser 2020). Several studies advised a blood collection with N-glycosylation as a biomarker testing (Behrens et al., 2018) for early detection as of 3 weeks post infection and a multiplex qPCR to identify occult form of multiple heartworms species (Laidoudi 2020). Microfilaria was not observed from a blood smear in this case. Nonetheless, explicit clinical presentations, echocardiography, and the rapid test kit were sufficient to make a diagnosis in this case report.

A long-term macrocyclic lactone is another acceptable alternative apart from the gold standard, surgical removal of heartworm and melarsormine adulticide (Jones 2016; AHS 2020). The latters can potentially cause lethal adverse effect from either post operation complications (Bov? et al., 2010) or arsenic poisoning symptoms and was abstained in this patient. Alberigi et al. (2020) had demonstrated the use of semi-annual therapy of 0.5 mg/kg moxidectin injection with oral 10 mg/kg doxycycline were effective against dogs with dirofilaremia and improved the pulmonary conditions with noticeable change within 30 days by dirofilaria counts (Alberigi et al., 2020). Hence, the application of a monthly 10% imidacloprid and 2.5% moxidectin (Advocate®) with 4 weeks of 10 mg/kg doxycycline were proved to be as effective in terms of clinical conditions in this case report.

Notwithstanding the fact that the owner had missed 2 visits and 2 inconsecutive Advocate® administrations, the dog was in an improvement. She expressed a more comfortable being. Due to the severity of worm infestation and the occurrence of right-side heart failure signs, it was not expected to fully recover. Even though the dog survived from a pulmonary thromboembolism and the clinical presentation has improved, it did not show a remarkable change in a cardiac remodeling. Considering a poor prognosis for this disease, the mortality rate of dogs with caval syndrome is expected to be 30-40% (Kittleson 1998) even with the worm extraction (Hoch and Strictland 2008). Nevertheless, improving the quality of life for this patient had been the primary goal. The dog was planned to continue using a longterm macrocyclic lactone for heartworm management protocol and then reassess the worm and possible thrombi through echocardiography and antigen testing after 6 months from the first treatment. Since microfilariae can live up to 2 years (AHS 2018), it could be considered as the point when the treatment outcome should be fully followed, otherwise after antigen test was negeative. Ames et al. (2020) had designed a treatment of topical imidacloprid-moxidectin and doxycycline in dog with heartworm infection, stage 1 and 2 (Ames et al., 2020). The study result that treated with the same protocol was promising with 96% no antigen detection cases and supporting this case report at stage 4 or caval syndrome.

Conclusions

A prescription of long-term macrocyclic lactone with adjunctive therapy in this case report had demonstrated a satisfying outcome. The dog exhibited an improvement in clinical signs each time of visit at the animal hospital. A monthly application of 10% imidacloprid and 2.5%

moxidectin had shown the efficacy in controlling heartworms of a dog with caval syndrome thus far, along with 4 weeks of 10 mg/kg doxycycline and exercise restriction to prevent complication from pulmonary thromboembolism. Reassessment of this patient was planned for a monthly health screening and a 3-month echocardiography for worms migration, thrombi, and antigen testing. The limitation was to make a substantial regime and an efficient client communication in very poor prognosis patients. Dedication from the owners is strongly supporting the treatment plan. It is important to control the infection of the patient as well as the other dogs in the same house to reduce a local transmission, by vector control and semi-annual heartworm protection regimen.

Acknowledgements

The authors would like to express their sincere gratitude to all staffs at Prasu-arthorn animal hospital, Assist. Prof. Dr. Somkiat Huaijantug, Dr. Phuttiphan Channgam, with the owner permission and this patient whose support and collaboration made this case report possible.

References

Alberigi B, Fernandes JI, Paiva JP, Mendes-de-Almeida F, Knackfuss F, Merlo A, et al. Efficacy of semi-annual therapy of an extended-release injectable moxidectin suspension and oral doxycycline in *Dirofilaria immitis* naturally infected dogs. Parasit Vectors. 2020;13(1):503.

American Heartworm Society. Current canine guidelines for the prevention, diagnosis, and management of Heartworm (*Dirofilaria immitis*) infection in dogs. 2020;1:4-29.

Ames MK VV, Plath C, Atkins C. Use of moxidectin/imidacloprid and doxycycline for non-arsenical heartworm adulticidal therapy. 2017 ACVIM Forum Research Report Program National Harbor, Maryland. J Vet Intern Med. 2017;31:1577.

Ames MK, VanVranken P, Evans C, Atkins CE. Non-Arsenical heartworm adulticidal therapy using topical moxidectin-imidacloprid and doxycycline: A prospective case series. Vet Parasitol. 2020; 282:109099.

Atkins CE. Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens. JAVMA. 2003;222(9):1221-3.

Barr MC, Boynton EP, Schmidt PL, Bossong F, Johnston GR. Diagnosis of Canine Heartworm Infection. Today Vet Prac J. 2011;(4):30-7.

Bazzocchi C, Mortarino M, Grandi G, Kramer LH, Genchi C, Bandi C, et al. Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. Int J Parasitol. 2008;38(12):1401-10.

Behrens AJ, Duke RM, Petralia LMC, Lehoux S, Carlow CKS, Taron CH, et al. Changes in canine serum N-glycosylation as a result of infection with the heartworm parasite Dirofilaria immitis. Sci Rep. 2018;8(1):16625.

Bobinnec GL. Mitral Valve Disease (MVD): Proposal for an Optimisation of the Left Atrium on Aorta Ratio (LA/AO). Proceedings of the 35th World Small Animal Veterinary Congress WSAVA 2010.Geneva, Switzerland; 2010.

Bové CM, Gordon SG, Saunders AB, Miller MW, Roland RM, Sarah EA, et al. Outcome of minimally invasive surgical treatment of heartworm caval syndrome in dogs: 42 cases (1999-2007). J Am Vet Med Assoc. 2010;236:187-92.

Bowman DD. Heartworms, macrocyclic lactones, and the specter of resistance to prevention in the United States. Parasit Vectors. 2012;5:138

Bowman DD., Atkins CE. Heartworm biology, treatment, and control. Vet Clin North Am Small Anim Pract. 2009;39(6):1127-58.

Courtney CH, and Zeng QY. Comparison of heartworm antigen test kit performance in dogs having low heartworm burdens. Vet Parasitol. 2001;96: 317-22.

DeFrancesco TC, Atkins CE, Miller MW, Meurs KM, Keene BW. Use of echocardiography for the diagnosis of heartworm disease in cats: 43 cases (1985ñ1997). JAVMA. 2001;218(1):66-9.

Esser LC, Borkovec M, Bauer A, Haggstrom J, Wess G. Left ventricular M-mode prediction intervals in 7651 dogs: Population-wide and selected breed-specific values. J Vet Intern Med. 2020;34(6): 2242-52.

Frank K, Heald RD. The Emerging Role of Wolbachia Species in Heartworm Disease. Compendium: Continuing Education for Veterinarians®. 2010;32(4):1-5.

Genchi M, Mangia C, Ferrari N, Loukeri S. Evaluation of a rapid immunochromatographic test for the detection of low burden *Dirofilaria immitis* (heartworm) in dogs and cats. Parasitol Res. 2018;117(1):31-4.

Hoch H, Strictland KN. Canine and feline dirofilariasis: prophylaxis, treatment, and complications of treatment. Compend Contin Educ Pract Vet. 2008;(30):146-51.

IDEXX. SNAP 4Dx Plus Test. [Internet]. IDEXX. 2016 [cited 2020 November 28] Available from https://www.idexx.com/en/veterinary/snap-tests/snap-4dx-plus-test/.

Jones SL. Canine Caval syndrome series part 3: Management of caval syndrome. Today Vet Prac J. 2016;(3):55-61.

Kamyingkird K, Junsiri W, Chimnoi W, Kengradomkij C, Saengow S, Sangchuto K, et al. Prevalence and risk factors associated with Dirofilaria immitis infection in dogs and cats in Songkhla and Satun provinces, Thailand. Agric Nat Resour. 2017;51:299-302.

Keith NS. Canine and Feline Caval Syndrome. Clin Tech Small Anim Pract. 1998;3(2):88-95.

Laidoudi Y, Davoust B, Varloud M, Niang EHA, Fenollar F, Mediannikov O. Development of a multiplex qPCR-based approach for the diagnosis of *Dirofilaria immitis*, *D. repens* and *Acanthocheilonema reconditum*. Parasit Vectors. 2020;13(1):319.

Lee AC, Bowman DD, Lucio-Forster A, Beall MJ, Liotta JL, Dillon R. Evaluation of a new in-clinic method for the detection of canine heartworm antigen. Vet Parasitol. 2011;177(3-4):387-91.

Liu J, Drexel J, Andrews B, Eberts M, Breitschwerdt E, Chandrashekar R. Comparative Evaluation of 2 In-Clinic Assays for Vector-Borne Disease Testing in Dogs. Top Companion Anim Med. 2018;33(4): 114-8.

McCall JW, Genchi C, Kramer L, Guerrero J, Dzimianski MT, Supakorndej P, et al. Heartworm and Wolbachia: therapeutic implications. Vet Parasitol. 2008;158(3):204-14.

Kittleson MD. Heartworm infestation and disease (dirofilariasis). In: Kittleson MD, Kienle RD, editors. Small Animal Cardiovascular Medicine. 1st ed. St. Louis: MO;1998. p. 370-401.

Romano AE, Saunders AB, Gordon SG, Wesselowski S. Intracardiac heartworms in dogs: Clinical and echocardiographic characteristics in 72 cases (2010-2019). J Vet Intern Med. 2021;35(1):88-97.

Taylor MA, Coop RL, Wall RL. Dirofilaria. In: Taylor MA, Coop RL, Wall RL, editors. Veterinary parasitology. 4th ed. Wiley: Blackwell;2016. p. 64-5.

Trancoso TAL, Lima NDC, Barbosa AS, Leles D, Fonseca ABM, Labarthe NV, et al. Detection of Dirofilaria immitis using microscopic, serological and molecular techniques among dogs in Cabo Frio, RJ, Brazil. Rev Bras Parasitol Vet. 2020; 29(1):e017219.

TroCCAP. Guidelines for the diagnosis, treatment and control of canine endoparasites in the tropics. Tropical Council for Companion Animal Parasites. 2017;1:40.

Yoon WK, Han D, Hyun C. Catheter-guided percutaneous heartworm removal using a nitinol basket in dogs with caval syndrome. J Vet Sci. 2011;12(2):199-201.

Yun SJ, Cheon HB, Han JI, Kang JH, Chang JH, Na KJ, et al. Portal Vein Thrombosis in a Dog with Dirofilariasis. J Vet Clin. 2010;27(5):600-4.

Case Report

Surgical Ligation of Patent Ductus Arteriosus in an Adult Pomeranian

Kornrawee Tharavanij* Nattapon Riengvirodkij

Prasu-Arthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University, Salaya, Phutthamonthon, Nakhon Pathom, 73170, Thailand

***Corresponding author, E-mail address: kornrawee.sir@gmail.com**

Received: 16 August 2021; Revised: 10 November 2021; Accepted: 11 November 2021

Abstract

Patent ductus arteriosus (PDA) is the most common congenital heart disease in dogs. The ductus arteriosus extends from the main pulmonary artery to the descending aorta, shunting blood away from the nonfunctional lungs in neonates. When it fails to constrict after birth, oxygenated blood can flow from the aorta to the pulmonary artery. Most of untreated patients develop congestive heart failure at one year of age. The treatment options include both minimally invasive and open surgical techniques. It is recommended that PDA be promptly closed after diagnosis as immature dogs are the best candidates for surgery. An adult Pomeranian with PDA was referred to Prasu-Arthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University. An open surgical ligation was performed at the animal hospital with the cooperation of a diplomate of the Thai Board of Thoracic Surgery from the Faculty of Medicine Ramathibodi Hospital, Mahidol University. No intraoperative complications occurred. Postoperative echocardiograms showed no residual flow, and there was a gradual decrease in heart size three days to two months after surgery. The dog was more energetic and healthier.

Keywords: PDA, Pomeranian, open surgical ligation

การผ่าตัดแก้ไขโรคหลอดเลือดหัวใจคงค้างในสุนัขปอมเมอราเนียนเต็มวัย

กรรวี ธาระวานิช* ณัฐพล เรียงวิโรจน์กิจ

โรงพยาบาลสัตว์ประศุอาทร คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
ถนนพุทธมณฑล สาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

***ผู้รับผิดชอบบทความ E-mail address: kornrawee.sir@gmail.com**

Received: 16 August 2021; Revised: 10 November 2021; Accepted: 11 November 2021

บทคัดย่อ

โรคหลอดเลือดหัวใจคงค้าง (Patent ductus arteriosus: PDA) เป็นโรคหัวใจพิการแต่กำเนิดที่พบได้บ่อยในสุนัข เส้นเลือดนี้เชื่อมต่อระหว่างเส้นเลือดพัลโมนารีอาร์เตอรีกับเส้นเลือดเอออร์ตาส่วนลง ทำหน้าที่เป็นทางผ่านของเลือดไม่ให้ไปยังปอดที่ยังไม่ทำงานในลูกสัตว์ หากเส้นเลือดนี้ยังคงค้างอยู่หลังจากคลอด เลือดที่มีออกซิเจนจะสามารถไหลจากเส้นเลือดเอออร์ตาส่วนลงไปสู่เส้นเลือดพัลโมนารีอาร์เตอรี ส่วนมากสัตว์ป่วยที่ไม่ได้รับการรักษาจะมีภาวะหัวใจล้มเหลวเมื่ออายุ 1 ปี ทางเลือกในการผ่าตัดมีทั้งแบบไม่เปิด และเปิดช่องอก การผ่าตัดเพื่อปิดเส้นเลือด PDA นั้น ควรทำให้เร็วที่สุด เนื่องจากการผ่าตัดได้ผลดีในลูกสัตว์ที่ยังไม่โตเต็มวัย สุนัขปอมเมอราเนียนโตเต็มวัยที่มีภาวะ PDA ถูกส่งตัวมาที่โรงพยาบาลสัตว์ประศุอาทร มหาวิทยาลัยมหิดล และได้รับการผ่าตัดเปิดช่องอกเพื่อผูกปิดเส้นเลือด โดยได้รับความร่วมมือจากอาจารย์แพทย์สาขาศัลยศาสตร์ทรวงอก คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล ทั้งนี้ ไม่พบข้อแทรกซ้อนระหว่างการผ่าตัด จากการบันทึกภาพหัวใจด้วยคลื่นเสียงความถี่สูงหลังผ่าตัด 3 วันจนถึง 2 เดือน พบว่าหัวใจมีขนาดเล็กลง และไม่พบการไหลของเลือดผ่านหลอดเลือดดังกล่าวเหลืออยู่อีก สุนัขแข็งแรงมากขึ้นหลังการผ่าตัด

คำสำคัญ : โรคหลอดเลือดหัวใจคงค้าง ปอมเมอราเนียน ผ่าตัดเปิดช่องอกผูกเส้นเลือด

Introduction

Patent ductus arteriosus (PDA) occurs when the ductus arteriosus muscle fails to constrict after birth, leaving a persistent opening between the aorta and pulmonary artery. It is the most common congenital heart disease in dogs and is overrepresented in female purebred dogs, including Poodles, Keeshonds, Maltese, Bichons, Yorkshire Terriers, Cocker Spaniels, Pekinese, Collies, Shelties, Welsh corgis, and Pomeranians. It can also occur in cats but not as frequent as dogs (Orton et al., 2018).

Young dogs with PDA may have no clinical signs. PDA is commonly identified at the time of routine vaccinations. Simple thoracic auscultation can reveal a characteristic continuous machinery murmur at the high left heart base. On the other hand, the patient can present with coughing, dyspnea, exercise intolerance, and stunted growth. Further diagnostic evaluations include thoracic radiography and echocardiography. Treating PDA requires medical management or occlusion of the duct or both. If left untreated, the mortality rate of an animal with PDA aged one year is 70% (Fossum 2013).

PDA allows blood to continuously flow from the systemic circulation to the pulmonary circulation (left-to-right shunting) (Figure 1). Chronic volume overload causes left-sided heart enlargement and, eventually, left-sided congestive heart failure. If the pulmonary hypertension is so severe that the pressure in the pulmonary artery exceeds that in the aorta, blood will reversely flow from the pulmonary artery into the aorta (right to left shunting), causing nonoxygenated blood to mix with oxygenated blood. Only a small percentage of dogs with PDA will develop this "reversal." The characteristic sign of reverse PDA is differential cyanosis most obvious in the caudal mucous membranes. The heart murmur will disappear, and the dogs may have intermittent hind limb weakness and seizures.

PDA can be treated using medical management or occlusion of the ductus arteriosus or both. Prostaglandin synthase inhibitors can stimulate ductus closure. Unfortunately, ductal smooth muscle hypoplasia is common in dogs, and the time of diagnosis is often weeks to months after birth. These factors make prostaglandin synthase inhibitors ineffective. As a supportive treatment, furosemide can improve pulmonary edema. Digoxin can control the ventricular response rate in dogs with atrial fibrillation.

PDA is mainly treated with physical occlusion of the ductus arteriosus, performed using minimally invasive techniques or open surgical management. Minimally invasive techniques include transvenous catheterization and thoracoscopic PDA occlusion. Thrombogenic coils and Amplatz canine duct occluder (ACDO) are the two main devices used in transvenous catheterization. Angiography is an essential and accurate measurement of the PDA, and device sizing is required.

In the retrospective study by Goodrich et al. (2007), using a thrombogenic coil had a reduced risk of major complications compared with surgical ligation, but it was associated with a lower initial success rate and the same mortality rate. Dogs with nontapering ductus are contraindicated for the thrombogenic coil because the device could slip into the pulmonary artery.

ACDO is a self-expanding plug made of nitinol wire. The dense wire mesh of the device obstructs the blood flow through a PDA. The device can be securely positioned in the ostium of PDA. In thoracoscopic PDA occlusion, titanium vascular clips are used to occlude a PDA. Accurately measuring PDA size and choosing the proper vascular clip size are crucial.

Minimally invasive techniques are recommended, if available, because of the reduced operative pain and

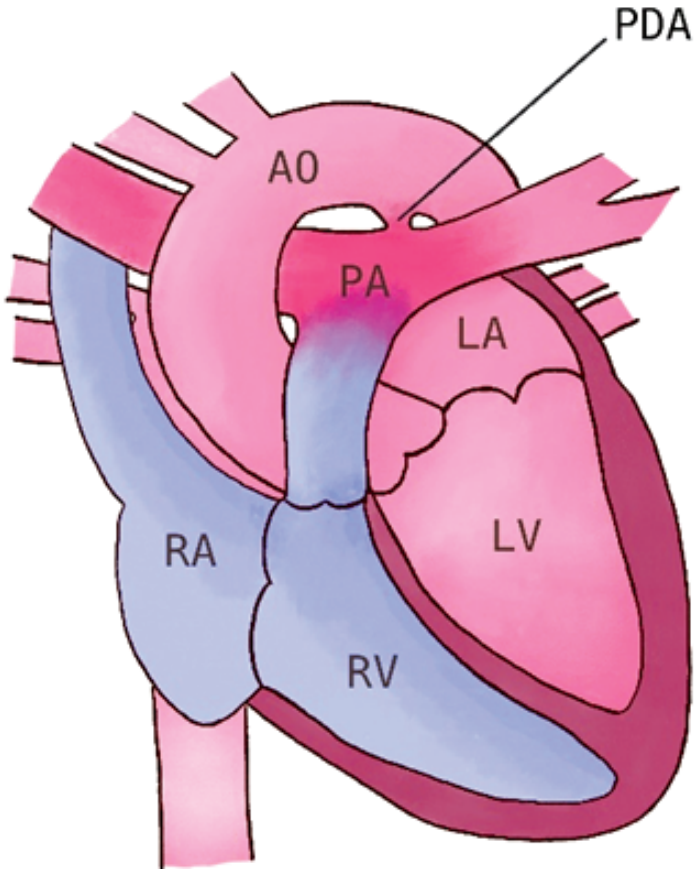


Figure 1. Diagram showing flow from systemic circulation to pulmonary circulation. PDA: patent ductus arteriosus; AO: aorta; PA: pulmonary artery; RA: right atrium; RV: right ventricle; LA: left atrium; LV: left ventricle.

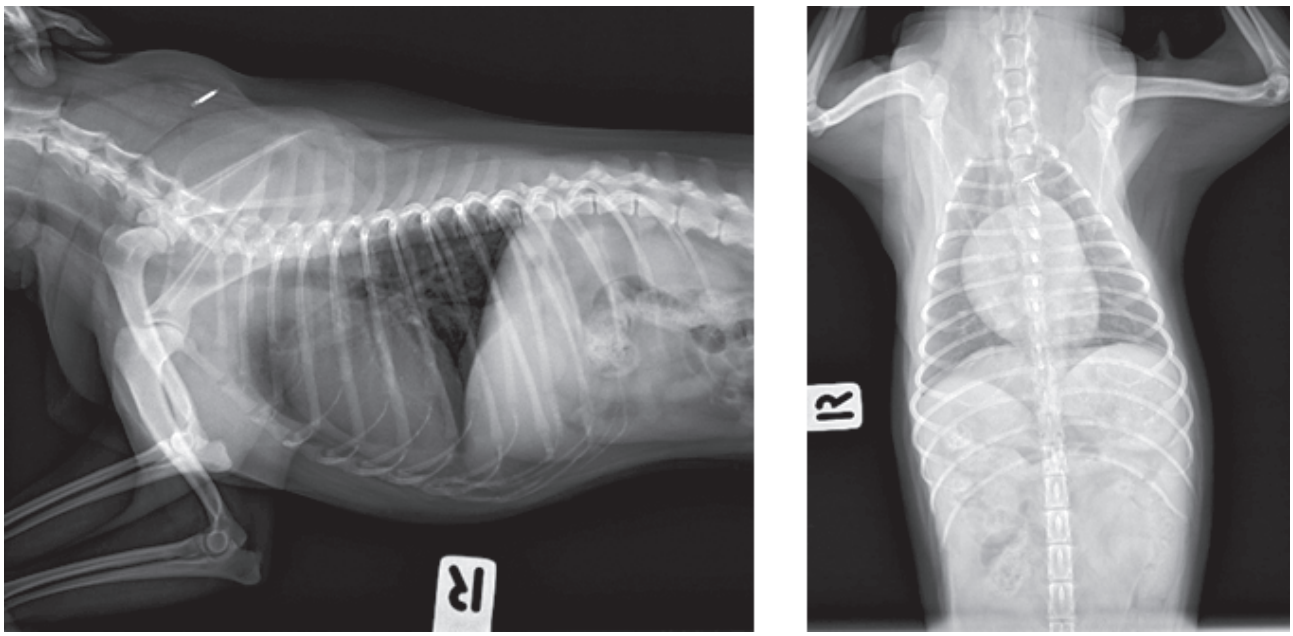


Figure 2. Thoracic radiograph of the lateral (left) and ventrodorsal (right) views showing pulmonary artery and vein dilation.

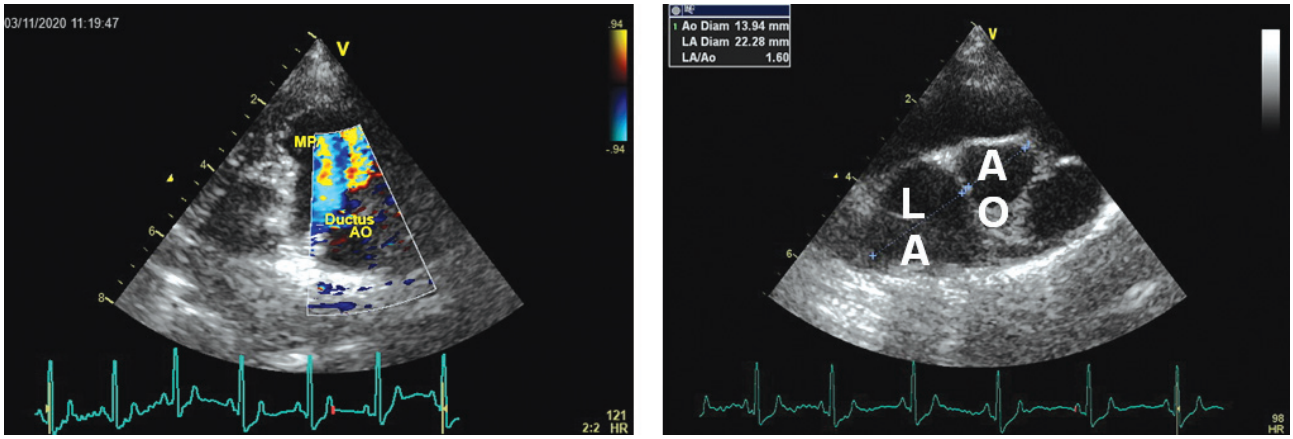


Figure 3. Echocardiography on the left parasternal cranial right outflow tract (left) view and right parasternal short axis (right) views showing turbulence flow through the pulmonary artery. Left atrial and ventricular enlargement. MPA: main pulmonary artery; LA: left atrium; AO: aorta.

recovery time. However, investment in a high-cost instrument and expertise in minimally invasive surgery are required.

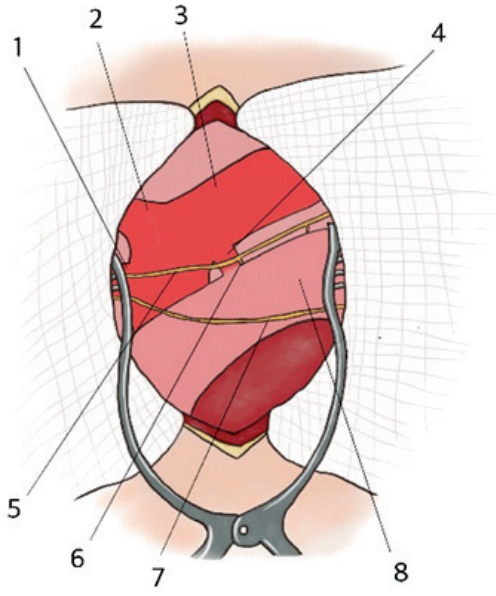
The ligation of the ductus is an open surgical technique, which is considered curative. Very-low-birth-weight, premature puppies are ideal candidates for surgery. Surgery should be promptly performed after the diagnosis. Broaddus and Tillson (2010) described the open surgical technique and reported the outcome of the surgery. An invited instructor, who is a diplomate of the Thai board of Thoracic Surgery from the Faculty of Medicine Ramathibodi Hospital, Mahidol University, performed the surgery at the Prasu-Arthorn Animal Hospital, Mahidol University. A veterinary clinician from Prasu-Arthorn Animal Hospital assisted the surgeon.

Case Descriptions

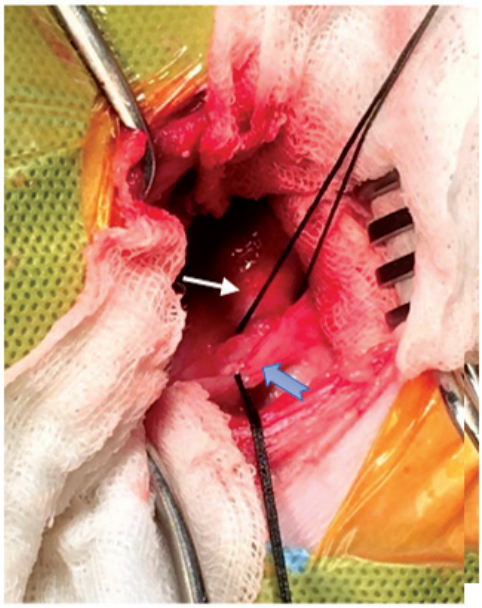
A three-year-old male Pomeranian was referred to Prasu-Arthorn Animal Hospital for further investigations of his cardiac murmur. On physical examination, the dog was healthy despite his grade IV/VI systolic murmur auscultated from the left chest wall at the level of the heart base. His body condition score was 4/9. Hematology and serum biochemical profiles were within normal limits. Thoracic radiographs showed pulmonary artery and vein dilation (Figure 2). There was no evidence of pulmonary edema. The vertebral heart score was 11.5. On echocardiogram, continuous turbulent flow was found through the pulmonary artery. PDA with an eight-millimeter diameter was identified. Color-flow Doppler revealed a blood flow velocity of 3.9 m/sec across the PDA, which indicated a pressure gradient at approximately 60 mmHg. There was a slight increase in the left atrium to the aorta (LA: AO) ratio and left ventricular internal dimension normalized by body weight (NLVIDd) at 1.6 and 1.9, respectively. No mitral or tricuspid regurgitation was found (Figure 3). To prepare for surgery, pimobendan at 0.25 mg/kg was prescribed twice daily to improve ventricular contractility. As there was no evidence of pulmonary edema and heart failure, diuretics and angiotensin-converting enzyme inhibitor were not required. Surgery for PDA ligation was then scheduled.

Preoperatively, the dog was premedicated with 0.3 mg/kg morphine and 0.3 mg/kg midazolam IM. General

anesthesia was induced with 2 mg/kg etomidate IV given titrated to effect. 25 mg/kg cefazolin was given IV. Acetate solution was administered intravenously at a rate of 5 ml/kg/h. Anesthesia was maintained with isoflurane in oxygen. As a mechanical ventilator was unavailable at the time of surgery, positive ventilation was applied manually after opening the thoracic cavity. Positive ventilation was necessary to aid the lung expansion since, during thoracotomy, normal negative pressure in the thoracic cavity was disrupted. The dog was placed in right lateral recumbency with a rolled towel tucked under the cranial thorax. The entire left side of the thorax was clipped and prepared. The surgeon stood on the patient's dorsal side. Thoracotomy was performed at the left fourth intercostal space. The underlying cutaneous trunci and latissimus dorsi muscles were incised. Intercostal spaces were recounted from the first rib before further incision. Scalenus muscle was incised at the fourth intercostal space. External and internal intercostal muscles were incised in the middle between the ribs, and then, the pleura was penetrated. Moistened gauze sponges were placed along the cranial and caudal rim of the ribs. Weitlaner retractor was placed and retracted to achieve sufficient exposure. The cranial lung lobe was packed caudally to expose the aorta, pulmonary artery, and phrenic and vagus nerves (Figure 4a). The vagus nerve was isolated and retracted ventrally. Care was taken to keep the left recurrent laryngeal nerve intact as it leaves the vagus nerve caudally to the PDA (Figure 4a). Usually, associated thrill can be palpated at the site of the PDA. However, in this patient, the thrill was palpated slightly cranial and ventral to the PDA. The PDA location was carefully verified using anatomical landmarks including the aortic arch and left subclavian artery. The ductus was



4a



4b

Figure 4a. Diagram showing important landmarks at the left heart base. (1) Brachiocephalic trunk; (2) left subclavian artery; (3) aorta; (4) patent ductus arteriosus; (5) left vagus nerve; (6) left recurrent laryngeal nerve; (7) phrenic nerve; (8) pulmonary trunk.

Figure 4b. Photograph showing intraoperative appearance. The vagus nerve was retracted ventrally using one strand of silk (notched arrow). The suture was passed around the PDA one by one (arrow).

bluntly dissected using right-angle forceps from cranial to caudal aspect. Two separate strands of USP size 1 silk were passed behind the ductus one by one (Figure 4b). Recurrent laryngeal nerve was observed, and care was taken to avoid the nerve during suturing. The suture close to the aorta was ligated first to stop blood flowing through the ductus, followed by the one close to the pulmonary artery. Heart rate and blood pressure at the time of ligation were closely monitored and were in significantly variable. The PDA area was then palpated again, and the thrill disappeared. A chest drain was placed using a feeding tube and three-way stop clock system. The thoracotomy closure was routinely performed. Air and fluid were aspirated until a negative pressure was achieved. The patient recovered well and was stable after surgery.

Postoperatively, the patient was administered with fentanyl, 3 μg/kg/hr CRI, to control the pain. It was tapered and then changed to tramadol injection, 4 mg/kg TID, on the second day after surgery. Carprofen, 4.4 mg/kg SID, was given for three days. Three days after the operation, the patient was comfortable and eating. Fluid from the chest drain at this time was minimal (mean production of 0.35 ml/kg/day), and thus, the chest drain was removed. When the pain score was 1/4 according to the Colorado State University pain scales, the patient was discharged after three days of hospitalization. There was no further need for intravenous fluid, medication injections, or oxygen therapy. On discharge, cephalexin, 25 mg/kg BID for seven days, was prescribed for the patient. An echocardiogram was performed before discharge. The size of the left side of the heart returned to normal. The ductus had a 7.9 mm diameter. However, no ductal flow through the pulmonary artery was detected. The skin suture was removed 14 days after the operation. Pimobendan was discontinued at this time. At one- and two-month follow-up, the echocardiogram showed that the left atrium reduced in size compared with before surgery. The LA: AO ratio was 1.3. No ductal recanalization was found (Figure 5). Echocardiographic parameters before and after surgery are shown in Table 1.

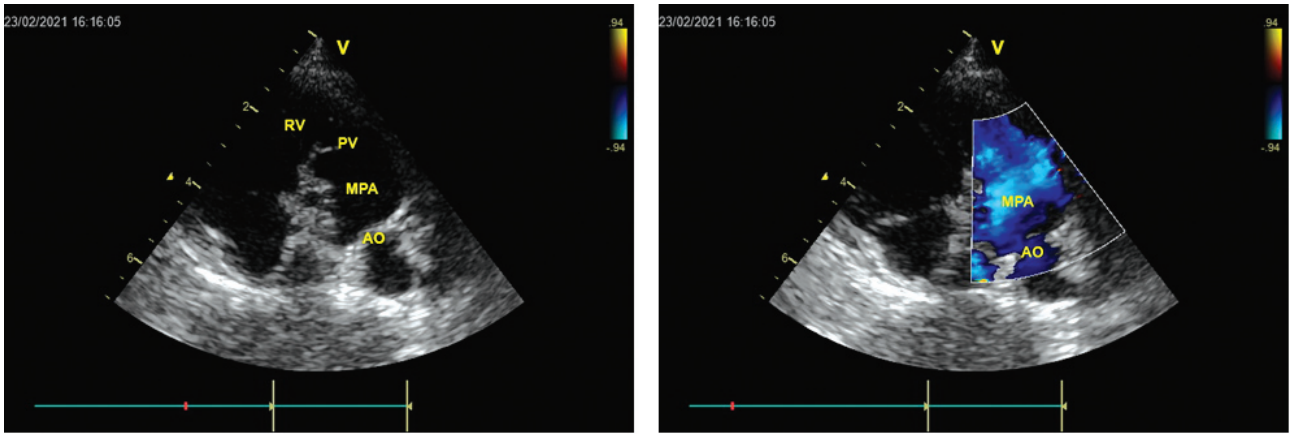


Figure 5. Echocardiography at two months after surgery on left parasternal cranial right outflow tract views showing no residual flow. RV: right ventricle; PV pulmonic valve; MPA: pulmonary artery; LA: left atrium; AO: aorta.

Table 1. Echocardiographic values before and after surgery.

Parameter	Before	3 days PO	30 days PO	60 days PO
AO diameter (cm)	1.4	1.6	1.3	1.3
LA diameter (cm)	2.2	2.0	1.6	1.6
LA/AO ratio	1.6	1.2	1.3	1.3
%FS	39	33	27	24
NLVIDd (cm/kg)	1.9	1.6	1.7	1.7
AVmax (m/s)	1.5	1.2	1.3	N/A
PVmax (m/s)	1.2	N/A	1.0	N/A

AO: aorta; LA: left atrium; FS: fractional shortening; NLVIDd: normalized left ventricular internal diameter in diastole; AVmax: aortic flow; PVmax: pulmonary artery flow.

Discussion

PDA is the most common congenital heart disease in dogs. Most dogs with PDA undergo surgery at a young age (Saunders et al., 2013). However, our patient presented with no clinical signs at three years of age. This conforms to the study by Israël et al. (2003), that stated that left-to-right shunt PDA is more common in older dogs than broadly recognized. They recommended the occlusion of the PDA regardless of age because it can relieve the clinical signs and should be promptly performed to avoid mechanical stress on the mitral valve, which can then develop to mitral valve endocardiosis. The risk of residual flow and complication rates are not greater in the adult population (Boutet et al., 2017).

Moreover, another report showed that increasing age was negatively associated with survival rate (Bureau et al., 2005). The authors further discussed that age may not directly increase the risk of death caused by PDA, but, in older animals, the duct could be more friable and could have more surrounding fibrous tissues. Thus, the risk of intraoperative hemorrhage and subsequent death may increase.

In our patient, the echocardiograms showed improvement in the size of the left atrium and ventricle after surgery. This contrasts with the results in the previously mentioned study by Israël et al. (2003), which mentioned that, in most older animals, cardiomegaly was irreversible. Our patient may represent the minor incidence that cardiac size was improved after occlusion. Although cardiomegaly persisted after occlusion, it may not affect the lifespan.

The study showed that the ductal diameters varied in size from 3.5 mm to 20 mm. An 8 mm ductus (like the one in our study) or wider was not always associated with congestive heart failure or poor survival. There was no correlation between ductal size and clinical signs.

Regarding the occlusion techniques, although both minimally invasive techniques and open surgical techniques have been described for a long time in the veterinary literature, the first case report of tranveneous catheterization technique using ACDO to close the PDA was in 2015 in Thailand (Buranakarl et al., 2015). The

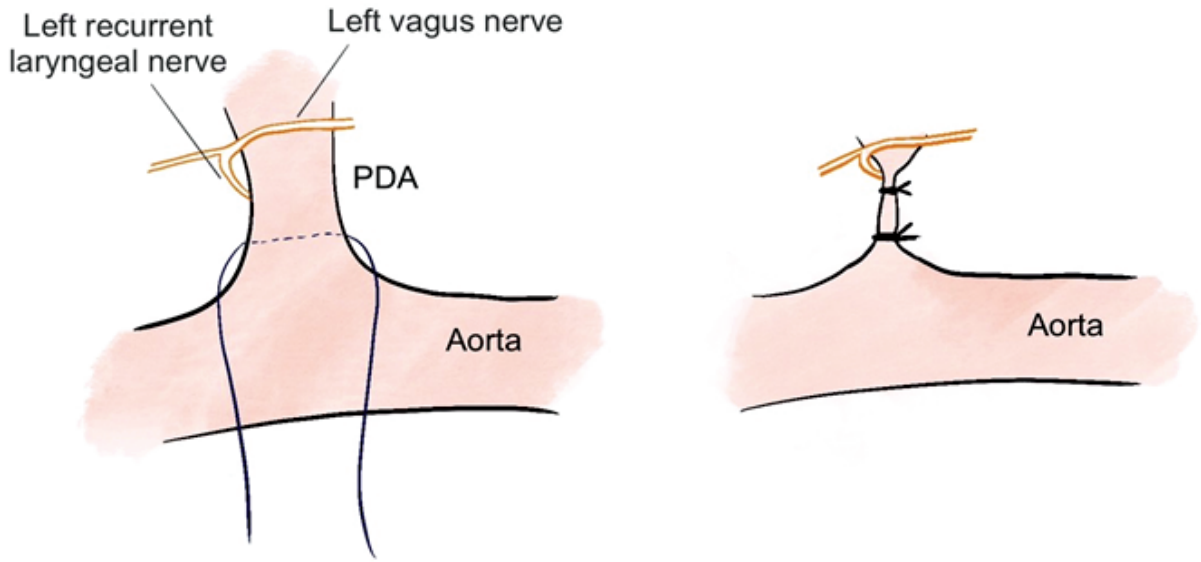


Figure 6. Diagram showing anatomical landmark from the standing position of the surgeon.

operation was successful. The dog had improved cardiac function and size. Thoracoscopic PDA occlusion was not reported in Thailand. Minimally invasive techniques are significantly less accessible than open surgical techniques because of device availability and high cost. The device must be compatible with the PDA in terms of size and shape. Also, the surgeon should be highly experienced in minimally invasive procedures. Although angiography is needed to determine the shape and the minimal internal diameter of the ductus, because of our patient's size, occlusion using transvascular catheterization technique may be possible. However, this option was unavailable at the authors' practice. Treatment options were already discussed with the owner before a decision was made for surgical ligation.

A standard technique and many variations were described for open surgery. The major goals of these techniques are to avoid damage to the ductus during dissection and suture passage (Broaddus and Tillson 2010). Attention should be paid to the medial side of the ductus, which is usually fragile and tearing it can cause catastrophic hemorrhage. In the study by Parchman (2008), the suture was tied and then the knot was grabbed while passing behind the duct to avoid closing the forceps during passage. Another goal is to avoid recanalization on the long term. Brockman et al. (2018), recommended that one strand of 5-0 polypropylene suture be placed between the silk sutures to prevent recanalization.

The technique performed in this report was similar to the standard technique. The differences were the position of the surgeon, the direction of dissection, and the suture passage. Although, in the veterinary field, the standing position of the surgeon is on the patientís ventral side, the surgeon in our report was standing on the patient's dorsal side. This standing position is common in surgical PDA ligation for infants. Thus, the surgeon was familiar with anatomical landmarks viewed in this position (Figure 6) rather than the ventral side position.

Before dissection around the ductus, the vagus nerve was retracted ventrally. Ventral retraction is described in both veterinary and human surgery for PDA (Mandhan et al., 2006; Valentik et al., 2007; Brockman et al., 2018).

Because the surgeon was standing on the dorsal side of the patient, retracting the vagus nerve ventrally toward the assistant, away from the surgeon, can give an advantage in terms of the working space.

The dissection of PDA was performed from the right to the left direction according to the surgeon's position. From his experience, since there is more space caudal to the ductus, it is easier to pass the tip of the right-angle forceps in that direction. Moreover, it is convenient to introduce the suture into the jaws of forceps when there is more room. Also, dissection from the right to the left might be more convenient for a right-handed surgeon. However, in this report, the direction of dissection and suture passage (although used in human surgery) is the opposite direction of the usual direction recommended in the veterinary field. The experienced surgeon contributed to successful ligation of the ductus without intraoperative complications. Authors encourage using methods that are well described and studied in small animals. Nevertheless, in older animals where the ductal surrounding tissue could be more fibrous, opening the cranial plane of the PDA is difficult. After creating a caudal dissection plane, changing the standing position can be beneficial to right-handed surgeons during the opening of the cranial plane since it aids with the hand direction during dissection.

Other methods to ligate PDA have been described and studied in small animals, including Jackson-Henderson and intrapericardial technique. Jackson-Henderson method avoids direct dissection of the PDA by passing the suture around the descending aorta on either side of the ductus. However, the incidence of residual flow was reported to be higher than that in the standard technique (Stanley et al., 2003).

Selmic et al. (2013), thoroughly described an intrapericardial technique. This technique provides a lower rate of residual flow than the others. In this technique, an incision is made into the pericardium and mediastinal pleura perpendicular to the ductus and immediately ventral to the insertion of fibrous pericardium. The location of the incision is limited because the fibrous pericardium is inseparable from the adventitia of the aorta at the level of the brachiocephalic trunk and from the adventitia of the pulmonary artery proximal to the bifurcation. This fibrous pericardium in the aorta and pulmonary artery adventitia was also noticed during surgery in our patient. The intrapericardial technique, when applicable, provides better visualization of the ventral aorta, pulmonary artery, and margins of the ductus. Blind dissection on the medial side of the ductus can be avoided. Moreover, less periductal tissue is included in the ligature. Thanks to these advantages, the rate of perioperative hemorrhage and echocardiographic residual flow are low.

In conclusion, our patient, a fully grown Pomeranian with left-to-right shunt PDA, underwent open surgical ligation. The surgical technique used in this study was similar to the human surgical PDA ligation technique (the details are mentioned in this report). Thanks to experience and skill of the surgeon, there were no intraoperative complications. Recovery was smooth, and the patient was discharged after three days of hospitalization. Cardiomegaly was improved after surgery. The long-term outcomes are to be monitored through clinical examination, thoracic radiography, echocardiography, and electrocardiography. Long-term outcome is expected to be good. A possible but rare, long-term complication is recanalization. It is 1%-3% for extrapericardial ligation and can develop six weeks to 37 months after surgery.

The overall result of the surgery was satisfactory up to the publication date.

Acknowledgments

The authors would like to express sincere appreciation to Dr. Piya Cherntanomwong for his kind and foremost cooperation. The authors would also like to thank Asst. Prof. Dr. Rungrote Osathanon for his valuable advice and the medical team at Prasu-Arthorn Animal Hospital for their great work in both pre- and postoperative medical management.

References

Boutet BG, Saunders AB, Gordon SG. Clinical characteristics of adult dogs more than 5 years of age at presentation for patent ductus arteriosus. J Vet Intern Med. 2017;31(3):685-90.

Broaddus K, Tillson M. Patent ductus arteriosus in dogs. Compendium. 2010;32(9):E3. Available from: https://www.vetfolio.com/learn/article/patent-ductus-arteriosus-in-dogs.

Brockman D, Holt D, Haar G. Surgery of the heart, pericardium and great vessels. In: BSAVA manual of canine and feline head, neck and thoracic surgery. 2nd ed. BSAVA; 2018. p. 195-8.

Buranakarl C, Kijtawornrat A, Udayachalerm W, Chuthathep S, Tungjitpeanpong R, Chaivoravitsakul N. Intervention technique using transvenous patent ductus arteriosus (PDA) occluder in a dog. Thai J Vet Med. 2015;45(4):593-602.

Bureau S, Monnet E, Orton EC. Evaluation of survival rate and prognostic indicators for surgical treatment of left-to-right patent ductus arteriosus in dogs: 52 Cases (1995-2003). J Am Vet Med Assoc. 2005;227(11):1794-9.

Fossum T. Surgery of the cardiovascular system. In: Small animal surgery. 4th ed. St. Louis: Mosby; 2013. p. 871-6.

Glaus TM, Martin M, Boller M, Stafford Johnson M, Kutter A, Flückiger M, Tofeig M. Catheter closure of patent ductus arteriosus in dogs: Variation in ductal size requires different techniques. J Vet Cardiol. 2003;5(1):7-12.

Goodrich KR, Kyles AE, Kass PH, Campbell F. Retrospective comparison of surgical ligation and transarterial catheter occlusion for treatment of patent ductus arteriosus in two hundred and four dogs (1993-2003). Vet Surg. 2007;36(1):43-9.

Van Israël NV, French AT, Dukes-McEwan J, Welsh EM. Patent ductus arteriosus in the older Dog. J Vet Cardiol. 2003;5(1):13-21.

Mandhan PL, Samarakkody U, Brown S, Kukkady A, Maoate K, Blakelock R, Beasley S. Comparison of suture ligation and clip application for the treatment of patent ductus arteriosus in preterm neonates. J Thorac Cardiovasc Surg. 2006;132(3):672-4.

Orton E, Monnet E, McCracken T, Borman M. Patent ductus arteriosus. In: Small animal thoracic surgery. John Wiley-Blackwell; 2018. p.177-82.

Parchman MB. A simple manoeuvre to aid in suture passage during ligation of patent ductus arteriosus. J Small Anim Pract. 2008;33(2):59-60.

Saunders AB, Gordon SG, Boggess MM, Miller MW. Long-term outcome in dogs with patent ductus arteriosus: 520 Cases (1994-2009). J Vet Intern Med. 2014;28(2):401-10.

Selmic LE, Nelson DA, Saunders AB, Hobson HP, Saunders WB. An intrapericardial technique for PDA ligation: Surgical description and clinical outcome in 35 dogs. J Am Anim Hosp Assoc. 2013;49(1):31-40.

Stanley BJ, Luis-Fuentes V, Darke PG. Comparison of the incidence of residual shunting between two surgical techniques used for ligation of patent ductus arteriosus in the dog. Vet Surg. 2003;32(3):231-7.

Valentík P, Omeje IC, Poruban R, Šagát M, Nosál M. Surgical closure of patent ductus arteriosus in pre-term babies. Images Paediatr Cardiol. 2007; 9(2):27-36.

หลักเกณฑ์และอัตราค่าบริการการตรวจวินิจฉัยทางห้องปฏิบัติการ ของศูนย์เฝ้าระวังและติดตามโรคจากสัตว์ป่า สัตว์ต่างถิ่นและสัตว์อพยพ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

หลักเกณฑ์และอัตราค่าบริการการตรวจวินิจฉัยทางห้องปฏิบัติการ ของศูนย์เฝ้าระวังและติดตามโรคจากสัตว์ป่า สัตว์ต่างถิ่นและสัตว์อพยพ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

การตรวจพยาธิภายนอก

รายการตรวจ	วิธีการตรวจ	ชนิดสิ่งส่งตรวจ	ระยะเวลาการรายงานผล	ค่าบริการ (บาท) ต่อตัวอย่าง*
ตรวจระบุชนิดพยาธิภายนอก	Morphology identification	พยาธิภายนอกที่เก็บในแอลกอฮอล์	ภายใน 5 วันทำการ	400

การตรวจเพาะเลี้ยงแบคทีเรีย

รายการตรวจ	วิธีการตรวจ	ชนิดสิ่งส่งตรวจ	ระยะเวลาการรายงานผล	ค่าบริการ (บาท) ต่อตัวอย่าง*
Leptospira spp.	เพาะเลี้ยงในอาหารเลี้ยงเชื้อ	ปัสสาวะ เลือด ชิ้นเนื้อไต ตัวอย่างสิ่งแวดล้อม (ดินและ/หรือน้ำ) (สิ่งส่งตรวจทุกชนิดห้ามแช่เย็น)	ภายใน 4 เดือน	300
Chlamydiales***	เพาะเลี้ยงในเซล์เพาะเลี้ยง	ตัวอย่างป้ายจากคอและ/หรือตา ชิ้นเนื้ออวัยวะภายใน (สมอง ตับ ไต หัวใจ ปอด ม้าม กระเพาะอาหาร หลอดลม)	-	3,000

การตรวจด้วยวิธีทางอิมมูโนฮิสโตเคมี

รายการตรวจ	วิธีการตรวจ	ชนิดสิ่งส่งตรวจ	ระยะเวลาการรายงานผล	ค่าบริการ (บาท) ต่อตัวอย่าง*
Canine distemper virus***	Immunohistochemistry (IHC)	Paraffin sections and cytology preparations on a slide with a positivecharge	-	200 (ไม่รวมค่าทำและตัดพาราฟินบล็อค)
Canine distemper virus***	Indirect Immunofluorescence Assay (IFA)	Tissue or tissue smear	-	200
Chlamydia spp.***	IHC	Paraffin sections and cytology preparations on a slide with a positive charge	-	200 (ไม่รวมค่าทำและตัดพาราฟินบล็อค)
Chlamydia spp.***	IFA	Tissue or tissue smear	-	200
Newcastle disease virus***	IHC	Paraffin sections and cytology preparations on a slide with a positive charge	-	200 (ไม่รวมค่าทำและตัดพาราฟินบล็อค)
Newcastle disease virus***	IFA	Tissue or tissue smear	-	200
Rabies virus antigen***	Direct Immunofluorescent Assay (DIFA)	Brain tissue or The brain smear	ภายใน 7 วันทำการ	200
Avian influenza A virus***	IHC	Paraffin sections and cytology preparations on a slide with a positive charge	-	-
Rabies virus (antibody detection)	Fluorescent antibody virus neutralization assay (FAVN)	Serum or plasma	15 วันทำการ	1-50 ตัวอย่าง ราคาตัวอย่างละ 2,500 บาท มากกว่า 50 ตัวอย่าง ราคาตัวอย่างละ 2,000 บาท

การทำสัตว์สตั๊ฟฟ์

รายการ	ราคา (บาท)	หมายเหตุ
สัตว์เลี้ยงลูกด้วยนม, สัตว์เลี้ยง		
1. น้ำหนักไม่เกิน 500 กรัม	700	
2. น้ำหนักตั้งแต่ 500 กรัม - 1 กิโลกรัม	1,000	รับสตั๊ฟฟ์เฉพาะสัตว์ที่มี
3. น้ำหนักตั้งแต่ 1.1 - 2 กิโลกรัม	1,400	น้ำหนักตัวไม่เกิน 5 กิโลกรัม
4. น้ำหนักตั้งแต่ 2.1 - 3 กิโลกรัม	2,100	(ในกรณีที่น้ำหนักตัวเกิน
5. น้ำหนักตั้งแต่ 3.1 - 4 กิโลกรัม	3,500	5 กิโลกรัม ราคาตามแต่ตกลง)
6. น้ำหนักตั้งแต่ 4.1 - 5 กิโลกรัม	5,000	
สัตว์ปีก		
1. ความยาวไม่เกิน 20 เซนติเมตร	1,000	
2. ความยาวตั้งแต่ 21 - 50 เซนติเมตร	2,000	คิดราคาตามขนาดความยาวตั้งแต่ปลายปาก - โคนหาง (ซม.)
3. ความยาวตั้งแต่ 51 ขึ้นไป	ราคาตามแต่ตกลง	
ปลา		
1. โชว์ด้านเดียว		
1.1 ความยาว 1 - 15 เซนติเมตร	500	คิดราคาตามขนาดความยาวตั้งแต่ปลายปาก - ปลายหาง (ซม.)
1.2 ความยาว 15 - 30 เซนติเมตร	2,000*	*คิดเพิ่ม 1,000/ความยาวที่เพิ่มขึ้น 15 เซนติเมตร
2. โชว์ 2 ด้าน		
2.1 ความยาว 1 - 15 เซนติเมตร	500	**คิดเพิ่ม 1,500/ความยาวที่เพิ่มขึ้น 15 เซนติเมตร
2.2 ความยาว 15 - 30 เซนติเมตร	3,000**	
สัตว์เลื้อยคลานและ		
สัตว์สะเทินน้ำสะเทินบก		
1. กลุ่ม Lizard และงู		
1.1 ความยาว 1-15 เซนติเมตร	700	คิดราคาตามขนาดความยาวตั้งแต่ปลายปาก - ปลายหาง (ซม.)
1.2 ความยาว 15-30 เซนติเมตร	1,000***	***คิดเพิ่ม 1,500/ความยาวที่เพิ่มขึ้น 15 เซนติเมตร
2. เต่า	1,000/10 เซนติเมตร****	****คิดเพิ่ม 1,000/ความยาวที่เพิ่มขึ้น 10 เซนติเมตร

หมายเหตุ

* ราคานี้เฉพาะค่าตรวจทางห้องปฏิบัติการเท่านั้น ไม่รวมค่าหัตถการสำหรับการเก็บตัวอย่างจากสิ่งมีชีวิต

** โปรดติดต่อเจ้าหน้าที่ห้องปฏิบัติการก่อนส่งตรวจ

*** รับตรวจเฉพาะงานวิจัย หากต้องการส่งตรวจโปรดติดต่อเจ้าหน้าที่ห้องปฏิบัติการ

**** ตัวอย่างตรวจ Lyssavirus หรือ Rabies virus จะต้องเก็บตัวอย่างโดยสัตวแพทย์เท่านั้น และโปรดติดต่อห้องปฏิบัติการก่อนส่งทุกครั้ง

***** รับเฉพาะสิ่งส่งตรวจเท่านั้นและติดต่อห้องปฏิบัติการก่อนส่งตรวจอย่างน้อย 1 วัน

รายละเอียดการติดต่อ

คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล

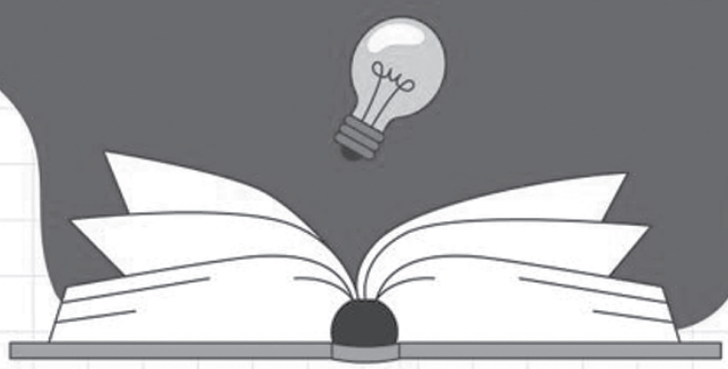
เลขที่ 999 ถ.พุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170 โทร. 02-441-5242

มหาวิทยาลัยมหิดล
คณะสัตวแพทยศาสตร์

#อยากเรียนต่อสัตวแพทย์

Graduate diploma program in clinical veterinary science

เปิดรับสมัครเข้าศึกษาต่อ ระดับประกาศนียบัตรบัณฑิต



เปิดรับสมัค
โดยคณะสัตวแพทยศาสตร์
2 รอบ
(อาจมีรอบ 3 หากมีที่ว่าง)

รับสมัครตั้งแต่วันที่ 1 ธันวาคม 2564
ผ่านระบบของบัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล
ปิดรับสมัครรอบที่ 1 วันที่ 30 เมษายน 2565
ปิดรับสมัครรอบที่ 2 วันที่ 25 มิถุนายน 2565

*สำหรับผู้สมัครรอบ 2 ก่อนชำระเงินค่าสมัคร
โปรดติดต่อประธานหลักสูตรก่อนเพื่อประเมินจำนวนรับนักศึกษาที่สามารถรับได้
ผ่านทาง e-mail: namphung.sue@mahidol.edu

เมื่อชำระค่าสมัครแล้ว ทางหลักสูตรสงวนสิทธิ์ไม่คืนเงินค่าสมัครในทุกกรณี

ดูรายละเอียดเพิ่มเติมได้ที่ www.grad.mahidol.ac.th

vs.mahidol.ac.th facebook.com/MUVetSci/ 02-441-5242

Mahidol University
Faculty of Veterinary Science

Study Veterinary Science at Thailand's Top University*

*Ranked by:
QS World University Rankings by Subject 2019
Times Higher Education Asia University Rankings 2019
The CWTS Leiden Ranking 2019
2019 Asian University Ranking by UniRank (4icu.org)
UI Green Metric World University Rankings 2018

Undergraduate Programme

Doctor of Veterinary Medicine (6 years programme)

Postgraduate Programme

Doctor of Philosophy (PhD) in Veterinary Science (International Programme)

- Production Medicine
- Companion Animal Medicine
- Pathology
- Epidemiology

Master of Science (MSc) in Veterinary Biomedical Science (International Programme)

- Veterinary Diagnostic Sciences
- Applied Veterinary Biomedical Sciences and Veterinary Public Health
- Zoo Animal and Wildlife Health Management

Graduate Diploma (Grad Dip) (International Programme)

- Clinical Veterinary Science

For more information please contact www.vs.mahidol.ac.th **or call** 02-441-5248 /MUVetSci