JOURNAL OF
APPLIED ANIMAL SCIENCE

ISSN 1906-2257 (Print)
ISSN 2985-1211 (Online)

VOL.16 NO.2 JULY-DECEMBER 2023

"Journal of Applied Animal Science" (JAAS)

Scope of the Journal

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

Journal Management

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. 081-826-5455 E-mail address: pointswift@yahoo.com

"Journal of Applied Animal Science" (JAAS)

สารจากคณบดี

เรียน ท่านผู้อ่านและผู้สนใจทุกท่าน

ในวารสาร Journal of Applied Animal Science (JAAS) Vol. 16 No. 2 (2023): July-December นี้มีเนื้อหาที่น่าสนใจและหลากหลายด้านทั้งจากในประเทศไทยและต่างประเทศ ที่จะสร้างองค์ความรู้และแนวทางในการประยุกต์ใช้ในวงการสัตวแพทย์ ซึ่งมีเนื้อหาสาระที่เป็นการอัปเดตความรู้ในเรื่องการวินิจฉัยและการรักษาใหม่ ๆ และขอถือโอกาสนี้ขอบคุณเนื้อหาจากผู้ประพันธ์ที่ได้มีส่วนร่วมในฉบับนี้ ประกอบด้วย Research Article 1 เรื่อง Short communication 1 เรื่อง และ case report 2 เรื่อง ดังนี้

Research article: Reproductive Potentials of Female Japanese Quails to Administration of Egg-Lime-Molasses Mixture

Short communication: Serological Detection of Brucella Infection in Small Ruminant in Nhong-Mamong District, Chainat Province, Thailand

Case report:

1. Metastasis of Thyroid Gland Carcinoma to Mandibular Lymph Node
2. Laser Acupuncture Treatment in a Ferret with Spinal Cord Injury

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

และขอถือโอกาสนี้ ส่งความสุขปีใหม่ ค.ศ.2024 ขอให้สิ่งศักดิ์สิทธิ์ทั้งหลายที่ท่านนับถือ โปรดอำนวยอวยพรให้ผู้อ่านทุกท่าน และครอบครัว ญาติ มิตร มีแต่ความสุข ความเจริญ คิดหวังสิ่งใดขอให้สมความมุ่งมาตร ปรารถนาที่ตั้งใจโดยทุกประการ

รองศาสตราจารย์ ดร.สัตวแพทย์หญิงวลาสินี ศักดิ์คำดวง
คณบดีคณะสัตวแพทยศาสตร์
มหาวิทยาลัยมหิดล

"Journal of Applied Animal Science" (JAAS)

ปีที่ 16 ฉบับที่ 2 กรกฎาคม-ธันวาคม 2566 Vol. 16 No. 2 July-December 2023

สารบัญ

คำแนะนำสำหรับผู้แต่ง

"Journal of Applied Animal Science" (JAAS)

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

ผู้สนใจส่งบทความเพื่อตีพิมพ์ในวารสารสัตวศาสตร์ประยุกต์กรุณาปฏิบัติตามข้อแนะนำและส่งพร้อมจดหมายนำ

1. ประเภทบทความ ที่รับพิจารณาได้แก่ รายงานการวิจัย รายงานฉบับย่อ บทความปริทัศน์และรายงานทางคลินิกเขียนด้วยภาษาไทยหรือภาษาอังกฤษ แต่บทคัดย่อต้องมีทั้งภาษาไทยและภาษาอังกฤษ

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

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

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

ก. รายงานฉบับย่อและรายงานทางคลินิก อาจเขียนโดยไม่แยกหัวข้อ หรืออาจรวมส่วนผลการวิจัยและวิจารณ์เป็นหัวข้อเดียว

ข. บทความปริทัศน์ ควรเริ่มด้วยบทนำ แล้วบรรยายโดยแยกตามหัวข้อที่ต้องการนำเสนอ พร้อมบทสรุป

5. ตาราง-รูปภาพ ตารางและรูปภาพให้แทรกไว้ท้ายสุดของบทความ คำบรรยายตารางพิมพ์ด้านบน คำบรรยายรูปภาพพิมพ์ใต้ภาพ และมีหมายเลขอาระบิกกำกับตามลำดับการอ้างถึง ตารางควรเข้าใจได้ง่าย ให้ส่งรูปภาพความละเอียดสูงแยกต่างหากมาพร้อมด้วย

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

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

7. ชื่อวิทยาศาสตร์ ให้พิมพ์เป็นภาษาอังกฤษตามประมวลนามศัพท์สากลและทำให้เด่นแตกต่างจากเนื้อหา

8. การถอดคำไทยเป็นภาษาอังกฤษ ใช้หลักเกณฑ์การถอดอักษรไทยเป็นอักษรโรมันแบบถ่ายเสียงของราชบัณฑิตยสถาน

9. อักษรย่อและสัญลักษณ์ หากเป็นที่รับรู้โดยทั่วกันอนุโลมให้ใช้ได้โดยไม่ต้องพิมพ์ตัวเต็มก่อน

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

อีเมลบรรณาธิการวารสาร editor.jaas2020@gmail.com

ที่อยู่ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล 999 ถนนพุทธมณฑล สาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จังหวัดนครปฐม 73170

Instructions to Authors

"Journal of Applied Animal Science" (JAAS)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

9. Standard abbreviations and symbols are acceptable without definition.

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

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

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

Editor Note

สวัสดีส่งท้ายปีท่านผู้อ่านและสมาชิกวารสาร Journal of Applied Animal Science (JAAS) ทุกท่าน พบกันอีกครั้งในฉบับสุดท้ายของปี พ.ศ. 2566 สำหรับเนื้อหาในฉบับนี้ยังคงประกอบด้วยบทความที่น่าสนใจได้แก่ บทความวิจัย 2 เรื่อง **"Reproductive Potentials of Female Japanese Quails to Administration of Egg-Lime-Molasses Mixture"** และ **ผลบวกทางซีรัมวิทยาต่อโรคติดเชื้อบรูเซลลาในสัตว์เคี้ยวเอื้องขนาดเล็กของอำเภอหนองมะโมง จังหวัดชัยนาท ประเทศไทย** รายงานสัตว์ป่วย 2 เรื่อง **"การแพร่กระจายของมะเร็งของต่อมพาราไทรอยด์ไปสู่ต่อมน้ำเหลืองที่บริเวณกราม"** และ **"การรักษาภาวะไขสันหลังบาดเจ็บในเฟอเรท โดยการฝังเข็มด้วยเลเซอร์"** ซึ่งทางกองบรรณาธิการหวังเป็นอย่างยิ่งว่าบทความทั้ง 4 เรื่องจะเป็นประโยชน์แก่ท่านผู้อ่านไม่มากก็น้อย อย่างไรก็ตาม หากท่านผู้อ่านมีข้อสงสัยหรือข้อเสนอแนะประการใด โปรดแจ้งมาที่วารสารเพื่อปรับปรุงให้ดียิ่งขึ้นในโอกาสต่อไป

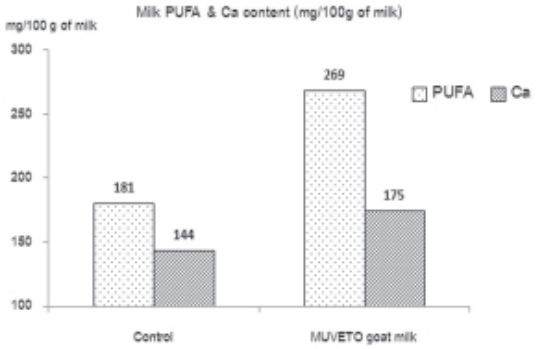
นอกจากนี้ ทางกองบรรณาธิการขอถือโอกาสแจ้งให้ทุกท่านทราบว่าในปี พ.ศ. 2567 ทางวารสารจะมีการยื่นประเมินเพื่อจัดกลุ่มคุณภาพวารสารใหม่ในรอบที่ 5 (พ.ศ. 2568-2572) ตามเกณฑ์การประเมินของศูนย์ดัชนีการอ้างอิงวารสารวิชาการไทย (TCI) ซึ่งมีเป้าหมายในการกระตุ้นให้เกิดการพัฒนาคุณภาพในการบริหารจัดการของวารสารไทย ทั้งในด้านเนื้อหาและรูปแบบให้มีมาตรฐานระดับสากล

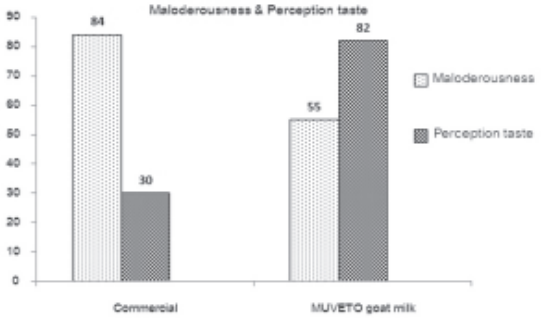
สุดท้ายนี้ ในนามหัวหน้ากองบรรณาธิการ ขอขอบพระคุณกองบรรณาธิการวารสาร ผู้ทรงคุณวุฒิที่ให้ความอนุเคราะห์ในการพิจารณาบทความและให้คำแนะนำอันเป็นประโยชน์ต่อการพัฒนาคุณภาพของบทความสู่วงการวิชาการ และที่สำคัญขอขอบพระคุณสมาชิกวารสารและผู้นิพนธ์บทความทุกท่านที่ให้การสนับสนุนการดำเนินงานของวารสารมาอย่างต่อเนื่องและหวังเป็นอย่างยิ่งว่าจะได้รับการสนับสนุนจากทุกท่านอีกในปีถัดไป

รองศาสตราจารย์ ดร.นายสัตวแพทย์ธนศักดิ์ ช่างบรรจง

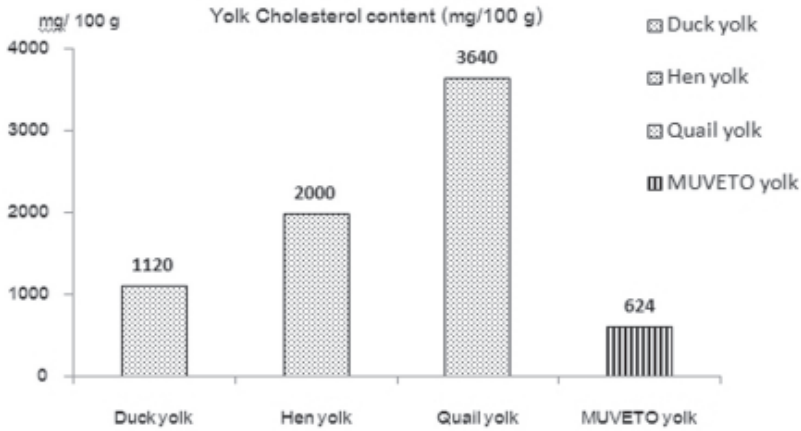
บรรณาธิการ (Editor-in-Chief)

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

Reproductive Potentials of Female Japanese Quails to Administration of Egg-Lime-Molasses Mixture

Adeyinka Oye Akintunde[1*] Lois Chidinma Ndubuisi-Ogbonna[1]
Mofiyinfoluwa Modupe Ladele[1] Oladapo Ayodeji Olorunfemi[1] Olayinka Abosede Ojo[2]
Olufunso Emmanuel Akinboye[1] Bolatito Adenike Shobo[1] Osagie John Afodu[1]

[1]Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria
[2]Department of Animal Production Fisheries and Aquaculture, Kwara State University, Malete, Kwara State, Nigeria

[*]Corresponding author, E-mail address: adeyinka.akintunde@gmail.com

Received: 29 September 2023; Revised: 1 November 2023; Accepted: 2 November 2023

Abstract

Japanese quails are hardy with huge potentials to bridge protein deficiency, hence the need for an assessment of their reproductive potentials. The egg-lime-molasses mixture (ELM) was prepared by placing 10 fresh chicken eggs (average weight of 58 g per egg) in a bowl, after which 1 liter of lime juice and 500 g of molasses were added, then it was covered and left for 10 days. The solution was then blended. Two hundred day-old Japanese quails were assigned to 5 treatments with 40 birds in a completely randomized design (CRD) and 4 replicates of 10 birds each. The control (T1) had no ELM, while treatments 2, 3, 4 and 5 (T2, T3, T4, and T5) had inclusions of 2%, 4%, 6%, and 8% ELM in water, respectively. Data were collected on the morphometrics, egg characteristics, follicle stimulating, and leutenizing hormone concentrations. The findings revealed that at the early stage of laying, the administration of ELM did not have a significant effect ($p > 0.05$) on egg parameters. However, at the latter phase of egg laying, there was a significant effect ($p < 0.05$) on shell weight and albumen percentage. ELM did not have a significant effect ($p > 0.05$) on the production performance. There was no significant effect ($p < 0.05$) on follicular characteristics, while birds administered 30ml ELM/500ml of water had the significantly highest ($p < 0.05$) weights of oviduct (1.01 ± 0.01 g) and ovary (3.995 ± 0.005 g). Birds administered higher levels of ELM had significantly higher concentrations ($p < 0.05$) of leutenizing and follicle stimulating hormones. The inclusion of aqueous administration of ELM in drinking water has no detrimental effect on the reproductive potentials of female Japanese quails.

Keywords: Fertilizing, Hormones, Ovary, Oviduct

Introduction

Raising poultry primarily serves the functions of producing meat, eggs, and feathers, as well as occasionally for religious rituals (Scanes et al., 2004). Poultry are effective food-to-feed converters. The highest nutritional benefits are provided by foods of poultry origin, with eggs being particularly high in all essential nutrients and both palatable and nutrient-dense (Scanes et al., 2004).

Japanese quails are noted for their early maturity. By the time they are 50 days old, they are fully developed and have been seen to lay their first eggs. Its production cycle takes between 300 and 320 days to complete. Hens could lay 200 eggs in their first year of laying. Only two to two and half years are considered to be the average lifespan. The typical egg weighs about 10g or 8% of the quail's overall weight. The fact that quail eggs are a source of protein, vitamins, and lipids such as phospholipids and polyunsaturated fatty acids makes them a functional food (Meluzzi et al., 2000). Quail eggs have a significantly better nutritional value than other eggs since they are rich in antioxidants, minerals, and vitamins and provide a lot more nutrients than other foods (Lalwani 2011).

Due to the health, nutritional, and economic advantages of quail, the market for quail birds and its products is rising, albeit very slowly, particularly in emerging nations like Nigeria. As a result, efforts to increase the level of quail production in the developing nations must be stepped up in order to keep up with this rising demand. One of the most promising methods for bridging the animal protein shortfall is quail farming. Due to modern applications like consumption of its goods and frequently its numerous by-products, the manufacturing of quail products is distinctive and has attracted particular attention. However, the potential of raising poultry has not been completely realized since most farmers have focused only on raising chickens, despite the fact that other birds have similar economic, social, and nutritional advantages. It is significant to note that numerous initiatives have been made to increase Nigeria's overall poultry production. However, it has been shown that a number of variables, including nutrition and genetics, affect egg production (Akintunde and Toye 2021, 2022).

According to Akintunde et al. (2023), the aqueous mixture of eggs, lime, and molasses had the following compositions: moisture content (19.60%), crude protein (15.20%), lipids (5.50%), ash (14.60%), crude fiber (9.60%), carbs (35.20%), fatty acid (4.40%), and energy (1060.30 Kcal/100 kg). Alkaloids, flavonoids, glycosides, saponin, steroids, phenols, terpenoides, tannin, and antraquinones were all found in ELM after phytochemical analysis. According to quantitative analysis of the phytochemicals, ELM contains alkaloids (8.46 mg/100 g), flavonoids (2.30 mg/100 g), glycosides (0.08 mg/100 g), saponin (5.25 mg/100 g), steroids (0.22 mg/l00 g), phenols (0.09 mg/100 g), terpenoides (0.56 mg/100 g), tannin (8.34 mg/100 g), and antraquinones (1.60 mg/100 g). The components of the vitamins are as follows: Vitamin A (3.20 mg/100 g), Vitamin B1 (280 mg/100 g), Vitamin B2 (880 mg/100 g), Vitamin B3 (340 mg/100 g), Vitamin C (15.40 mg/100 g), and Vitamin E (0.015 mg/100 g). According to the mineral analysis, it contains silicon (22.70 ppm), calcium (29.95%), magnesium (4.08%), potassium (23.20%), sodium (0.38%), phosphorus (6.90%), chlorine (0.30%), manganese (1.44 ppm), iron (3.60 ppm), aluminum (5.35%), titanium (2.10 ppm), and magnesium (4.08%) (Akintunde et al., 2023). The study was aimed at determining the reproductive potentials of female Japanese quails to oral administration of egg-lime-molasses mixture.

Materials and Methods

Experimental Site

This experiment was performed at a standard poultry unit (approved for research by the Department of Agriculture and Industrial Technology, Babcock University) at the farm house of Babcock University, Ilishan-Remo Ogun State, Nigeria. Babcock university is located at latitude 6.8920 °N and longitude 3.7181 °E. It is covered predominantly by rain forest and has wooden savanna in the northwest. with an annual rainfall of about 1500 mm, with a mean temperature range of 27 - 31 degree census and a very high relative humidity (above 87%).

Preparation of Egg Lime Molasses Solution

The eggs of chickens were first checked to make sure they were incredibly fresh; this was done by submerging them in water. Following the placement of 10 fresh eggs in a bowl, 1 liter of lime juice and 500 g of molasses from sugarcane were then added. The bowl was then carefully covered and left for 10 days at a temperature of 27 °C and a relative humidity of 61%. The egg shells had broken down into solution after 10 days. The entire mixture was then blended together.

Experimental Birds

The day-old of the Japanese quail were obtained from a local farmer in Lagos State, Nigeria. The pen was cleaned, disinfected, and given time to air dry two weeks prior to the arrival of the 200 Japanese Quails. Also thoroughly cleaned and disinfected were the drinkers and feeders. To provide heat and illumination at night for continuous feed intake, 100 watt electric lamps were fitted in the cages. A completely randomized design (CRD) was utilized to randomly assign the birds to five treatments, each of which had four replicates and ten birds per replication.

Experimental Treatments

The development of five oral medications was done. The control (T1), which had no egg lime molasses solution in the water while the treatment groups 2, 3, 4 and 5 (T2, T3, T4, and T5) had 2%, 4%, 6% and 8% ELM/ drinking water respectively. In the first week, the third week, the fifth week, and the seventh week, the birds were administered the egg-lime-molasses solution five days each. The starter phase of the experiment was from day one to day 28 while the finisher phase was from day 28 to day 49.

Data Collection

Data were collected on egg production parameters, egg quality (internal and external qualities) and morphometrics of the female reproductive tracts.

Egg Production Parameters

At day 28, sexing was performed and 28 female birds were used per treatment (7 birds per replicate) for the study. The following data were then taken:

i. Age at first lay: The number of days from point of lay to the day the first egg is laid.

ii. Body weight of hen at first lay: This was measure as weight of each live pullet average over the number of pullets' weight per group at first lay.

iii. Weight of first egg: weight of each first egg average over the weight of first eggs laid per group using a balance sensitivity of 0.01 g.

iv. Hen day production: Number of eggs collected divided by number of birds alive as at that day multiplied by 100%.

Egg Quality Evaluation

Two eggs per replicate were sampled for egg quality evaluation at first lay, and then weekly till day 49.

Internal Egg Qualities

The following measurements were determined:

i. Albumen height: The eggs were softly cracked, and a tripod spherometer was used to measure the maximum albumen height (Doyon et al., 1986).

ii. % Albumen weight: This was calculated as the percentage of the albumen weight to the egg weight.

iii. Yolk weight: This was measured using Mettler top-loading weighing balance.

iv. Haugh Unit (HU): This was calculated using the values obtained for the egg weight and albumen height as expressed by Haugh (1937) and enunciated by Asuquo et al. (1992) in the formula shown below:

HU = 100log (H+7.5-1.7W0.37)

Where, H = Albumen height in mm

W = Egg weight in gram

Table 1. Composition for experimental starter and finisher diets (Kg/100 kg).

Ingredients	Starter (%)	Finisher (%)
Maize	48.00	59.00
Soybean meal	33.00	30.00
Wheat offal	6.00	5.64
Fishmeal	4.00	-
Palm oil	-	3.00
Vegetable oil	4.00	-
Meat - bone meal	2.50	-
Limestone	1.00	-
Dicalcium phosphate	0.50	1.56
Oyster shell	-	1.00
Salt	0.40	0.25
Methionine	0.20	0.25
Lysine	0.10	0.05
Avatec (Coccidiostat)	-	0.06
%CP	15.20	20.00

External Egg Qualities

i. Egg weight: This was measured using Mettler® top-loading sensitive scale (manufactured by Mettler Toledo, Canada).

ii. Egg length and width: The length and width of each egg were measured using vernier calipers. The width was measured as the distance between two ends of the egg at the widest cross-sectional region using vernier calipers. The length was measured as the distance between the broad and narrow ends of the eggs.

iii. Egg shape Index (ESI): This was calculated as the percentage of the egg breadth (width) to the egg length (Panda 1996). This formula is as follows:

$$\text{Egg Shape Index} = \frac{\text{Width of egg (mm)} \times 100}{\text{Length of egg (mm)} \times 1}$$

iv. Shell thickness: The thickness of individual air-dry shells was measured to the nearest 0.01mm using micrometer screw gauge (Chowdury 1987).

v. Shell weight: Egg shells were air-dried in the crates. The relative shell weight was calculated by relating the shell weight to the weight of the egg.

Determination of Leutenizing Hormone and Follicle Stimulating Hormone Levels

Blood samples were collected from 8 birds per treatment at day 42. Blood collected was harvested into heparinized plastic tubes and the tubes were labeled accordingly. Hormones assay was done for luteinizing hormone (LH) and follicle stimulating hormone (FSH). These were determined by enzyme immunoassay method, using commercial kits Stratus (DADE International Incorporation in immune fluorescence apparatus BAXTER STRATUS II).

Morphometrics of Female Reproductive Organs

After the birds had been slaughtered (12 birds per treatment), the reproductive tract was removed, trimmed free of fat and adhering connective tissue, the body weight, the female reproductive tract, each ovary that had been taken out of the ovarian bursa, the infundibulum, and the oviduct were measured, and morphometric evaluations were carried out using a highly sensitive digital balance. A thread was used to measure the oviduct and infundibulum, which were then promptly remeasured on a ruler and noted.

The relative ovary weight was calculated as ovary weight (wet weight, mg)/BW (g) x 100% where, BW is body weight.

Statistical Analysis

Data collected were presented as mean±standard error, the comparison was done using one-way analysis of variance using statistical package for social science version 22.0 and the treatment means were separated using Duncan Multiple Range Test (Steel and Torries, 1990) at 95% confidence limit.

Results

Table 2 showed that there was no significant difference ($p > 0.05$) in the production performance of the Japanese quails to aqueous administration of egg-lime-molasses mixture (ELM). The administration of ELM did not significantly influence ($p > 0.05$) day at first lay, weight of first egg, total number of eggs laid and hen day production.

Table 3 showed the means of external and internal egg parameters measured of Japanese quails to different concentrations of the egg-lime-mollases solution from day 35 to day 42. There was no significant difference ($p > 0.05$)

among all the parameters across the treatments. Different concentrations of ELM solution did not affect ($p > 0.05$) the internal and external characteristics of the Japanese quail eggs.

Table 4 showed the egg parametes of Japanese quails to administration of ELM from day 42 to day 49. Significant difference ($p < 0.05$) was observed in shell weight, shell percentage and albumen percentage of the Japanese quails where the control had the highest shell weight (1.35 ± 0.07 g) and birds administered 0.40 ml/ 500 ml of water had the least shell weight (1.09 ± 0.03 g). however, birds administered the highest concentration of ELM had the least significant ($p < 0.05$) value for albumen percentage.

Table 5 showed the reproductive morphometrics and hormonal responses of Japanese quails to the administration of ELM. The administration of ELM significantly influenced ($p < 0.05$) the weight of oviduct, weight of infundibulum, ovary weight and relative ovary weight with birds with the administration of 30 ml (T_4) having the significantly highest ($p < 0.05$) weight of oviduct (1.01 ± 0.01 g) and relative ovary weight (3.995 ± 0.005%) while the birds in the control (0ml ELM) had the significantly highest ($p < 0.05$) weight for infundibulum (4.19 ± 0.49 g). Also, the administration of ELM significantly influenced the reproductive hormones (LH and FSH) where birds with the highest concentration of ELM (40 ml per 500 ml of water) had significantly highest ($p < 0.05$) concentrations for leutenizing hormones and follicle imulating hormones.

Table 2. Egg production parameters of Japanese quails to aqueous administration of egg-lime-molasses mixture.

	T1	T2	T3	T4	T5
Day at First Lay	37.25 ± 1.03	39.25 ± 1.60	36.25 ± 1.11	36.75 ± 0.95	36.75 ± 1.49
Weight of First Egg (g)	6.74 ± 0.64	8.23 ± 0.51	7.69 ± 0.76	6.41 ± 0.64	7.31 ± 0.55
Eggs Laid Till Day Day 49	21.25 ± 4.96	16.25 ± 8.68	22.50 ± 5.95	29.25 ± 3.90	24.50 ± 7.10
Hen Day Production (%)	54.25 ± 5.75	55.00 ± 18.48	72.08 ± 4.62	56.69 ± 2.55	62.33 ± 13.95

No significant difference was found between the treatment groups ($p > 0.05$). T1 - 0% ELM; T2 - 2% ELM; T3 - 4% ELM; T4 - 6% ELM; T5 - 8% ELM.

Table 3. Egg properties and qualities of Japanese quails to aqueous administration of egg-lime-molasses mixture (Day 35-42).

	T1	T2	T3	T4	T5
Egg Weight (g)	8.08 ± 0.36	8.33 ± 0.45	8.71 ± 0.24	8.85 ± 0.22	8.81 ± 0.18
Egg Length (mm)	12.95 ± 0.51	12.66 ± 0.81	13.61 ± 0.39	13.35 ± 0.32	13.74 ± 0.29
Egg Width (mm)	7.21 ± 0.3	6.96 ± 0.53	7.74 ± 0.35	8.1 ± 0.33	7.56 ± 0.32
Shell Thickness (mm)	0.18 ± 0.01	0.17 ± 0.01	0.21 ± 0.02	0.16 ± 0.01	0.19 ± 0.02
Shell Weight (g)	1.14 ± 0.04	1.1 ± 0.07	1.16 ± 0.11	1.13 ± 0.03	1.17 ± 0.07
Albumen Height (mm)	2.21 ± 0.08	2.28 ± 0.08	2.3 ± 0.04	2.35 ± 0.05	2.39 ± 0.04
Yolk Height (mm)	8.61 ± 0.12	8.56 ± 0.17	8.64 ± 0.08	8.65 ± 0.08	8.71 ± 0.1
Albumen Weight (g)	4.88 ± 0.25	5.06 ± 0.31	5.26 ± 0.2	5.48 ± 0.17	5.48 ± 0.2
Yolk Weight (g)	2.07 ± 0.09	2.17 ± 0.13	2.3 ± 0.1	2.23 ± 0.08	2.17 ± 0.16
Shell Percentage (%)	14.1 ± 0.23	13.33 ± 0.83	13.33 ± 1.3	12.81 ± 0.21	13.29 ± 0.64
Albumen Percentage (%)	60.23 ± 0.53	60.64 ± 1.16	60.28 ± 1.11	61.92 ± 0.99	62.08 ± 1.62
Yolk Percentage (%)	25.67 ± 0.49	26.03 ± 0.85	26.38 ± 0.93	25.27 ± 0.82	24.64 ± 1.8
Haugh Unit	78.57 ± 0.29	78.73 ± 0.34	78.42 ± 0.22	78.62 ± 0.14	78.92 ± 0.15
Egg Shape Index	0.56 ± 0.01	0.55 ± 0.02	0.57 ± 0.02	0.61 ± 0.03	0.55 ± 0.02

No significant difference was found between the treatment groups ($p > 0.05$). T1 - 0% ELM; T2 - 2% ELM; T3 - 4% ELM; T4 - 6% ELM; T5 - 8% ELM.

Table 4. Egg properties and qualities of Japanese quails to aqueous administration of egg-lime-molasses mixture (Day 42-49).

	T1	T2	T3	T4	T5
Egg Weight (g)	9.41 ± 0.41	9.73 ± 0.39	9.43 ± 0.33	9.59 ± 0.3	9.43 ± 0.33
Egg Length (mm)	13.99 ± 0.61	14.83 ± 0.49	14.09 ± 0.45	14.28 ± 0.42	14.33 ± 0.54
Egg Width (mm)	8.2 ± 0.43	8.48 ± 0.32	8.24 ± 0.3	8.26 ± 0.26	8.4 ± 0.31
Shell Thickness (mm)	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
Shell Weight (g)	1.35 ± 0.07^{b}	1.15 ± 0.05^{a}	1.09 ± 0.03^{a}	1.15 ± 0.05^{a}	1.24 ± 0.05^{ab}
Albumen Height (mm)	2.63 ± 0.11	2.89 ± 0.13	2.94 ± 0.08	2.28 ± 0.44	2.29 ± 0.29
Yolk Height (mm)	9.9 ± 0.21	9.34 ± 0.16	9.36 ± 0.18	9.68 ± 0.22	9.81 ± 0.22
Albumen Weight (g)	5.52 ± 0.27	5.85 ± 0.22	5.76 ± 0.22	5.87 ± 0.17	5.46 ± 0.24
Yolk Weight (g)	2.54 ± 0.11	2.73 ± 0.17	2.59 ± 0.18	2.57 ± 0.16	2.74 ± 0.09
Shell Percentage (%)	14.38 ± 0.55^{c}	11.94 ± 0.59^{ab}	11.67 ± 0.54^{a}	11.96 ± 0.27^{ab}	13.17 ± 0.24^{bc}
Albumen Percentage (%)	58.61 ± 0.7^{ab}	60.2 ± 0.83^{ab}	61.06 ± 1.26^{b}	61.31 ± 1.2^{b}	57.76 ± 0.85^{a}
Yolk Percentage (%)	27.02 ± 0.67	27.86 ± 1.01	27.27 ± 1.3	26.74 ± 1.09	29.07 ± 0.75
Haugh Unit	79.9 ± 0.89	81.34 ± 0.98	82 ± 0.58	76.3 ± 3.61	77.04 ± 2.61
Egg Shape Index	0.59 ± 0.02	0.57 ± 0.01	0.59 ± 0.02	0.58 ± 0.01	0.59 ± 0.01

^{a,b,c} means within a row with difference superscripts are significantly different ($p < 0.05$). T1 - 0% ELM; T2 - 2% ELM; T3 - 4% ELM; T4 - 6% ELM; T5 - 8% ELM.

Table 5. Morphometrics of reproductive organs and reproductive hormomal responses of Japanese quails to aqueous administration of egg-lime-molasses mixture.

	T1	T2	T3	T4	T5
Liveweight (g)	163.50 ± 1.50	178.00 ± 7.00	191.00 ± 35.00	146.50 ± 17.50	157.00 ± 9.00
Weight of Oviduct (g)	0.87 ± 0.07^{ab}	0.63 ± 0.11^{a}	0.81 ± 0.09^{ab}	1.01 ± 0.01^{b}	0.68 ± 0.06^{a}
Length of Oviduct (mm)	6.25 ± 0.15	7.40 ± 0.40	6.15 ± 1.35	5.90 ± 0.10	5.95 ± 0.95
Weight of Infudibulum (g)	4.19 ± 0.49^{b}	3.04 ± 0.28^{ab}	3.83 ± 0.60^{ab}	3.15 ± 0.15^{ab}	2.40 ± 0.04^{a}
Length of Infudibulum (mm)	19.05 ± 4.55	17.05 ± 2.75	16.75 ± 0.35	18.40 ± 0.40	13.85 ± 3.35
Ovary Weight (g)	1.87 ± 0.23^{a}	2.66 ± 0.20^{a}	3.59 ± 0.36^{b}	3.99 ± 0.00^{b}	2.64 ± 0.12^{a}
Follicle Number	33.00 ± 3.00	33.00 ± 6.00	29.50 ± 7.50	46.00 ± 3.00	49.50 ± 9.50
Relative ovary weight (%)	1.143 ± 0.13^{a}	1.50 ± 0.17^{a}	1.98 ± 0.55^{ab}	2.77 ± 0.33^{b}	1.68 ± 0.02^{ab}
LH (mIU/ml)	4.65 ± 0.05^{c}	4.10 ± 0.10^{b}	3.75 ± 0.05^{a}	5.00 ± 0.00^{d}	5.40 ± 0.10^{e}
FSH (mIU/ml)	2.15 ± 0.05^{a}	2.10 ± 0.10^{a}	1.85 ± 0.05^{a}	2.75 ± 0.25^{b}	3.10 ± 0.10^{b}

^{a,b,c} means within a row with difference superscripts are significantly different ($p < 0.05$). LH ñ Luteinizing hormone; FSH ñ Follicle-stimulating hormone. T1 - 0% ELM; T2 - 2% ELM; T3 - 4% ELM; T4 - 6% ELM; T5 - 8% ELM.

Discussion

The results showed that the administration of ELM did not have significant effect on age at first lay, weight of first egg, total number of eggs laid till day 49 and hen day production. It is well established that nutrition has a significant impact on reproductive processes in the majority of domestic animals (Attia 2018; Akintunde et al., 2020). From an economic perspective, sexual maturity age is a very important characteristic. The initial oviposition age is important since it affects the production in the first year, and the earlier a pullet begins to lay eggs, the sooner money is made. The outcomes concurred with Bolukbasi et al. (2009), who found no increase in laying hen performance with supplementation of black cumin seed oil (1, 2 or 3 ml/kg diet). In a study similar to this one, Cabuk et al. (2006) found that the essential oil mixture made of fennel seed oil and oregano oil did not significantly affect feed intake, egg production, or egg mass. Additionally, laying rate and weight of settable eggs did not change in response to an essential oil mixture supplementation at 24, 36, or 48 mg/kg that included essential oil from oregano, laurel, sage, myrtle, fennel, and citrus (Bozkurt et al., 2009). Similar to this, when diets were supplemented with 200 mg/kg of thyme, sage, or rosemary over a period of 12 weeks, Bolukbasi et al. (2008) reported no effect on laying rate but an increase in egg weight. Additionally, dietary garlic and thyme in laying hens did not affect egg production, egg mass, or FCR, according to Ghasemi et al. (2010). This outcome is consistent with prior findings published by Chowdhury et al. (2012), who found that the addition of 10% garlic paste had no appreciable positive impact on egg production rate. The outcomes concurred with the findings of Akintunde and Toye (2022), who found no discernible difference between Isa Brown chickens and Yoruba Ecotype Nigerian Local Chickens in response to the addition of *Moringa oleifera* seed meal.

The morphological characteristics of quail eggs have a significant impact on embryo growth and hatchability. These factors may be able to provide the nutrition, physical safety, and subsequent smooth hatching process that fast-growing quail embryos require to develop into viable chicks (Bai et al., 2016).

The phrase çegg qualityé refers generally to a number of standards that describe both internal and exterior quality. In contrast to internal quality, which refers to egg white (albumen) clarity and viscosity, air cell size, yolk shape, and yolk membrane strength, external egg quality is concerned with the cleanliness, texture, and shape of the eggshell. These characteristics are frequently used to assess egg quality from a number of perspectives, including egg nutritional content, egg integrity for commercialization, storage and incubation, as well as preservation during storage, in addition to their functional significance. The egg's main organic components are the albumen and yolk, which provide 16 and 11% of the protein and 32 and 1% of the fat, respectively (Willems et al., 2014).

Egg weight in the current experiment was not affected by the various treatments. The results of Bozkurt et al. (2012) and Manafi et al. (2016) are in agreement with these findings. In contrast, Bolükbasi et al. (2008) observed that adding essential oils boosted the egg weight of layer chickens. The findings of this study were also consistent with Manafi et al. (2018)'s observation that dietary treatments had no effect on egg shell thickness. However, in contrast to their findings regarding egg shell weight, which was increased by the addition of phytogenic feed additives (PFA), and albumen and Haugh unit scores, which were significantly decreased by the addition of PFA, the present study found that the addition of ELM had no significant impact on these parameters. According to a study, adding essential oils to the daily diet of laying quails did not significantly affect their Haugh unit scores, shell thickness, eggshell weight, yolk color, yolk weight, or albumen and yolk indices (Bozkurt et al., 2012). The results of Kaya et al. (2013), who reported that plant extract of Origanum vulgare, Thymus vulgaris, thyme oil, origanum oil, garlic oil, anise oil, and fennel oil boosted eggshell strength and eggshell thickness. However, Bozkurt et al. (2012) found that supplementing with essential oils had no impact on laying hens' eggshell strength and thickness. Due to the high energy value of ELM present in molasses and chicken egg shells, there may have been a considerable impact on egg shell weight, shell percentage, and albumen percent. In contrast to Ding et al. (2017), who found that adding the commercial supplement enviva essential oil significantly increased the thickness of the eggshell but had no effect on the shell's resistance when compared to the control group. Considering the fact that ELM was employed in the current study, the contrasting results could be due to variations in the test ingredients. This study also contradicted Nasiroleslami and Torki (2010), who claimed that adding a herbal supplement to the diet of laying birds increased egg weight, shell weight, and shell thickness. The discrepancy could be due to various test substances.

The ovary was significantly affected by the administration of ELM. The ovary weights in this study were between 1.87 and 3.99 g, which was greater than the 0.381 g reported by Akinloye et al. (2014) but lower than the 5.02 g reported by Kashmiri and Oreta (2011) for Japanese quails. The production of sex hormones and the growth of germ cells are the two primary purposes of the ovary in birds (Berg 2000). Leutenizing hormone and follicle stimulating hormone concentrations were significantly higher in the birds administered 6% of

ELM; this result may have been impacted by ovary weight because it was also true for the birds with the smallest ovary weights. Additionally, the number of follicles was largest in birds with substantial highest ovary weights and highest levels of LH and FSH. Thus, it is inferred that the administration of ELM may have a considerable impact on female Japanese quails' capacity for reproduction.

Similar to other bird species, the egg is fertilized in the oviduct by sperm that have been squeezed out by the passage of an egg after being stored in the lower end of the tract. Then, as the next egg approaches, they swim up the tract to meet it. The egg is propelled along by movements of the body and muscular oviduct contractions (Ogwuegbu and Aire 1990; Aughey and Frye 2001). During ovulation, the first order ovocyte travels into the oviduct where it undergoes further maturation, fertilization, the creation of egg shells, and the early phases of embryonic development (Subedi et al., 2008). The oviduct weights of the birds given 30ml of ELM were much higher, and this suggests that giving ELM at this dosage could have effect on female Japanese quail's capacity to reproduce.

According to research by Johnston and Gous (2003), the addition of follicle stimulating hormone (FSH) raised oestrogen levels in laying hens, leading to an increase in the quantity of tiny follicles and the stimulation of their rapid development phase. The high oviduct weight of female Japanese quails in T4 may be correlated with elevated FSH levels. These findings support a study by Musa-Azara et al. (2014) that suggested that oestrogenic compounds from Moringa oleifera inhibited the release of leutinizing hormone and FSH, which in turn decreased levels of endogenous oestrogen and progesterone. The limited number of follicles and low weight of the ovary and oviduct in birds may be related to the lowering of these sexual hormone levels. According to this study, administering larger concentrations of ELM has a follicle-stimulating effect, increasing the number of follicles in the ovaries of birds and resulting in noticeably higher weights for the ovary and oviducts.

Conclusion

It can be concluded from this study that the administration of aqueous solution of egg lime molasses mixture in drinking water of Japanese quails did not alter the egg production parameters of Japanese quails however birds that received 6% ELM had the best reproductive parameters hence administration of 6% is recommended for optimum reproductive performance of Japanese quails.

Competing interest

There is no conflict of interests of any sort between authors or elsewhere.

Acknowledgements

The authors sincerely appreciate the supports of the staff and students of Animal Science Unit, Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria under the leadership of Prof. M.D. Olumide and Dr. A.O. Olarinmoye for approving the use of facilities for the study.

References

Akinloye AK, Oyenekan IO, Okandeji ME, Mustapha OA, Olude MA, Adebayo AO. Gross morphometric study on the reproductive system of Japanese quail (*Coturnix coturnix japonica*). Trop Vet. 2014;32(3-4).

Akintunde AO, Toye AA, Ademola AA, Chimezie VO, Ajayi OA. Sperm Characteristics of Nigeria Local Cocks and Exotic Strain of Cocks fed Graded Levels of *Moringa oleifera* Seed Meal. Trop Anim Prod Inv. 2020;23(2):1-10.

Akintunde AO, Toye AA. Egg Characteristics and Prediction of Egg Weight in Chickens Fed Graded Levels of *Moringa oleifera* Seed Meal. Niger J Genet. 2021;35(1):141-51.

Akintunde AO, Toye AA. Comparative Study on Egg Characteristics of Yoruba Ecotype Nigerian Local Chickens and Isa Brown Chickens Fed Graded Levels of *Moringa oleifera* Seed Meal. Agric Sci Dig. 2022;DOI:10.18805/ag.DF-430.

Akintunde AO, Ndubuisi-Ogbonna LC, Olorunfemi OA, Ladele MM, Shobo BA, Adewole SA, et al. Nutritional and Ethno-Medicinal Potentials of Egg-Lime-Molasses Mixture in Livestock Production. Asian J Dairy Food Res. 2023;42(3):298-306.

Attia FA. The influence of supplementing chamomile and turmeric powder on productive performance and egg quality of laying hens. Egypt Poult Sci. 2018;38(2):451-63.

Aughey E, Frye FL. Comparative veterinary histology with clinical correlates. London: Manson Publishing; 2001.

Bai J, Pang Y, Zhang X, Li Y. Study on the morphological development of quail embryos. Rev Bras Ciênc Avic. 2016;18:91-3.

Berg C. Environmental pollutants and the reproductive system in birds: developmental effects of estrogenic compounds. Uppsala: Acta Universitatis Upsaliensis; 2000.

Bolukbasi SC, Erhan MK, Kaynar O. The effect of feeding thyme, sage and rosemary on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in faeces. Arch Geflugelkd. 2008;72:231-37.

Bolukbasi SC, Kaynar O, Erhan MK, Uruthan H. Effect of feeding Nigella sativa oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in faeces. Arch Geflugelkd. 2009;73:167-72.

Bozkurt M, Alcicek A, Cabuk M, Kucukyilmaz K, Catli AU. Effect of an herbal essential oil mixture on growth, laying traits, and egg hatching characteristics of broiler breeders. Poult Sci. 2009;88:2368-74.

Bozkurt M, Küçükyilmaz K, Catli AU, Çinar M, Bintas E, Çöven F. Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poult Sci J. 2012;91(6):1379-86.

Çabuk M, Bozkurt M, Alcicek A, Çatli AU, Baser KHC. Effect of a dietary essential oil mixture on performance of laying hens in the summer season. S Afr J Anim Sci. 2006;36(4):215-21.

Chowdhury SR, Chowdhury SD, Smith TK. Effects of dietary garlic on cholesterol metabolism in laying hens. Poult Sci. 2012;81:1856-62.

Ding X, Yu Y, Su Z, Zhang K. Effects of essential oils on performance, egg quality, nutrient digestibility and yolk fatty acid profile in laying hens. Anim Nutr. 2017;3(2):127-31.

Doyon G, Bernier-Cardou M, Hamilton RMG, Castaigne F, Randall CJ. Egg Quality. 2. Albumen Quality of Eggs from Five Commercial Strains of White Leghorn Hens during One Year of Lay. Poult Sci. 1986;65(1):63-6.

Ghasemi R, Zarei M, Torki M. Adding medicinal herbs including garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) to diet of laying hens and evaluating productive performance and egg quality characteristics. Am J Anim Vet Sci. 2010;5(2): 151-4.

Haugh RR. The Haugh unit for measuring eggquality. US Poult Mag. 1937;43:552-5.

Johnston SA, Gous RM. Modelling the changes in the proportions of the egg components during a laying cycle. Br Poult Sci. 2007;48(3):347-53.

Kashmiri LA, Oreta S. Role of body weight on reproductive and physiological trait in Japanese quail layers (*Coturnix japonica*). Int J Poult Sci. 2011;10(8):640-3.

Kaya A, Kaya H, Macit M, Celebi S, Esenbuga N, Yoruk MA, Karaoglu M. Effects of dietary inclusion of plant extract mixture and copper into layer diets on egg yield and quality, yolk cholesterol and fatty acid composition. Kafkas Üniv Vet Fak Derg. 2013;19:673-9.

Lalwani P. Quail Egg Nutrition [Internet]. 2011 [cited 2023 Sep 29]. Available from: http://www.buzzle.com/articles/quailegg-nutrition.html.

Manafi M, Hedayati M, Khalaji S. Effectiveness of Phytogenic Feed Additive as Alternative to Bacitracin Methylene Disalicylate on Hematological Parameters, Intestinal Histomorphology and Microbial Population and Production Performance of Japanese Quails. Asian-Australasian J Anim Sci. 2016;29(9):1300-8.

Meluzzi A, Sirri F, Manfreda G, Tallarico N, Franchini A. Effects of dietary vitamin E on the quality of table eggs enriched with n-3 longñchain fatty acids. Poult Sci. 2000;79:539-45.

Musa-Azara SI, Jibrin M, Hassan DI, Yakubu A. Effects of oral administration of *Moringa oleifera* seed on blood chemistry and reproductive performance of female rabbits. Int J Agric Sci Vet Med. 2014;2: 18-21.

Nasiroleslami M, Torki M. Including essential oils of fennel (*Foeniculum vulgare*) and ginger (*Zingiber officinale*) to diet and evaluating performance of laying hens, white blood cell count and egg quality characteristics. Adv Environ Biol. 2010;4:341-5.

Ogwuegbu SO, Aire TA. Ultrastructural studies of the magnum and isthmus of the active oviduct of the indigenous helmeted guinea fowl (*Numida meleagris galeata*, Pallas). Veterinarski Arhiv. 1990;60:101-8.

Omoikhoje SO, Bamgbose AM, Aruna MB. Replacement value of unpeeled cassava root meal (UCRM) for maize in weaner Rabbit Diets. Niger J Anim Prod. 2008;35:63-8.

Scanes CG, Brant G, Ensminger ME. Poultry Science. 4th ed. NewJersey: Pearson Prentice Hall; 2004.

Steele RGD, Torrie JH. Principles and Procedures of Statistics. 2nd ed. New York: McGraw-Hill Book Co Inc.; 1990.

Subedi K, Isobe N, Yoshimura Y. Changes in the localization of immunoreactive avian β-defensin-12 in ovarian follicles during follicular growth and in response to lipopolysaccharide. J Poult Sci. 2008;45:210-4.

Willems E, Decuypere E, Buyse J, Everaert N. Importance of albumen during embryonic development in avian species, with emphasis on domestic chicken. World's Poult Sci J. 2014;70(3):503-17.

Short Communication

Serological Detection of *Brucella* Infection in Small Ruminant in Nhong-Mamong District, Chainat Province, Thailand

Sineenard Jiemtaweeboon[1*] Yupaporn Lanumtiang[2]
Alongkot Tochaeng[3] Anuwat Wiratsudakul[1]

[1]Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand 73170
[2]Livestock and Wildlife Hospital Pasu-Palan, Faculty of Veterinary Science, Mahidol University, Kanchanaburi, Thailand 71150
[3]Department of Livestock Development, Nhong-mamong district, Chainat, Thailand 17120

[*]Corresponding author, E-mail address: sineenard.jie@mahidol.edu

Received: 30 September 2023; Revised: 13 November 2023; Accepted: 14 November 2023

Abstract

Brucellosis stands as a significant zoonotic disease of concern. In Thailand, the first case of human brucellosis emerged in 2003, attributed to goat milk consumption. Subsequently, in 2007, there was a resurgence of brucellosis, which heightened awareness of its implications for public health. Our study was undertaken as part of the 17th Veterinary Volunteer Project in the Nhong-mamong district, Chainat province from June 21 to June 30, 2023. The aim was to assess the seropositivity of brucellosis in goats and sheep in this area. A total of 649 serum samples were collected from 15 farms and examined using the Rose Bengal test. Our findings revealed that the seropositivity at the farm and individual levels was 13.33% (2/15) and 0.77% (5/649), respectively. Consequently, it became evident that the study area remained at risk for brucellosis infection, posing a threat to both humans and animals, particularly concerning *Brucella melitensis* in goats and sheep, which could lead to severe illness in humans. Consequently, an early warning surveillance system is essential to protect both animals and public health by providing timely knowledge and control measures.

Keywords: Brucellosis, Seropositive, Small ruminant, Chainat province

ผลบวกทางซีรัมวิทยาต่อโรคติดเชื้อบรูเซลลาในสัตว์เคี้ยวเอื้องขนาดเล็กของอำเภอหนองมะโมง จังหวัดชัยนาท ประเทศไทย

สินีนาถ เจียมทวีบุญ[1*] ยุภาภรณ์ ลาน้ำเที่ยง[2] อลงกต โตแฉ่ง[3] อนุวัตน์ วิรัชสุดากุล[1]

[1]ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล จังหวัดนครปฐม ประเทศไทย 73170
[2]โรงพยาบาลปศุสัตว์และสัตว์ป่า ปศุปาลัน คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล จังหวัดกาญจนบุรี ประเทศไทย 71150
[3]ปศุสัตว์อำเภอหนองมะโมง จังหวัดชัยนาท ประเทศไทย 17120

[*]ผู้รับผิดชอบบทความ E-mail address: sineenard.jie@mahidol.edu

Received: 30 September 2023; Revised: 13 November 2023; Accepted: 14 November 2023

บทคัดย่อ

โรคบรูเซลโลซีสเป็นโรคติดต่อระหว่างสัตว์และคนที่สำคัญ ประเทศไทยมีการรายงานผู้ป่วยโรคบรูเซลโลซีสรายแรกเมื่อปี พ.ศ. 2546 จากการสอบสวนโรคผู้ป่วยได้รับเชื้อจากการบริโภคนมแพะ ต่อมาในปี พ.ศ. 2550 มีการรายงานผู้ป่วยติดเชื้อโรคบรูเซลโลซิสอีกครั้ง ทำให้หน่วยงานสาธารณสุขเกิดความตระหนักถึงผลกระทบของโรคนี้ต่อสุขภาพของประชาชนมากขึ้น การศึกษาครั้งนี้ได้จัดทำขึ้นภายใต้โครงการสัตวแพทย์อาสา คณะสัตวแพทยศาสตร์ ครั้งที่ 17 ระหว่างวันที่ 21-30 มิถุนายน พ.ศ. 2566 ณ อ. หนองมะโมง จ. ชัยนาท เพื่อศึกษาผลบวกทางซีรัมวิทยาของโรคบรูเซลโลซีสในแพะและแกะ ตัวอย่างซีรัมแพะและแกะทั้งหมด 649 ตัวอย่าง จากฟาร์มแพะและแกะใน อ. หนองมะโมง จำนวน 15 ฟาร์ม ตัวอย่างซีรัมตรวจด้วยวิธี Rose Bengal test ผลปรากฏว่า ผลบวกทางซีรัมต่อโรคบรูเซลโลซีสรายฟาร์มและรายตัวมีค่าเท่ากับ ร้อยละ 13.33 (2/15) และ ร้อยละ 0.77 (5/649) ตามลำดับ แสดงให้เห็นว่าพื้นที่ที่ศึกษายังมีโอกาสเกิดโรคบรูเซลโลซีส ซึ่งเป็นอันตรายต่อทั้งมนุษย์และสัตว์โดยเฉพาะเชื้อ ***Brucella melitensis*** ในแพะและแกะ ซึ่งอาจนำไปสู่การเจ็บป่วยในมนุษย์ที่รุนแรงได้ ดังนั้นการมีระบบเฝ้าระวัง มาตรการการป้องกัน และการให้ความรู้แก่เกษตรกรจึงมีความจำเป็นในการป้องกันโรคทั้งในสัตว์และคน

คำสำคัญ : โรคบรูเซลโลซีส ผลบวกทางซีรัมวิทยา สัตว์เคี้ยวเอื้องขนาดเล็ก ชัยนาท

Introduction

Brucellosis is a highly contagious zoonotic disease with significant public health and economic implications (Wongsawang et al., 2014; Hull and Schumaker 2018). The World Organization for Animal Health (formerly known as OIE) recognizes brucellosis as one of the important zoonotic diseases responsible for human and animal illnesses, as well as detrimental effects on livestock production and trade. This underscores the need for robust public health monitoring (OIE 2019).

In Thailand, the Department of Livestock Development (DLD) has launched the "Brucellosis Free" campaign, which covers various livestock categories, including dairy cattle, goats, and sheep (Colombe et al., 2018). Additionally, they have established regulations for certifying farms as brucellosis-free, necessitating that goat and sheep farms be free from brucellosis.

Brucellosis is caused by small, gram-negative, non-motile, non-spore-forming coccobacilli bacteria in the genus *Brucella*, which are resilient in various environmental conditions, including cold temperatures, yet susceptible to destruction. In cattle, brucellosis is primarily caused by *B. abortus*. *B. melitensis*, and *B. ovis* are the main responsible pathogens in goats and sheep, respectively. In addition, *B. suis* is mostly found in swine, dogs can be infected with *B. canis* (Corbel 2006) and *B. neotomae* is the *Brucella* spp. frequently found in rodents (Villalobos-Vindas et al., 2017). *Brucella* infection can occur through ingestion via contaminated food and water, contact with vaginal discharge, or milk. Furthermore, mating with an infected animal, particularly a male goat, can swiftly spread the infection among female animals within the herd (Godfroid et al., 2011). Once inside the body, *Brucella* pathogens disseminate to lymph nodes and vital target organs, including the uterus and placenta, resulting in diverse manifestations, including abortion in females, retained placenta, arthritis, orchitis in males, and epididymitis (Ekgatat 2009; Kladkempetch et al., 2018).

Brucella melitensis is the most common *Brucella* pathogen that causes human brucellosis, followed by *B. abortus*, *B. suis*, and *B. canis*, respectively (Wallach et al., 1998). The incubation period of human brucellosis is varied, with the most frequent clinical signs being intermittent or irregular fever, headaches, weakness, joint pain, depression, and weight loss (Corbel 2006). *Brucella* infection in humans is mainly linked to occupational exposure, such as laboratory work or jobs involving direct contact with animals, like veterinarians and farmers (Ekgatat 2009). Studies on risk factors have revealed that direct contact with calving discharges, contact through wounds, exposure via the conjunctival sac of the eyes, inhalation of infectious aerosols, and even the consumption of dairy products, such as milk and cheese, pose a high risk of infection (Burns et al., 2023). Lewis et al. documented a case of a 51-year-old UK resident who was infected with *B. melitensis* after returning from Thailand in 2015 (Lewis et al., 2016), drawing attention to the risks associated with brucellosis and prompting efforts to educate the public about relevant knowledge and risk factors, both in the medical and veterinary fields.

In Thailand, brucellosis had not been reported since 1970 until 2003, when the Ministry of Public Health (MOPH) identified and documented the re-emergence of human brucellosis. The affected patient worked at a goat farm in Ratchaburi province and had a history of consuming goat milk and raw goat meat (Laosiritaworn et al., 2007). In 2007, three cases were reported in Chainat province and one in Uttaradit

province. All patients had a history of animal contact and consumption of animal products, particularly from small ruminants. Furthermore, in 2010, 11 brucellosis cases were reported across Nakhon Pathom, Ratchaburi, Rayong, Chainat, and Phetchaburi provinces. Nine were diagnosed via blood culture, while three were examined using the Rose Bengal Test (Annual epidemiological surveillance report 2010). Additionally, direct contact with goat secretions or blood, especially without protective equipment, or consuming goat meat, can transmit brucellosis to humans (Wongphruksasoong et al., 2012; Kanitpun et al., 2022). Consistent with Kanitpun et al. (2022), this study found that goats and sheep had the highest percentage of seropositive results for brucellosis, indicating that they are susceptible to the disease. However, the DLD's efforts in promoting goats and sheep farming, with a focus on brucellosis prevention and control, have had a positive impact, improving the situation of brucellosis in Thailand (Kanitpun et al., 2022).

In Chainat province, goat and sheep farming promotion was aligned with the DLD's policies. A goat farmers' group was formed and provided farmers with five female and one male goat each. Consequently, the population of goats and sheep in Chainat province experienced a rapid increase, accompanied by the detection of brucellosis in meat goats in Chainat (Suddee et al., 2011). However, the province in the central and western region of Thailand with the highest number of goat and sheep tests from 2013 to 2015 was Kanchanaburi (Peck et al., 2018).

This study thus aimed to examine the serological status of goats and sheep regarding *Brucella* infection in the Saphan Hin subdistrict of Nhong-mamong district, Chainat province, and raise awareness on prevention and control of the disease among relevant authorities and farmers.

Materials and methods

Study location

We performed a cross-sectional study to explore the serological status of *Brucella* infection in goats and sheep in Saphan Hin subdistricts, Nhong-mamong district, Chainat province, Thailand with a previous history of outbreak event (Suddee et al., 2011). The study was conducted on 21st-30th June 2023 under the 17th Veterinary Volunteer Project by the Faculty of Veterinary Science, Mahidol University, with the collaboration of the Nhong-mamong District Livestock Office, Chainat province.

Sample collection and laboratory diagnosis

We collected serum samples from goats and sheep older than six months on 14 goat farms and one sheep farm (Table 1) in Saphan Hin subdistrict, Nhong-mamong district, Chainat province (Figure 1). We collected 5 ml blood samples from the jugular vein of each animal. The samples were then examined for antibodies against *Brucella* spp. infection using the Rose Bengal Test (RBT). This test is based on the principle of agglutination between the antigen and antibody under acidic conditions, with a sensitivity and specificity of 99% and 97%, respectively (Thuamsuwan and Yoopracham 2021). The ratio of serum to the Rose Bengal solution is 75 µl:25 µl. The serum and solution were mixed thoroughly on a clean glass plate, which was gently shaken before the results were read after four minutes. Test results were recorded as either negative (no agglutination) or positive (+1 to +4, depending on the degree of agglutination).

Descriptive statistics and percent positivity at a 95% confidence interval (CI) were calculated using an online platform available at https://www.statskingdom.com/proportion-confidence-interval-calculator.html.

Table 1. Number of tested farms and animals for brucellosis in the Saphan Hin subdistrict, Chainat province.

Level	Results	Total tested samples	Moo1	Moo4	Moo6	Moo7	Moo9
Farm	Overall	15	2	7	3	2	1
	Goats	14	2	7	2	2	1
	Sheep	1	0	0	1	0	0
Individual	Overall	649	34	324	165	47	79
	Goats	598	34	324	114	47	79
	Sheep	51	0	0	51	0	0

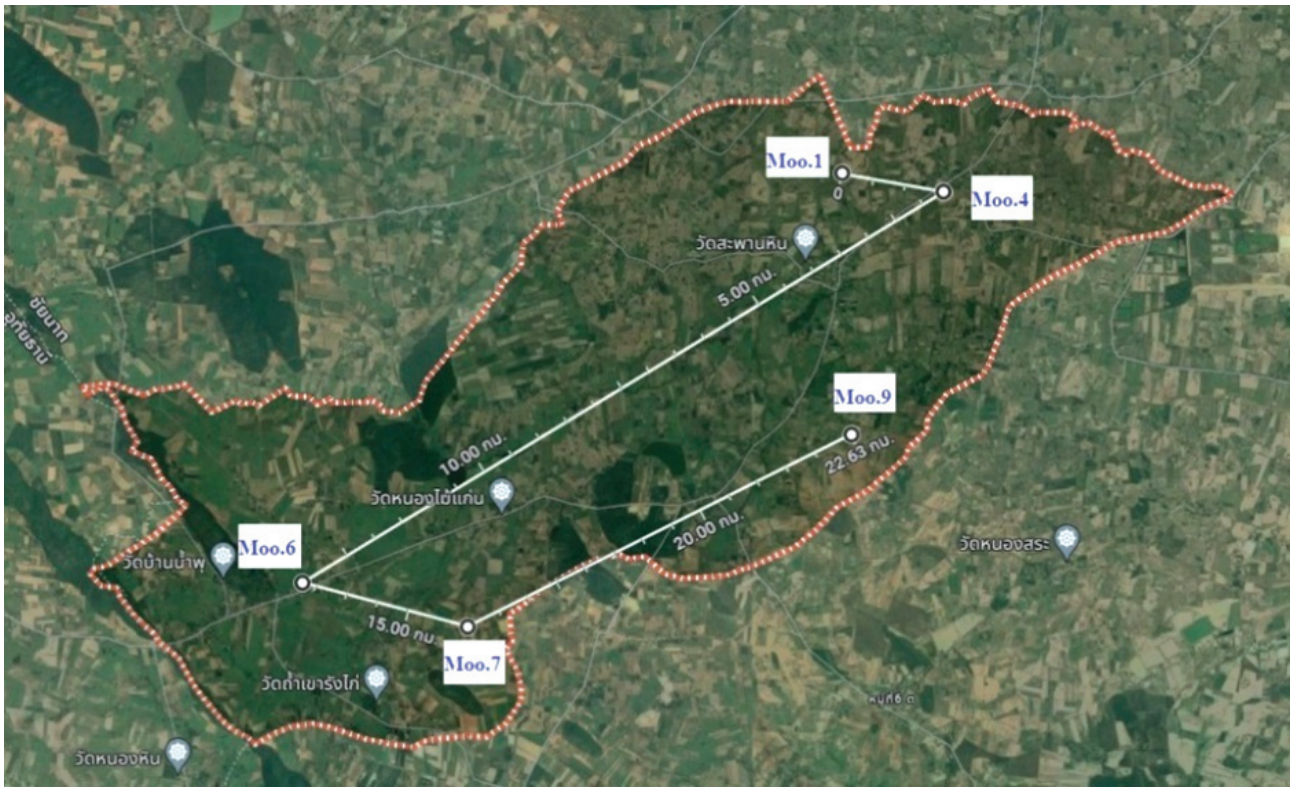


Figure 1. Locations of goat and sheep farms included in the study in Saphan Hin subdistrict, Nhong-mamong district, Chainat province, Thailand. (Google maps 2023).

Results

In total, 649 samples were collected from 14 goat farms and one sheep farm, in which two farms were found to have seropositivity (farm-level seropositivity: 2/15; 13.33%; (95% CI = 3.7-38.0). At the individual level, five samples were found seropositive (individual-level seropositivity: 5/649; 0.77%; (95% CI = 0.33-1.80). Moo (Village) 6 found the highest seropositive level, whereas no positive samples were found in Moo 1, Moo 7, and Moo 9 (Table 2). Concerning the species of the animals, sheep showed a higher seropositive level on both farm and individual levels (Table 3).

Table 2. Seropositive results of *Brucella* infection in small ruminants in each village (Moo) within Nhong-mamong district, Chainat province.

Level	Results	Nhong-mamong district, Chainat province					
		2023	Moo1	Moo4	Moo6	Moo7	Moo9
Farm	Overall	15	2	7	3	2	1
	Positive	2	0	1	1	0	0
	Seropositive	13.33%	0	14.29%	33.33%	0	0
	95%Cl	3.7-38	0	2.6-51	6.1-79	0	0
Individual	Overall	649	34	324	165	47	79
	Positive	5	0	1	4	0	0
	Seropositive	0.77%	0	0.30%	2.42%	0	0
	95%Cl	0.33-1.8	0	0.05-1.7	0.95-6.1	0	0

Table 3. Seropositive results of *Brucella* infection in each animal species in Nhong-mamong district, Chainat province.

Level	Results	Total tested samples	Positive	Seropositive	95%Cl
Farm	Overall	15	2	13.33%	3.7-38
	Goats	14	1	7.14%	1.3-31
	Sheep	1	1	100%	21-100
Individual	Overall	649	5	0.77%	0.33-1.8
	Goats	598	1	0.16%	0.03-0.94
	Sheep	51	4	7.84%	3.1-18

Discussion

Our study on brucellosis seropositivity in Saphan Hin subdistrict, an area densely populated with goat farms within Nhong-mamong district of Chainat province, revealed that most of these farms are small-scale and operate on a free-range basis, with an average of 44 goats per farm. There is a mix of both old and new farmers raising goats and sheep. Specifically, the newly raised farms are the first survey of brucellosis.

The seropositive tests revealed at least one farm and one animal within a goat herd tested positive in Nhong-mamong district, Chainat, Thailand. Additionally, a sheep herd had the highest seropositive result. It was 100% (95%Cl:21-100) at the herd level and 7.84% (95%Cl:3.1-18) at individual level. It might cause only one sheep farm in Nhong-mamong district, Chainat and brucellosis screening and quarantine information remain unclear. It is essential to conduct further testing and investigation to confirm the presence of brucellosis and assess the extent of the infection. Proper diagnosis and control measures are crucial to prevent the spread of the disease within the herd and to other animals or potentially to humans.

Our study also noted a higher trend of seropositivity in Nhong-mamong district compared to the findings in a previous study conducted in 2011 by Suddee et al. According to the previous study, the percentage of positive results at the herd level was 11.11% with 0.14% at the individual level (Suddee et al., 2011). This increase in seropositivity is a concerning trend as it may indicate a higher prevalence of the disease in Nhong-mamong district. Brucellosis outbreaks in goats and sheep across the entire country are primarily attributed to insufficient preventive measures and the introduction of new animals into herds from farms with unknown brucellosis status can be a risk factor for the spread of the disease (Kanitpun et al., 2022). As the movement of livestock and human workforces increases, there is a higher risk of brucellosis spreading to new areas (Colombe et al., 2018). These factors significantly contribute to disease incidence among animals. Given that *Brucella* pathogen can be directly transmitted from animals to humans through unhygienic practices such as consuming unpasteurized milk or raw meat products (Godfroid et al., 2011). Direct contact with infected animals or their calving secretions without the use of protective gloves or gear, and farmers' limited knowledge about zoonotic diseases, a public educational campaign should be urgently initiated in the area to prevent the occurrence of possible human infection (Annual epidemiological surveillance report 2010). Moreover, it is crucial to enhance awareness of brucellosis and conduct annual surveillance in both livestock and humans to successfully eliminate brucellosis from Chainat province. Achieving this goal will require ongoing collaboration between DLD, the medical community, veterinary schools, and private farmers to sustain brucellosis surveillance and prevention programs. Vaccination and treatment are possible for brucellosis in

animals, but the use of live vaccines presents significant safety concerns, such as the potential to induce abortion, pathogenicity for humans, interference with serodiagnosis, and antibiotic resistance. (Elbehiry et al., 2023) As a result, the preferred approach for brucellosis control and prevention involves regular monitoring and culling of infected animals. This strategy helps reduce the risk of disease transmission while safeguarding both animal and human health (Ullah et al., 2020).

We acknowledge some limitations faced in this study. Firstly, the study was conducted as part of the Veterinary Volunteer Project, which may have led to an inadequate sample size calculation. For future studies, we highly recommend employing more robust sampling techniques and accurately calculating the sample size to ensure the study population is proper representation. Additionally, we suggest considering a longitudinal study to continuously monitor the disease incidence trend in the study area, enabling early detection of any unexpectedly high incidence rates.

In conclusion, *Brucella* infection was serologically detected in goats and sheep in a certain area in Chainat province. Hence, it is imperative to establish comprehensive control and prevention measures, along with an annual surveillance campaign, in alignment with the "Brucellosis Free" initiative by the DLD to work towards eradicating brucellosis from these herds.

Acknowledgements

We extend our gratitude to Ms. Waruja Korkijthamkul for her assistance with language editing.

References

Burns RJL, Le KK, Siengsanun-Lamont J, Blacksell SD. A review of coxiellosis (Q fever) and brucellosis in goats and humans: Implications for disease control in smallholder farming systems in Southeast Asia. One Health. 2023;16:100568.

Colombe S, Watanapalachaigool E, Ekgatat M, Ko AI, Hinjoy S. Cross-sectional study of brucellosis and Q fever in Thailand among livestock in two districts at the Thai-Cambodian border, Sa Kaeo province. One Health. 2018;6:37-40.

Corbel MJ. Brucellosis in humans and animals. Geneva: WHO Press; 2006.

Ekgatat M. Diagnosis of brucellosis. Bangkok: National Institute of Animal Health; 2009.

Elbehiry A, Aldubaib M, Marzouk E, Abalkhail A, Almuzaini AM, Rawway M, et al. The Development of Diagnostic and Vaccine Strategies for Early Detection and Control of Human Brucellosis, Particularly in Endemic Areas. Vaccine 2023;11:654.

Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, Fretin D, et al. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Prev Vet Med. 2011;102:118-31.

Google. Map (Nhong-mamong) [Internet]. Google 2023 [cited 2023 Oct 24]. Available from: http://maps.google.com.co.th/.

Kanitpun R, Ekgatat M, Arunvipas P. The Seroprevalence Rates and Approach Management of Brucellosis in Thailand. Indian J Anim Res. 2022;56(12): 1530-6.

Kladkempetch D, Somtua N, Maktrirat R, Punyapornwithaya V, Sathanawong A. Seroprevalence and factors affecting brucellosis in goats in Chiang Mai Province. Vet Integr Sci. 2018;15(2): 99-107.

Laosiritaworn Y, Hinjoy S, Chuxnum T, Vagus A, Choomkasien P. Re-emerging Human Brucellosis, Thailand 2003. Bull Dep Med Serv. 2007;32(4): 415-23.

Lewis J, Folb J, Kalra S, Squire S, Taegtmeyer M, Beeching N. *Brucella melitensis* prosthetic joint infection in a traveller returning to the UK from Thailand: Case report and review of the literature. Travel Med Infect Dis. 2016;14:444-50.

Hull NC, Schumaker BA. Comparisons of brucellosis between human and veterinary medicine. Infect Ecol Epidemiol. 2018;8(1):1500846.

OIE. Terrestrial Manual [Internet]. OIE. 2019 [cited 2023 Sep 1]. Available from: https://rr-europe.woah.org/wp-content/uploads/2020/08/oie-terrestrial-code-1_2019_en.pdf.

Peck ME, Chanachai K, Jenpanich C, Amonsin A, Alexander BH, Bender JB. Seroprevalence of Brucellosis in Goats and Sheep in Thailand: Results from the Thai National Brucellosis Surveillance System from 2013-2015. Transbound Emerg Dis. 2018;65(3):799-805.

Suddee W, Opaschaitat P, Sontiphun S, Boonyo K, Kasemsuwan S, Rukkwamsuk T. Prevalence and risk factors of brucellosis seropositivity of meat goats in Chainat province. J Kasetsart Veterinarians. 2011;21(1):42-50.

Thuamsuwan N., Yoopracham B. Seroprevalence of brucellosis in meat goat farms in Singburi province during 2015-2019 [Internet]. 2021 [cited 2023 Sep 1] Available from: https://pvlo-sir.dld.go.th/webnew/index.php/th/news-menu-2/61-health/338-2558-2562-seroprevalence-of-brucellosis-in-meat-goat-farms-in-singburi-province-during-2015-2019-64-2-0116-1-099.

Ullah Q, Jamil T, Melzer F, Saqib M, Hussain MH, Aslam MA, et al. Epidemiology and Associated Risk Factors for Brucellosis in Small Ruminants Kept at Institutional Livestock Farms in Punjab, Pakistan. Front Vet Sci. 2020;7:526.

Villalobos-Vindas JM, Amuy E, Barquero-Calvo E, Rojas N, Chacón-Díaz C, Chaves-Olarte E, Guzman-Verri C, Moreno E. Brucellosis caused by the wood rat pathogen *Brucella neotomae*: two case reports. J Med Case Rep. 2017;11(1):352.

Wallach JC, Samartino LE, Efron A, Baldi PC. Human infection by *Brucella melitensis*: an outbreak attributed to contact with infect goats. FEMS Immunol Med Microbiol 1998;19:315-21.

Wongphruksasoong V, Santayakorn S, Sitthi W, Chuxnum T, Pipatjaturong N, Kunthu A, et al. An Outbreak of *Brucella melitensis* among Goat Famers in Thailand, December 2009. OSIR. 2021;5(1):14-21.

Wongsawang W, Sanyutitham S, Nakthong C. Seroprevalence of *Brucella* spp. Infection in Beef Cattle in Sai-Yok District, Kanchanaburi Province. J Appl Anim Sci. 2014;7(1):27-32.

Case Report

Metastasis of Thyroid Gland Carcinoma to Mandibular Lymph Node

Tipayawadee Jooypan[1] Pruksa Julapanthong[1] Surachart Benjathammarak[1] Kripitch Sutummaporn[2*]

[1]Prasu-arthorn Small Animal Hospital, Faculty of Veterinary Science, Mahidol University, Phuttamonthon 4 Rd., Salaya, Nakhon Pathom 73170, Thailand

[2]Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Phuttamonthon 4 Rd., Salaya, Nakhon Pathom 73170, Thailand

[*]Corresponding author, E-mail address: kripitch.sut@mahidol.edu

Received: 7 September 2023; Revised: 16 December 2023; Accepted: 18 December 2023

Abstract

Thyroid neoplasms encompass a range of abnormal growths of the neoplastic cells ranging from benign adenomas to malignant carcinomas and are one of the lists for the differential diagnoses from the thyroid gland or parathyroid neoplasm. These tumors cause various clinical signs in dogs, including breathing difficulties, swallowing issues, and compressing the surrounding tissues. Diagnosis involves a combination of physical examinations, radiography, ultrasound, hematology, clinical pathology investigation, and histopathological analyses. Treatment options depend on tumor types and stages, with surgical resection being common for benign tumors and more aggressive approaches such as surgery, radiation, and chemotherapy for malignancies. Even if the metastasis occurs to the regional lymph nodes, the mandibular lymph node metastasis is uncommon. The prognosis varies, with carcinomas posing greater challenges, particularly if they metastasize. Continuous monitoring and follow-up are crucial to assess treatment outcomes and tumor recurrences. Early detection and comprehensive veterinary management are essential in addressing thyroid neoplasms in dogs effectively.

Keywords: Thyroid gland carcinoma, Metastasis, Mandibular lymph node

การแพร่กระจายของมะเร็งของต่อมไทรอยด์ไปสู่ต่อมน้ำเหลืองที่บริเวณกราม

ทิพยวดี จุ้ยปาน[1] พฤกษา จุฬพันธ์ทอง[1] สุรชาติ เบญจธรรมรักษ์[1] ไกรพิชญ์ สุธรรมมาภรณ์[2*]

[1]โรงพยาบาลสัตว์ประศุอาทร คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จ.นครปฐม 73170 ประเทศไทย
[2]ภาควิชาปรีคลินิกและสัตวศาสตร์ประยุกต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล
ถนนพุทธมณฑลสาย 4 ตำบลศาลายา อำเภอพุทธมณฑล จ.นครปฐม 73170 ประเทศไทย

[*]ผู้รับผิดชอบบทความ E-mail address: kripitch.sut@mahidol.edu

Received: 7 September 2023; Revised: 16 December 2023; Accepted: 18 December 2023

บทคัดย่อ

เนื้องอกของต่อมไทรอยด์เป็นเนื้องอกที่เกิดจากความผิดปกติในการเจริญเติบโตของเซลล์ที่บริเวณตำแหน่งของไทรอยด์และพาราไทรอยด์ซึ่งสามารถพบได้ทั้งเนื้องอกที่ไม่ใช่มะเร็งและเนื้องอกที่เป็นมะเร็ง โดยเนื้องอกดังกล่าวมักได้รับการวินิจฉัยแยกจากเนื้องอกของเนื้อเยื่อข้างเคียง เช่น พยาธิสภาพการโตของต่อมไทรอยด์และพาราไทรอยด์เนื่องจากการแบ่งตัวเพิ่มจำนวนของเซลล์ เนื้องอกของต่อมไทรอยด์ ต่อมพาราไทรอยด์ มะเร็งของต่อมไทรอยด์และพาราไทรอยด์ อย่างไรก็ตามเนื้องอกในตำแหน่งดังกล่าวมักส่งผลให้เกิดอาการทางคลินิกในเรื่องของการหายใจลำบาก การกินได้ลำบากและการกดทับของเนื้องอกสู่เนื้อเยื่อข้างเคียง ซึ่งโดยทั่วไปแล้วการวินิจฉัยเนื้องอกดังกล่าวจำเป็นต้องได้รับการตรวจร่างกาย การฉายภายรังสี การทำอัลตราซาวนด์ การตรวจทางพยาธิวิทยาคลินิกและการตรวจทางจุลพยาธิวิทยา ในส่วนของการรักษาขึ้นอยู่กับลักษณะชนิดของเนื้องอกและระยะ โดยการทำการผ่าตัด การฉายรังสีและการทำเคมีบำบัด นอกจากนั้นเนื้องอกดังกล่าวสามารถแพร่กระจายไปสู่ต่อมน้ำเหลืองข้างเคียงแต่มีรายงานการพบที่ไม่บ่อยหนัก ดังนั้นการพยากรณ์โรคจึงอาจขึ้นอยู่กับการแพร่กระจายของเนื้องอกไปยังต่อมน้ำเหลือง การเฝ้าระวังการแพร่กระจายจึงมีความสำคัญต่อการรักษาและการจัดการ โดยการตรวจพบมะเร็งในระยะเริ่มต้นจึงส่งผลต่อประสิทธิภาพในการรักษา

คำสำคัญ : มะเร็งต่อมไทรอยด์ การแพร่กระจายไปยังต่อมน้ำเหลือง ต่อมน้ำเหลืองบริเวณกราม

Introduction

Thyroid gland carcinoma in dogs is a rare and aggressive tumor that originates in the thyroid glands (Meuten 2020). The thyroid glands are small endocrine glands, and play a particularly crucial role in producing the thyroxine for metabolism and regulating the calcium level in the body together with the parathyroid gland (Stanley and Martin 2023). Thyroid carcinoma originates from uncontrolled growth of the neoplastic cells and might cause abnormal levels of hormone from thyroid gland cells, especially thyroxine, calcitonin, and also other hormones from the parathyroid gland such as parathyroid hormone (Worley 2023). The clinical signs might be associated with hypercalcemia, including polyuria and polydipsia, weakness, loss of appetite, vomiting, constipation, muscle tremors, and renal failure. The diagnosis involves to hematological, serum biochemistry test, and serum hormonal level for identifying the clinical disorders. Moreover, the imaging analysis such as radiography or CT-scan for locating the tumor, histopathological examination, and/or immunohistochemistry for confirming the presence of carcinoma (Morris and Dobson 2008; Dobson and Lascelles 2011).

For the specific treatment, the complete surgical removal of the affected thyroid gland, parathyroid gland, and metastatic tissues is undeniable, and clinicians might need to consider additional therapy such as radiation therapy or chemotherapy (Dobson and Lascelles 2011). The prognosis is usually guarded as a malignant and aggressive tumor because the tumor behavior can cause metastasis to regional lymph nodes and lungs with abnormal levels of the hormone level for metabolism and calcium level regulation. Most dogs usually develop clinical signs related to hypothyroidism and abnormal calcium levels after surgical removal, especially, hypocalcemia. Therefore, tumor monitoring and clinical follow-up are necessary.

In this case report, we introduce the clinical case of the dog which was diagnosed with the neoplasm of the thyroid carcinoma, which is a rage and aggressive neoplasm, and metastasis to the mandibular lymph nodes. Moreover, the Immunohistochemistry panels (chromogranin A, calcitonin, parathyroid hormone, thyroglobulin, thyroid transcription factor-1; TTF-1) have been used for differentiating the thyroid carcinoma from other neoplasms (Doss et al., 1998; Meuten 2020).

Case description

A 10-year-old female beagle dog, 4.1 kilograms in body weight was presented at the Prasuarthorn Animal Hospital, Faculty of Veterinary Science, Mahidol University. The dog had a history of enlargement of tissues around the thyroid and parathyroid glands. The vaccination and parasitic prevention program were complete, and there were no other abnormal medical conditions.

On initial physical examination, the dog was bright, alert, and responsive. The heart and lung sounds were normal, the thoracic radiography illustrated the interstitial lung pattern, and the vertebral heart score was 10.0. (Figure 1). The metastatic nodules and abdominal masses were not found from radiography.

For clinical diagnosis, a clinical pathology examination was performed to rule out other systemic diseases when the first visit to our animal hospital. The complete blood count presented mild normocytic normochromic anemia, and there were no other remarkable abnormalities from hematology. The ALT (four times from the upper reference range) and ALP

(three times from the upper reference ranges) were highly elevated from the serum biochemistry investigation. The total T4 hormone level was elevated when compared to the reference range. The full results of the blood examination are shown in Table 1.

The computed tomography suggested that there were heterogeneously contrasted enhancing with 150 HU of the left thyroid gland which illustrated the irregular shape with approximately dimension 2.0 x 1.5 x 2.0 cm, and non-presence of iodine uptake within gland parenchyma with 45 HU. The right thyroid is normal size with non-presence of iodine uptake within the gland. The post-contrast images illustrate the left thyroid gland with heterogeneous contrast-enhancing its 150 HU, and no evidence of vessels invasive. The regional lymphadenopathy was observed from both the mandibular lymph nodes and the right superficial cervical lymph nodes. The multiple mediastinal lymphadenopathies were observed; however, there was no evidence of pulmonary metastasis. The multiple masses vary in size and were located from the right liver lobes.

Based on the suggestion from computed tomography and ultrasonography, the cytology was performed at the left thyroid and parathyroid glands. Then, the cytological findings found a cluster of neoplastic cells and mixed inflammatory cells. The cluster of neoplastic cells is a polyhedral cell with basophilic cytoplasm and hyperchromatic round to oval nuclei indicating a tumor of epithelial origin such as adenoma or adenocarcinoma (Figure 2). Therefore, complete surgical removal and histopathological examination of the thyroid and parathyroid were recommended. The gross appearance of the neoplasm was not described in the clinical record. After the surgical removal of the parathyroid and thyroid glands, the samples were sent to the Center for Veterinary Diagnosis, Faculty of Veterinary Science, Mahidol University for histopathological diagnosis. The samples were trimmed, embedded as paraffin-embedded tissues, and sectioned at 3 μm. The sections were stained for routine hematoxylin and eosin staining for histopathological diagnosis. The histopathological findings (Figure 3) showed the location of the neoplasm was closed to the thyroid glands and parathyroid gland, and the neoplasm was well-circumscribed, well-differentiated, encapsulated, and the neoplastic cells formed as sheets of densely packed polygonal cells. The neoplasm was separated by a thin fibrovascular stroma. Each neoplastic cell showed moderate pleomorphism, anisocytosis, and anisokaryosis. The cells varied from round to polygonal and have round nuclei with hyperchromatic nuclei. The cytoplasm was eosinophilic cytoplasm. Moreover, the neoplastic cells also infiltrated the subcapsular sinus of the mandibular lymph nodes. The histopathological diagnosis suggests for thyroid carcinoma or parathyroid carcinoma. Therefore, immunohistochemistry was performed for differentiating diagnosis. Briefly, the samples were sectioned at 6 μm coated on the positively charged glass slides and sent to the Institute of Pathology, Ministry of Public Health, Thailand. The sets of the mouse monoclonal antibodies were used as primary antibodies (TTF-1, thyroxine, calcitonin, PTH receptor, chromogranin A) for differentiating thyroid carcinoma and parathyroid carcinoma (Table 2). The neoplasm was strongly positive for chromogranin A (Figure 4) and TTF-1 (Figure 5) indicating the thyroid origin of the neoplastic cells.

After the total thyroid and parathyroid surgical removal operation, the dog was routinely followed up by clinicians for monitoring the hematological results, serum biochemistry, blood gas, and electrolytes in Table 3.

The hematological results, serum biochemistry, and total T4 level were returned to be within the reference ranges after the operation. Although the acid-base balance is slightly metabolic alkalosis with physiological compensation (normal bicarbonate, but increased base excess). The serum electrolyte was slightly changed. For example, the ionized calcium was slightly low level but still was within the lower limit of the reference range and mild hyperkalemia in Table 3. Therefore, routinely followed up of the electrolytes should be intensively monitored. After the complete surgical removal of the tumors, the chemotherapy was performed according to the 2016 American Hospital Association Oncology guidelines for dogs and cats (Biller et al., 2016). The carboplatin was intravenously administered as chemotherapy once every three weeks for a total of four treatments after surgical removal of the thyroid carcinoma.

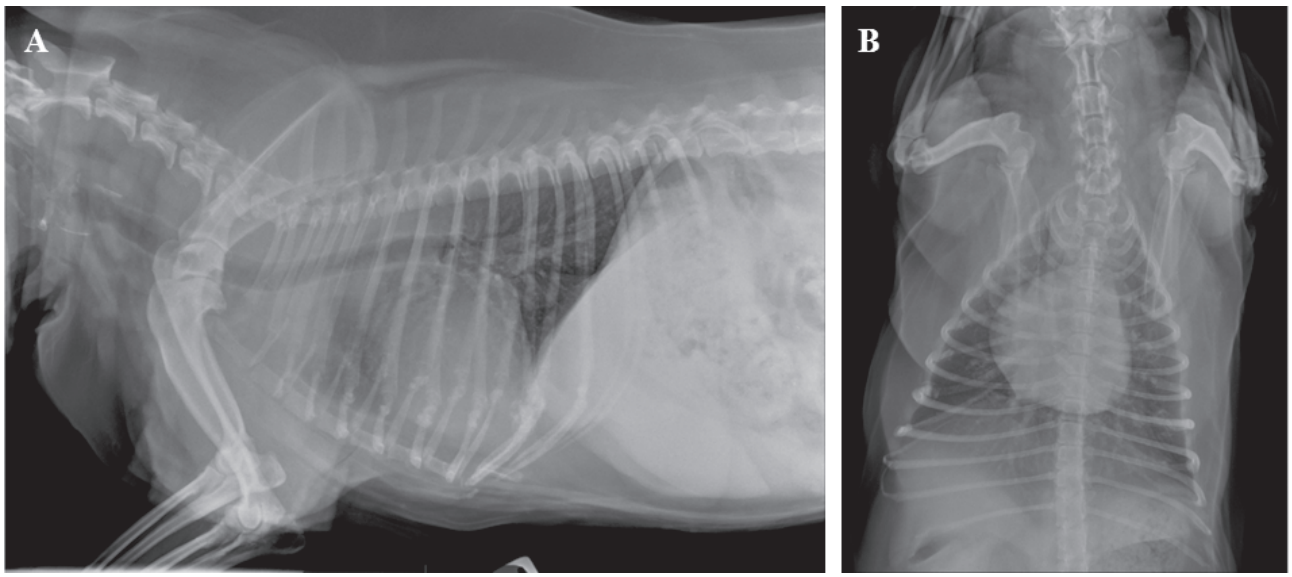


Figure 1. Right lateral recumbency (A) and ventrodorsal (B) view of thoracic radiography.

Table 1. Results of blood examination on 21 June 2023, the first day of the visit before surgical removal of thyroid and parathyroid gland.

Complete blood count			
Parameters	**21/06/23**	**Reference**	**Unit**
Total white blood cells	14.48	6.0-17.0	$10^3/\mu L$
Monocytes	1.16	0.15-1.35	$10^3/\mu L$
Neutrophils	11.29	3.00-11.50	$10^3/\mu L$
Band neutrophils	0	0-3	%
Lymphocytes	2.03	1.00-4.80	$10^3/\mu L$
Eosinophils	0.14	0.10-1.25	$10^3/\mu L$
Basophils	0	0.00-0.10	$10^3/\mu L$
Red blood cells (RBC)	4.91	5.0-9.0	$10^6/\mu L$
Hemoglobin (Hb)	10.7	10.0-18.0	g/dL
Hematocrit (Hct)	32.6	35-55	%
MCV	66.5	60-77	fL
MCH	21.8	20-25	pg
MCHC	32.8	32-36	g/dL
Platelet count (PLT)	482	200-500	$10^3/\mu L$
RDW	15.1	12-15	%
Plasma protein	7.6	6.0-7.5	g/dL
Platelet smear	Aequate		
Blood morphology			
Polychromasia	Few		
Anisocytosis	Few		
Macrocyte	Few		
Blood chemistry			
Parameters	**21/06/2023**	**Reference**	**Unit**
BUN	11	7-27	mg/dL
Creatinine	0.96	0.5-1.8	mg/dL
ALT	488	10-100	U/L
ALP	781	23-212	U/L
Total protein	7.0	5.2-8.2	g/dL
Albumin	3.1	2.7-3.8	g/dL
Glucose	100		
Total T4	6.48	1.0-4.0	µg/dL

Abbreviation: Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width Blood urea nitrogen (BUN), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total T4 (Total thyroxine 4).

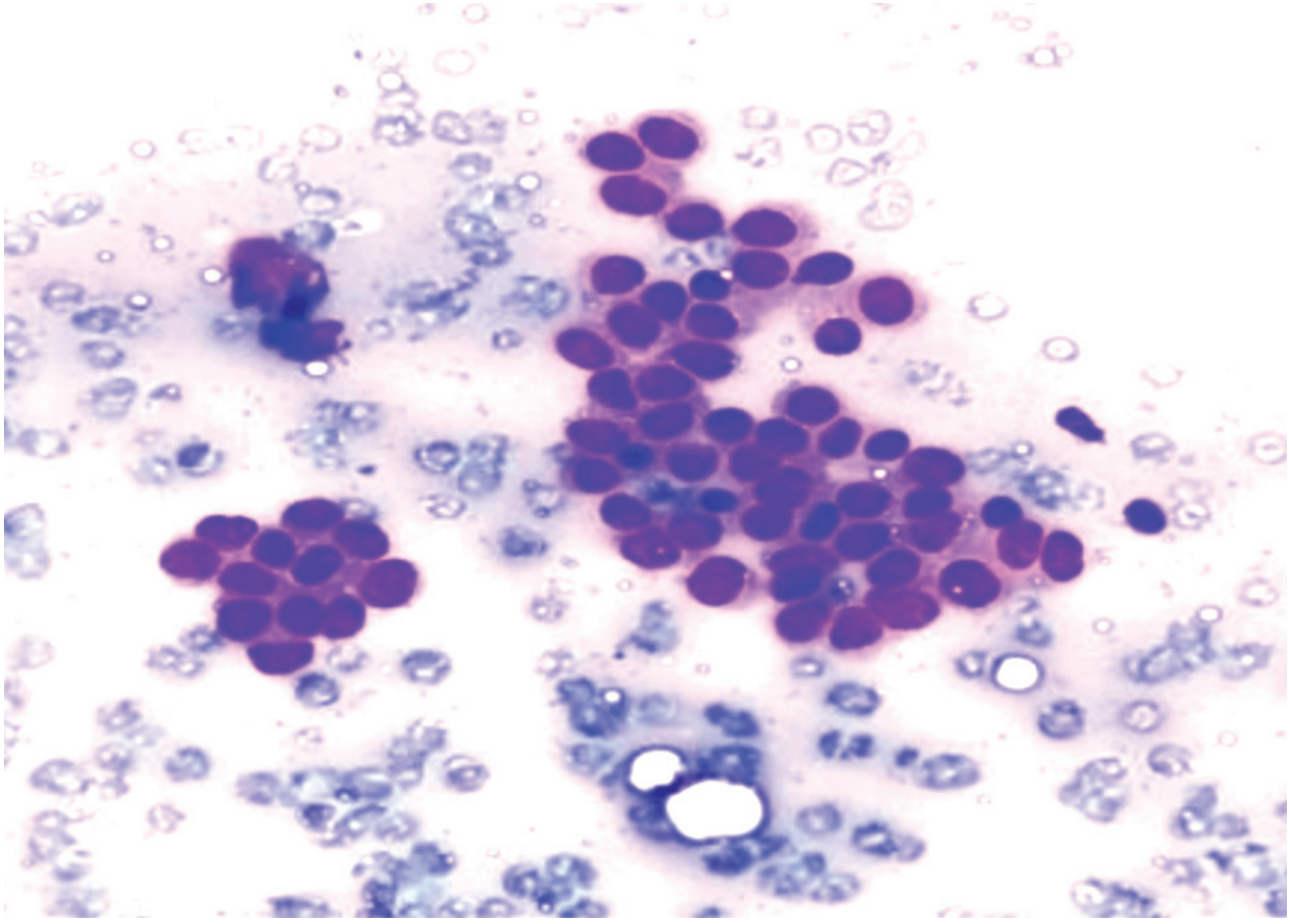


Figure 2. The cytological finding shows a cluster of epithelial-origin neoplastic cells showing pleomorphism, anisocytosis, and anisokaryosis. The cells are polygonal, and the nucleus is round with hyperchromatic nuclei (40x).

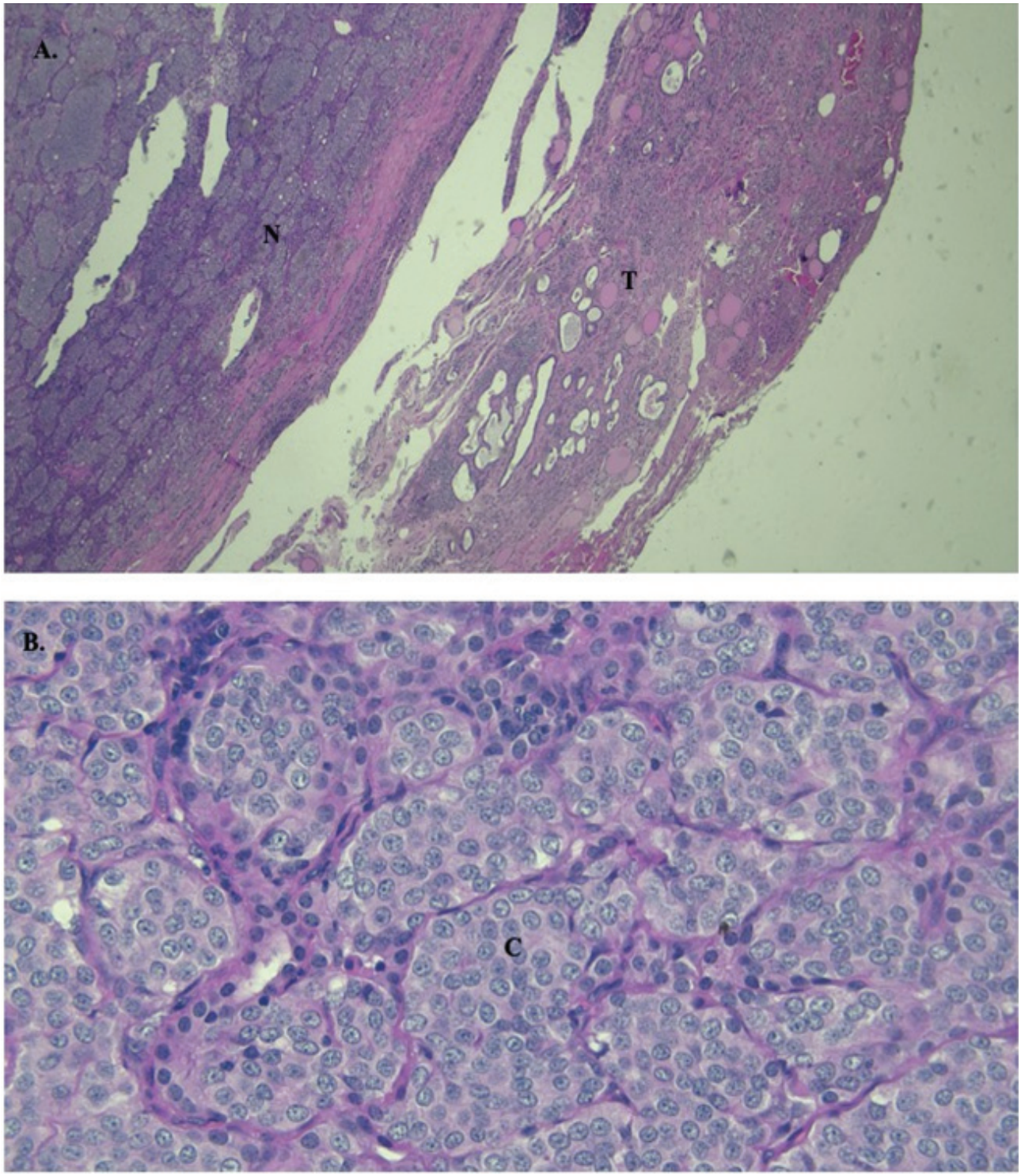


Figure 3. Thuroid carcinoma with H&E stain 4x and 40x respectively. A. Neoplasm (N) and thyroid gland (T) 4x. B. Thyroid carcinoma (C) proliferated as a lobular pattern, and the cells showed moderate pleomorphism anisocytosis, and anisokaryosis 40x.

Table 2. Immunohistochemistry for differentiating the thyroid carcinoma from other neoplasms.

Immunohistochemistry	Results
Chromogranin A	Positive
Calcitonin	Negative
Parathyroid hormone	Negative
Thyroglobulin	Negative
TTF-1	Positive

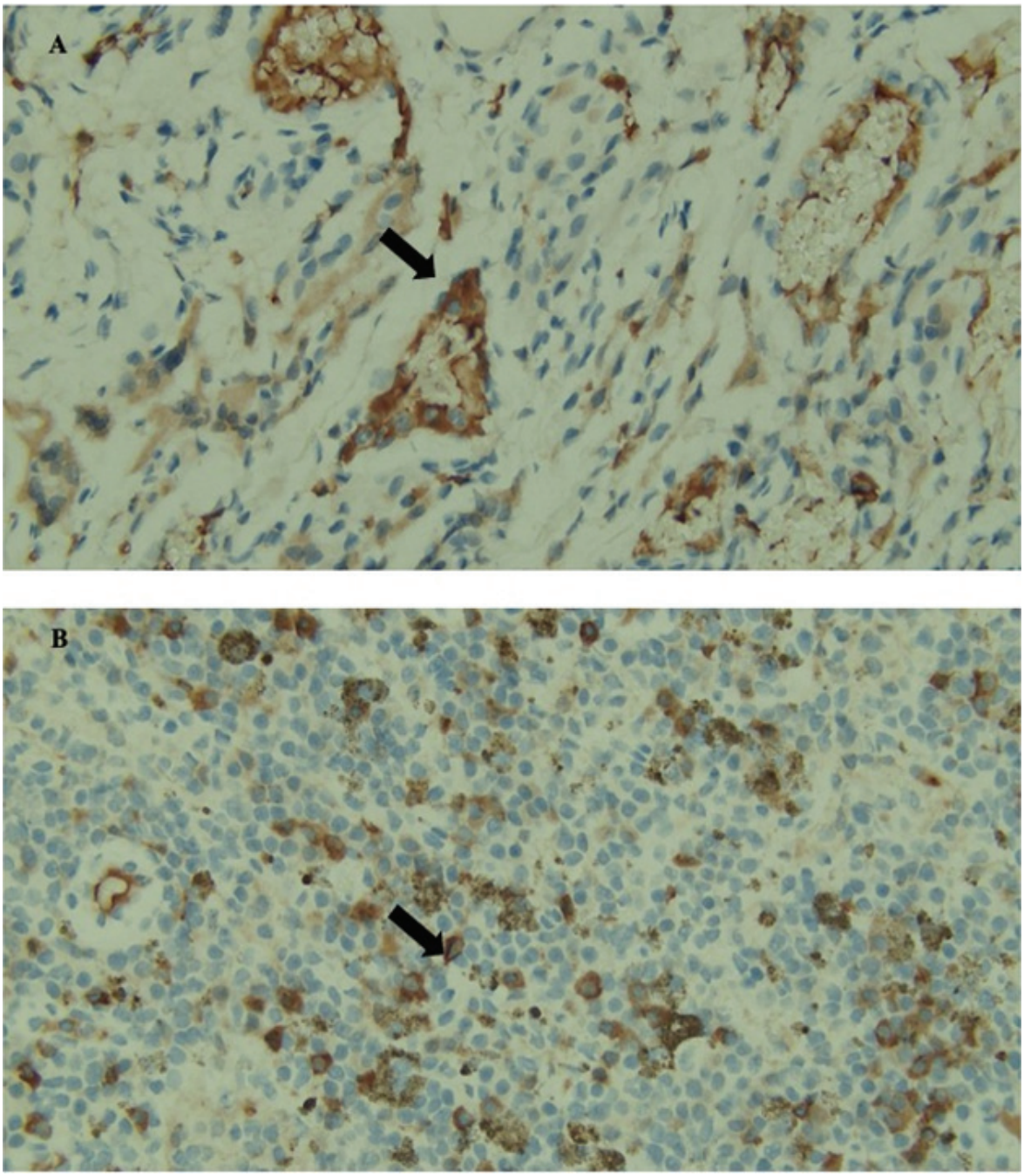


Figure 4. Chromogranin A immunohistochemistry 40x of thyroid and parathyroid gland (A) and submandibular lymh node (B), respectively. The brown-stained positive cells are neoplastic cells (arrows).

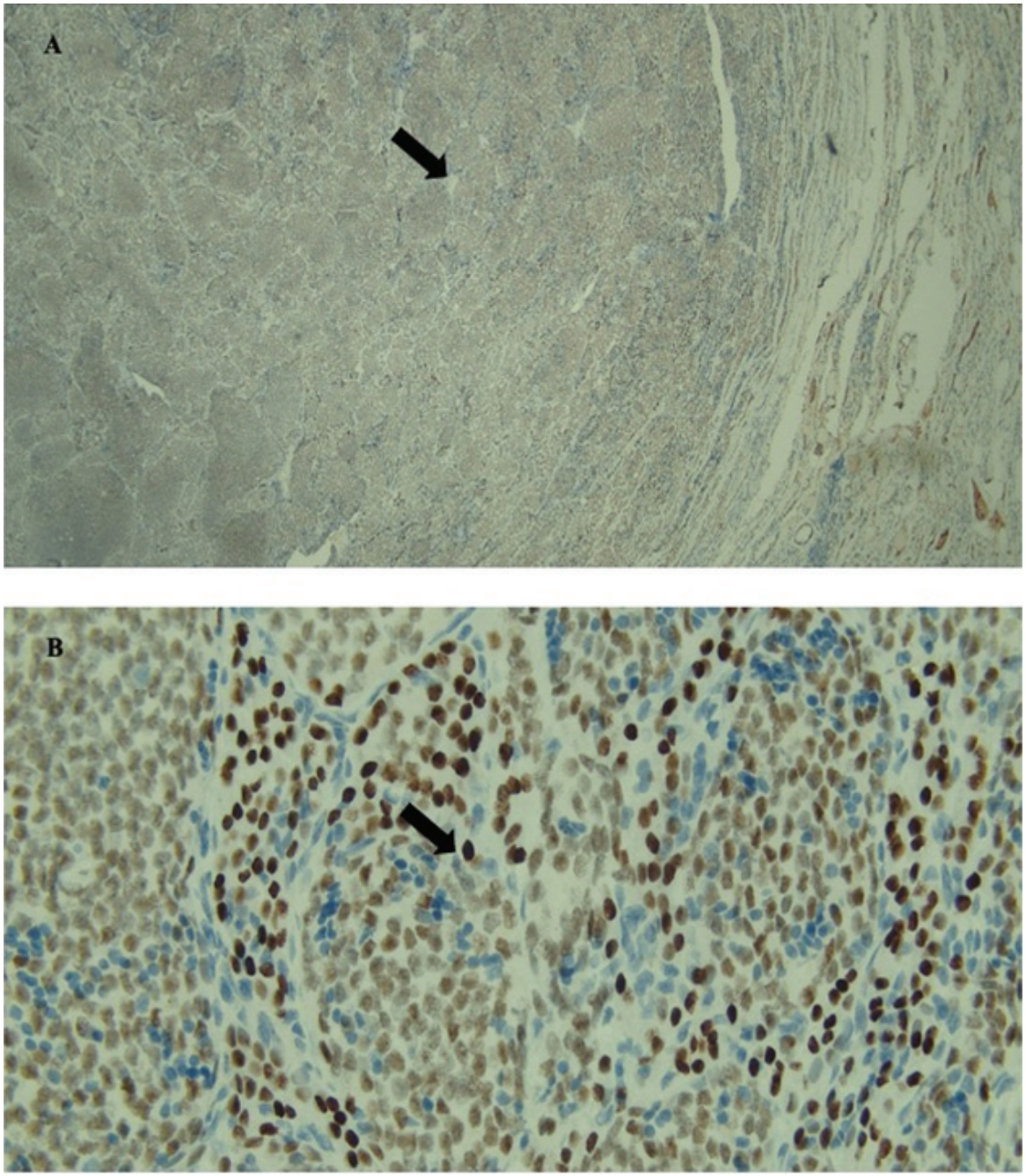


Figure 5. TTF-1 immunohistochemistry 10x of thyroid neoplasm (A) and 40x (B) respectively. The brown-stained positive cells are thyroid-origin neoplastic expressing the TTF-1.

Table 3. Results of blood examination on 10 August 2023, after surgical removal of thyroid and parathyroid gland.

Complete blood count			
Parameters	**10/08/23**	**Reference**	**Unit**
Total white blood cells	13.61	6.0-17.0	$10^3/\mu L$
Monocytes	0.82	0.15-1.35	$10^3/\mu L$
Neutrophils	9.39	3.00-11.50	$10^3/\mu L$
Band neutrophils	0	0-3	%
Lymphocytes	3.40	1.00-4.80	$10^3/\mu L$
Eosinophils	0	0.10-1.25	$10^3/\mu L$
Basophils	0	0.00-0.10	$10^3/\mu L$
Red blood cells (RBC)	5.43	5.0-9.0	$10^6/\mu L$
Hemoglobin (Hb)	12.3	10.0-18.0	g/dL
Hematocrit (Hct)	36.9	35-55	%
MCV	68.0	60-77	fL
MCH	22.7	20-25	pg
MCHC	33.3	32-36	g/dL
Platelet count (PLT)	552	200-500	$10^3/\mu L$
RDW	14.6	12-15	%
Plasma protein	7.2	6.0-7.5	g/dL
Platelet smear	Increase		
Morphology			
Anisocytosis	Few		
Macrocyte	Few		
Blood chemistry			
Parameters	**10/08/23**	**Reference**	**Unit**
BUN	16	7-27	mg/dL
Creatinine	0.98	0.5-1.8	mg/dL
ALT	77	10-100	U/L
ALP	207	23-212	U/L
Total protein	6.9	5.2-8.2	g/dL
Albumin	2.9	2.7-3.8	g/dL
Glucose	105		
Total T4	2.50	1.0-4.0	µg/dL

Table 3. Results of blood examination on 10 August 2023, after surgical removal of thyroid and parathyroid gland. (Cont.)

Blood gas (Venous)	10/08/2023	Reference	Unit
pH	7.464	7.34-7.46	
pCO2	37.1	32-49	
pO2	114.4	24-48	mmHg
Na+	149	144-160	mmHg
K+	6.39	3.5-5.8	mmHg
iCa	1.25	1.25-1.50	mmoL/L
Hematocrit (Hct)	44	35-55	mmoL/L
HCO3	26.0	20.0-29.0	mmoL/L
BE	2.4	-	
TCO2	27.1	21.0-31.0	mmoL/L
O2SAT	98.4	93-100	%
Hemoglobin (Hb)	14.8	12.0-18.0	g/dL

Abbreviation: Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Blood urea nitrogen (BUN), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total T4 (Total thyroxine 4), partial pressure of carbon dioxide (pCO2), partial pressure of oxygen (O2), Sodium (Na+), Potassium (K+), ionized Calcium (iCa), Bicarbonate (HCO3), Base excess (BE), Total carbon dioxide (TCO2), Saturated oxygen (O2SAT).

Discussion

Thyroid neoplasm in dogs is the neoplasm that presents various gross pathological features depending on the type of tumor, its size, and locations and causes various clinical conditions such as upper respiratory tract and digestive tract compression (Morris and Dobson 2008; Dobson and Lascelles 2011; Meuten 2020). The common types of thyroid neoplasms in dogs include adenomas (benign tumors) and thyroid carcinomas (malignant tumors) (Meuten 2020). The appearance of thyroid neoplasm is discrete nodules or masses within the thyroid gland tissue and varies in color ranging from white to tan to brown (Jubb et al., 2012). According to the aggressiveness of tumors, the neoplasm usually invades the surrounding tissues, such as nearby lymph nodes, blood vessels, and adjacent structures in the neck causing hemorrhage, necrosis, or calcification. The compression effects lead to difficulty breathing, and swallowing difficulties. Therefore, total surgical removal is necessary for the survival of the dogs (Erickson et al., 2021). The histopathological examination is compulsory for the definitive diagnosis of the tissue samples obtained during surgery or biopsy (Meuten 2020). In this case report, we used a set of the mouse monoclonal antibodies including, TTF-1, thyroxine, parathyroid hormone receptor, calcitonin, and chromogranin-A to identify the origin of the neoplastic cells (Pessina et al., 2016). Based on the histopathological examination and immunohistochemistry, the neoplasm is strongly positive for TTF-1 and chromogranin A. The TTF-1 is usually expressed in thyroid carcinoma; however, the chromogranin A is expressed in the secretory granules of endocrine cells and plays a role in hormone packaging and secretion. The immunohistochemistry of chromogranin identifies the origin of the C cells of the thyroid gland and parathyroid chief cells (Doss et al., 1998).

Generally, after surgical removal of the thyroid and parathyroid, a retrospective multi-institutional study of 100 cases of dogs (2010-2019 reported that thirty-four percent of dogs developed hypocalcemia within one week after surgery; moreover, the estimated one, two-, and three-year survivals were 84%, 65%, and 51% respectively. The most common clinical signs after surgical removal are polyuria, polydipsia, hindlimb paresis, lethargy, and hypoxia. The median survival time is 2 years (Erickson et al., 2021).

In our case, the clinical signs and the clinical pathological abnormalities data before surgical removal were not obvious such as mild normochromic and normocytic anemia, high level of total T4. However, the additional imaging analysis especially CT-scan suggest the abnormal growth of the neoplasm located at the thyroid and parathyroid gland. Moreover, the multiple masses at the liver are also related to the markedly elevated level of ALT and ALP. Therefore, further investigation of the multiple masses in the liver should be performed. After surgical removal, all clinical pathological data, electrolytes, and hormone level were returned to normal except hypocalcemia, hyperkalemia, and metabolic alkalosis. The possible pathogenesis suggests the hypothyroidism and hypoparathyroidism which cause abnormalities of the metabolism and calcium regulation. Therefore, hormonal supplementation might necessary as adjunctive therapy together with the full course of chemotherapy.

Conclusion

The thyroid neoplasm is a rare neoplasm and the development of abnormal growths or tumors in the parathyroid gland, a vital endocrine organ located in the neck. These neoplasms can encompass both benign (non-cancerous) and malignant (cancerous) tumors. The clinical signs associated with thyroid neoplasms in dogs can vary depending on the size and location of the tumor. The common clinical signs include difficulty breathing and swallowing. The clinical diagnosis typically involves a combination of physical examination, imaging studies (such as radiography ultrasound or computed tomography), clinical pathology levels, and tissue biopsy for histopathological examination. The complete surgical removal is necessary for aggressive tumors with additional radiation therapy and chemotherapy. Overall, the prognosis is guarded, and ongoing monitoring by clinicians to assess for recurrence or metastasis with regular follow-up appointments is compulsory.

Acknowledgements

We would like to thank clinician, staffs, and veterinary technicians from the Prasu Arthorn Veterinary Teaching Hospital and The Center for Veterinary Diagnosis from Faculty of Veterinary Science, Mahidol University for kindly supports for all facility and laboratory supports.

References

Biller B, Berg J, Garrett L, Ruslander D, Wearing R, Abbott B, et al. 2016 AAHA oncology guidelines for dogs and cats. J Am Anim Hosp Assoc. 2016;52(4): 181-204.

Dobson JM, Lascelles BDX. BSAVA manual of canine and feline oncology. Quedgeley: British Small Animal Veterinary Association; 2011.

Doss JC, Grone A, Capen CC, Rosol TJ. Immunohistochemical localization of chromogranin A in endocrine tissues and endocrine tumors of dogs. Vet Pathol. 1998;35(4):312-5.

Erickson AK, Regier PJ, Watt MM, Ham KM, Marvel SJ, Wallace ML, et al. Incidence, survival time, and surgical treatment of parathyroid carcinomas in dogs: 100 cases (2010-2019). J Am Vet Med Assoc. 2021;259(11):1309-17.

Jubb KVF, Kennedy PC, Palmer N. Pathology of domestic animals. San Diego: Academic press; 2012.

Meuten DJ. Tumors in domestic animals. Iowa: John Wiley & Sons; 2020.

Morris J, Dobson J. Small animal oncology. Iowa: John Wiley & Sons; 2008.

Pessina P, Castillo V, Sartore I, Borrego J, Meikle A. Semiquantitative immunohistochemical marker staining and localization in canine thyroid carcinoma and normal thyroid gland. Vet Comp Oncol. 2016;14(3):e102-e12.

Stanley S, Martin A. Thyroid, parathyroid hormones and calcium homeostasis. Anaesth Intensive Care. 2023;24(10):639-43.

Worley DR. Canine Thyroid Neoplasia. In: Monnet E, editor. Small Animal Soft Tissue Surgery. 2nd ed. New Jersey: John Wiley & Sons; 2023. p. 779-84.

Case Report

Laser Acupuncture Treatment in a Ferret with Spinal Cord Injury

Nijanan Siriarchawattana* Benchapol Lorsunyaluck

Panalai Veterinary Hospital, 100/5 M.5, Tiwanon Road, Pakkret, Nonthaburi 11120, Thailand

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

Received: 21 September 2023; Revised: 8 December 2023; Accepted: 11 December 2023

Abstract

Acupuncture is the use of a small needle to treat numerous diseases in traditional Chinese medicine. It has been performed on domestic and non-domestic animals as a main, alternate, or multimodal treatment that synergizes with other therapies. Currently, ferrets have become more popular in various countries, including Thailand. Spinal cord injury is one of the common diseases observed in ferrets. According to this presenting ferret, she visited Panalai Veterinary Hospital with posterior paresis after falling from the 8th floor of a building 3 days ago. The physical and neurological examination results showed normal vital signs except for posterior paresis grade 3. The radiograph and abdominal ultrasound results were unremarkable. The differential diagnosis included spinal cord injury from trauma or Spinal *Qi*/Blood Stagnation and Kidney *Qi* Deficiency as the traditional Chinese veterinary medicine diagnosis. Laser acupuncture treatment is the primary treatment for this case. Each acupoint was treated with laser therapy (M-Vet, MLS®) in Laserpuncture mode. The parameter of laser acupuncture was 611 Hz, intensity 1%, total time 5 seconds, 4.44 J/cm^2 and repeating 3 times per point. Treatment sessions were twice a week for 3 treatments, then once a week for 9 treatments. As a result, the patient achieved complete recovery in two months after 12 therapies. This successful treatment enabled the patient to regain the ability to walk normally, even on slippery surfaces. This case report describes the effectiveness of laser acupuncture treatment in a ferret with posterior paresis resulting from a spinal cord injury.

Keywords: Ferret, Laser acupuncture, Posterior paresis, Traditional Chinese Veterinary Medicine

การรักษาภาวะไขสันหลังบาดเจ็บในเฟอเรท โดยการฝังเข็มด้วยเลเซอร์

นิจนันท์ ศิริอาชาวัฒนา* เบญจพล หล่อสัญญาลักษณ์

โรงพยาบาลสัตว์พนาลัย 100/5 หมู่ 5 ถนนติวานนท์ ตำบลปากเกร็ด จังหวัดนนทบุรี 11120 ประเทศไทย

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

Received: 21 September 2023; Revised: 8 December 2023; Accepted: 11 December 2023

บทคัดย่อ

การฝังเข็มเป็นศาสตร์การแพทย์แผนจีน ที่ใช้เข็มฝังไปตามจุดฝังเข็มตามร่างกายเพื่อฟื้นฟูร่างกายและบำบัดโรค โดยการฝังเข็มได้มีการนำมาใช้บำบัดในสัตว์หลายชนิดรวมถึงสัตว์เลี้ยงชนิดพิเศษ เช่น เฟอเรท โรคในกลุ่มระบบประสาทที่พบได้บ่อยในเฟอเรท ได้แก่ ภาวะไขสันหลังบาดเจ็บ ดังเช่นเฟอเรท เพศเมีย อายุ 4 ปี น้ำหนัก 0.77 กิโลกรัม นี้ ได้เข้ารับการรักษา ณ โรงพยาบาลสัตว์พนาลัย ด้วยภาวะขาหลังเป็นอัมพฤกษ์หลังจากตกตึก 8 ชั้น จากการตรวจร่างกายและระบบประสาท พบว่าเฟอเรทมีภาวะขาหลังเป็นอัมพฤกษ์ ระดับ 3 และไม่พบความผิดปกติในผลการวินิจฉัยด้วยรังสีเอกซเรย์และอัลตราซาวด์ จึงสามารถวินิจฉัยแยกโรคได้เป็น ภาวะไขสันหลังบาดเจ็บจากอุบัติเหตุ ในทางแพทย์แผนตะวันตก และภาวะชี่หรือเลือดของไขสันหลังติดขัด และภาวะพร่องชี่ของไต (Spinal *Qi*/Blood Stagnation and Kidney *Qi* Deficiency) ในแพทย์แผนจีน ซึ่งการรักษาหลักของเฟอเรทนี้คือการฝังเข็มด้วยเลเซอร์ อุปกรณ์เลเซอร์ที่ใช้ได้แก่ MLS® Laser รุ่น M-Vet โดยแต่จะจุดฝังเข็ม ตั้งค่าในโหมด Laserpuncture ความถี่ 611 เฮิรตซ์ ความเข้ม 1% พลังงาน 4.44 J/cm^2 ครั้งละ 5 วินาทีและทำซ้ำ 3 ครั้งต่อจุด แผนการรักษาได้แก่ฝังเข็ม 2 ครั้งต่ออาทิตย์ จำนวน 3 ครั้ง และเปลี่ยนเป็นอาทิตย์ละครั้ง จำนวน 9 ครั้ง ผลการรักษาพบว่าเฟอเรทสามารถกลับมาใช้ขาได้อย่างสมบูรณ์ทั้งการเดินบนพื้นผิวขรุขระและพื้นลื่นภายในเวลา 2 เดือน ผ่านการฝังเข็มทั้งหมด 12 ครั้ง ดังนั้นในรายงานกรณีศึกษาผู้ป่วยฉบับนี้จึงได้แสดงถึงผลลัพธ์และความสำเร็จของการรักษาเฟอเรทที่มีภาวะขาหลังเป็นอัมพฤกษ์จากไขสันหลังบาดเจ็บโดยการฝังเข็มด้วยเลเซอร์

คำสำคัญ : เฟอเรท ฝังเข็มด้วยเลเซอร์ ภาวะขาหลังเป็นอัมพฤกษ์ การแพทย์แผนจีนทางสัตวแพทย์

Introduction

Traditional Chinese Veterinary Medicine (TCVM) is a medical system grounded in the principles of Daoist philosophy. The cosmic laws and forces govern the external world and also govern the body's internal environment (Xie and Preast 2013). The fundamental theories of Daoist and Traditional Chinese Medicine (TCM) or TCVM are *Qi* (known as vital energy) and Yin-Yang. *Qi* flows through the body, which maintains the proper function of the body system. Yin-Yang theory is described as the opposite and complementary forces that balance and harmonize the body's function. Disharmony or imbalance of *Qi* and/or Yin Yang can cause the disease and illness of the host (Xie and Preast 2013). Moreover, *Wu Xing* or Five Elements is another theory of TCM and TCVM, which are represented all the fundamental elements constituting the universe as well as the body. Body functions and structures are categorized as wood, fire, earth, metal and water. Therefore, the process of therapy involves restoring the disharmony back into harmony (Xie and Preast 2013; Koh and Harrison 2023).

Acupuncture is the part of TCM and TCVM that uses fine needles inserted through the skin at specific loci or acupoints (Xie and Preast 2013). This practice aims to alleviate various ailments, discomfort, and pain, ultimately enhancing overall well-being and quality of life. Acupoints are located in or near muscles, blood vessels, lymph vessels, or nerves (Xie and Preast 2007). When the acupoints are stimulated, inflammatory mediators like endorphins, enkephalins, and corticosteroids are released through the nervous system by changing the way neurotransmitters work (Koh and Harrison 2023). Acupuncture in animals was initially used in equine treatment in ancient Chinese times (Harrison and Churgin 2022). Currently, acupuncture is widely used throughout various countries, which could be the main treatment or alternative treatment for domestic and non-domestic animals such as dogs, cats, rabbits, elephants, horses, primates, or ferrets (Srionrod 2021; Harrison and Churgin 2022; Koh and Harrison 2023). The methods for stimulating acupuncture points are dry needle acupuncture, hemoacupuncture, aquapuncture, pneumoacupuncture and moxibustion. In addition, the electrical devices which can stimulate include electroacupuncture, laseracupuncture, magnetic therapy and infrared therapy (Xie and Preast 2007).

Laser acupuncture (LA) has been defined as "Photonic stimulation of acupuncture points and areas to initiate therapeutic effects similar to those of needle acupuncture and related therapies, together with the benefits of laser biological benefits" (Yang et al., 2021). The laser radiation elicits anti-inflammatory and anti-edema effects via the vasodilation and activation of microcirculation, which washout the pro-inflammatory molecules. Laser inhibits the neural activity and induces some opioids resulting in analgesic effects. In addition, laser radiation is effective in stimulating tissue repair through vasodilation, which can increase the supply of oxygen, nutrients, and growth factors, modulate inflammation to trigger wound healing, stimulate fibroblast migration and activation, increase ATP production and improve cell energy metabolism (Bockstahler et al., 2019). The noninvasive and painless treatment of laser acupuncture is more feasible than needle acupuncture, which can stimulate difficult or uncomfortable needle areas like auricular acupoints and reduce the risk of organ puncture, infection or bleeding complications (Chon et al., 2019). And the laser acupuncture treatment length (10-60 seconds per acupuncture site) is significantly shorter

than needle acupuncture (10-30 minutes retention time) (Yang et al., 2021). Therefore, laser acupuncture can be used in animals that cannot tolerate needle acupuncture or uncooperative animals that cannot restraint for a long time (Petermann 2011).

Ferret (*Mustela furo*) is one of the exotic pets that is widespread in various countries, including Thailand. Ferrets have neurological diseases similar to dogs and cats (Huynh and Piazza 2021). Although neurological examination in ferrets follows the same principle as in domestic animals, the skeletal anatomy of ferrets differs from dogs and cats, with 7 cervical vertebrae, 15 thoracic vertebrae, 5-7 lumbar vertebrae, and 3 sacral vertebrae (Mitchell and Tully 2009). The different anatomy of the ferret would make it aware of acupoint locations that were transposed from acupoints in dogs. Posterior paresis, or paraparesis, is a common presentation in ferrets from many causes, including immune-mediated, infectious, toxicity, spinal defects, metabolic, neoplasia or degenerative disease cats (Huynh and Piazza 2021). Posterior paresis can involve the upper motor neuron (UMN) or lower motor neuron (LMN). Upper motor neuron deficits reflect a T3-L3 spinal cord lesion, while lower motor neuron deficits reflect an L4-S2 spinal cord lesion (Huynh and Piazza 2020). Treatment depends on the cause of the lesion. Recently, acupuncture has played a role in palliative care or alternative treatment for various diseases in ferrets (Lao et al., 1995; Kordupel 2023). However, most ferrets do not tolerate holding still with restraining or staying still with accepting needles to be placed. Therefore, laser acupuncture or aquapuncture would be a proper acupuncture technique for ferrets (Koh and Harrison 2023). But there is still a little literature about laser acupuncture treatment in ferrets.

This case report describes laser acupuncture treatment for posterior paresis in ferrets, which is a successful, painless treatment.

Case description

A 0.77 kg, 4-year-old female ferret fell from an 8th-floor building 3 days before visiting Panalai Veterinary Hospital. After the accident, she could not use her hindlimb. She was referred to Panalai Veterinary Hospital and treated with meloxicam 0.2 mg/kg via subcutaneous, butorphanol 0.1 mg/kg via subcutaneous, gabapentin 10 mg/kg per oral, anti-oxidant per oral, and vitamin B complex per oral from the previous animal hospital. The oral medicines were continued for only 3 days because the owner could not give her medicine. The ferret's appetite was still good. Her mental status was bright, alert and responsive. The pain score was 1/4 by flinching when touching her back. The physical examination results of this patient at the first visit were normal heart sound and normal lung sound, mucous membrane was pale pink, capillary refilled time was normal and there was normal hydration. The neurological examination results of all visits are demonstrated in Tables 1-3. All cranial nerve reflex results are normal, and she had no problem with micturition disorder. As a consequence, the ferret manifested posterior paresis grade 3.

Table 1. Neurological examination of this patient, postural reactions result of 1st - 12th visit.

Postural reaction	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Proprioception												
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Lt HL	0	0	0	**+1**	**+1**	**+1**	**+2[a]**	**+2**	**+2**	**+2**	**+2**	**+2[b]**
Rt HL	0	0	0	**+1**	**+1**	**+1**	**+2[a]**	**+2**	**+2**	**+2**	**+2**	**+2[b]**
Hopping												
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Lt HL	0	0	0	**+1**	**+1**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**
Rt HL	0	0	0	**+1**	**+1**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**
Wheel barrowing												
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Ext. post. thrust												
Lt HL	0	0	0	**+1**	**+1**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**
Rt HL	0	0	0	**+1**	**+1**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**
Visual placing												
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Tactile placing												
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Lt HL	0	0	0	**+1**	**+1**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**
Rt HL	0	0	0	**+1**	**+1**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**	**+2**

Neurological examination in 1st visit is before treatment. Lt FL: Left forelimb, Rt FL: Right forelimb, Lt HL: Left hindlimb, Rt HL: Right hindlimb. a is the patient could walk normally on non-slippery surface, b is the patient could walk normally on slippery surface. Results: 0 = Absent reflex, +1 = Decreased reflex, +2 = Normal reflex

Table 2. Neurological examination of this patient, spinal reflexes result of 1st-12th visit.

Spinal reflex	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Flexor reflex												
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Lt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Rt FL	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
Ext. Carp. radialis												
Lt FL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rt FL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Patella reflex												
Lt FL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rt FL	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Perineal reflex												
Lt	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Rt	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Cross extensor												
Lt	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Rt	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Panniculus												
	Absented around L1-L3			+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Neurological examination in 1st visit is before treatment. Lt FL: Left forelimb, Rt FL: Right forelimb, Lt HL: Left hindlimb, Rt HL: Right hindlimb, Lt: Left, Rt: Right, +ve: positive, -ve: negative, N/A: Not applicable.

Table 3. Neurological examination of this patient, Sensation result of 1st-12th visit.

Test	Result
Superficial pain at hindlimb	Positive
Deep pain at hindlimb	Positive
Hyperesthesia	Negative
Self mutilation	Negative

A whole-body radiograph from the previous hospital showed an unremarkable lesion of bone. In addition, abdominal ultrasonography revealed an abdominal focused assessment with sonography for trauma, triage, and tracking (AFAST) is 0/4 (no abdominal fluid). Because of the limited equipment and patient budget, the ferret was not performed magnetic resonance imaging (MRI) to rule out the abnormality of the spinal cord. The results of the neurological examination indicated that the spinal lesion is located around T3-L3 and is especially suspected at T14-T15. Because the ferret hindlimb was paresis, but the flexor reflex of the spinal reflex in both hindlimbs was normal, which means the upper motor neuron sign. Panniculus reflex was absent around L1-L3, which indicates spinal cord lesion would be above one or two segments the absence. The differential diagnosis of a spinal cord lesion was a spinal cord injury from trauma.

The ferret's treatment was only laser acupuncture because of the difficulty of taking the medicine. According to the TCVM examination and diagnostic system, her examination results were that her body and feet felt warm when touched, her tongue was pale pink to purple, and her femoral pulse was wiry on the right side. She had a history of traumatic spinal cord injury and paraparesis. Consequently, Spinal *Qi*/Blood Stagnation and Kidney *Qi* Deficiency was her TCVM diagnosis. Because of trauma led *Qi*/Blood accumulated or Stagnation in the spinal cord, which showed a pale pink to purple tongue color appearance. When the Spinal cord *Qi*/Blood was Stagnation, *Qi* of Kidney, the one of Five Elements that controls the brain and spinal cord (Xie and Preast 2013), would be deficient and result in paresis/paralysis caudal to the lesion (Xie 2011). Based upon the TCVM diagnosis, the following acupuncture points were selected for treatment (Table 4; Figures 1.1-1.3). Each acupoint had been treated with laser therapy (M-Vet, MLS®) in Laserpuncture mode. The parameter of laser acupuncture in every acupoint is 611 Hz, intensity 1%, total time 5 seconds with 4.44 J/cm^2 and repeat 3 times per point (Petermann 2017; Ruffoni and Pozzi 2020). Multiwave Locked System (MLS®) laser therapy is medical equipment which has two different and synchronized emission lasers, including 905 nm as pulsed emission and 808 nm as continuous/frequenced emission (Ruffoni and Pozzi 2020). The ferret was restrained by gently holding it, as shown in Figure 4. The treatment process per session was 10 minutes. The treatment sessions were 12 sessions. The frequency of the first three sessions was twice a week for 3 treatments, then once a week for 9 treatments. After the first three sessions of treatment, the ferret still had decreased proprioception (+1) at both hindlimbs. She gradually improved in each session. She had normal proprioception (+2) at both

hindlimbs when walking on the non-slip floor after 6 sessions of treatment. This successful treatment outcome enabled the patient to regain the ability to walk normally, even on slippery surfaces, after 11 sessions of treatment (Tables 1-3) and was enhanced in the overall quality of life.

Table 4. Acupoints used to treat the ferret presenting with posterior paresis and TCVM Pattern diagnoses is Spinal cord *Qi*/Blood Stagnation with Kidney *Qi* Deficiency (Xie 2011).

Acupoint	Location	Technique	Indications and Actions
LI-10 (*Qian-san-li*)	Located 2 cun distal to LI-11 (1/6 of the distance between the elbow and carpus) between the extensor carpi radialis and the common digital extensor muscles.	Laser acupuncture	Immune regulation, general tonic, skin itching, diarrhea, Wind-Heat, elbow pain thoracic limb and pelvic limb weakness.
ST-36 (*Hou-san-li*)	Located 3 cun distal to ST-35, 0.5 cun lateral to the cranial crest of the tibia; in the belly of the cranial tibialis muscle. This is a long linear point.	Laser acupuncture	He-sea (Earth) Master point for gastrointestinal tract and abdomen. Nausea, vomiting, gastric pain, gastric ulcer, food stasis, general tonic
BL-11 (*Da-zhu*)	Located 1.5 cun lateral to the caudal border of the dorsal spinous process of the 1st thoracic vertebra. (The point may be punctured by a needle inserted midway between the spinous process and the medial border of the scapula directing the needle slightly laterally).	Laser acupuncture	Influential point for bone, cough, fever, cervical stiffness, shoulder pain, degenerative joint disease, intervertebral disk disease, back pain.
BL-23 (*Shen-shu*)	Located 1.5 cun lateral to the caudal border of the dorsal spinous process of the 2nd lumbar vertebra.	Laser acupuncture	Back-shu (Association) point for Kidney. Kidney Yin/*Qi* Deficiency, urinary incontinence, impotence, deafness, sore and weak back.

Table 4. Acupoints used to treat the ferret presenting with posterior paresis and TCVM Pattern diagnoses is Spinal cord *Qi*/Blood Stagnation with Kidney *Qi* Deficiency (Xie 2011). (Cont.)

Acupoint	Location	Technique	Indications and Actions
BL-24 (*Qi-hai-shu*)	Located on the dorsolateral aspect of the spine, 1.5 cun lateral to the caudal border of the dorsal spinous process of the third or fourth lumbar vertebra (L3 or L4).	Laser acupuncture	Sea of *Qi* point. *Qi* deficiency, thoracolumbar intervertebral disk disease, abdominal pain, uterine disease, diarrhea, hemorrhage.
BL-26 (*Guan-yuan-shu*)	Located 1.5 cun lateral to the caudal border of the dorsal spinous process of the 6th lumbar vertebra.	Laser acupuncture	Gates of original/source *Qi*. Kidney Yang/*Qi* Deficiency, urinary incontinence or dribbling, impotence, back pain, diarrhea.
BL-40 (*Wei-zhong*)	Located in the center of the popliteal crease. The point is found by directing the needle cranially towards the patella.	Laser acupuncture	He-sea point (Earth), Master point for caudal back/hips. Dysuria, urinary incontinence, hip joint, back problems, autoimmune disease, vomiting, diarrhea.
KID-1 (*Yong-quan*)	Located on the plantar surface of the pelvic limb paw, between the 3rd and 4th metatarsal bones underneath the central pad of the rear foot.	Laser acupuncture	Jing-well point (Wood), Son points for Excess. Rear weakness, coma, sore throat, urinary dribbling, aphonia (loss of voice).
KID-7 (*Fu-liu*)	Located 2 cun proximal to the tip of the medial malleolus, on the cranial border of the Achilles tendon.	Laser acupuncture	Jing-river point (Metal), Mother point for Deficiency. Anhidrosis, diarrhea, edema, abdominal fullness, paralysis of pelvic limbs.

Table 4. Acupoints used to treat the ferret presenting with posterior paresis and TCVM Pattern diagnoses is Spinal cord *Qi*/Blood Stagnation with Kidney *Qi* Deficiency (Xie 2011). (Cont.)

Acupoint	Location	Technique	Indications and Actions
GB-34 (*Yang-ling-quan*)	Located in the depression, just distal and cranial to the head of fibula on the lateral side of the pelvic limb.	Laser acupuncture	He-sea point (Earth). Influential point for ligaments/tendons. Vomiting, biliary disorders, pelvic limb weakness, weakness of ligaments/tendons.
Bai-hui	Located between L7-S1 on dorsalmidline.	Laser acupuncture	Yang Deficiency, diarrhea, constipation, intervertebral disk disease, Pelvic limb paresis or paralysis.
Shen-shu	Located 1 cun lateral to *Bai-hui*.	Laser acupuncture	Source *Qi*, Kidney *Qi*/Yang Deficiency, diarrhea, constipation, intervertebral disk disease, pelvic limb paresis or paralysis.
Shen-peng	Located 1 cun cranial to *Shen-shu*.	Laser acupuncture	Yang Deficiency, diarrhea, constipation, intervertebral disk disease, pelvic limb paresis or paralysis.
Shen-jiao	Located 1 cun caudal to *Shen-shu*.	Laser acupuncture	Yang Deficiency, diarrhea, constipation, intervertebral disk disease, pelvic limb paresis or paralysis.
Liu-feng	Located at the skin fold on the dorsal aspect of the metatarsophalangeal joints between digits 2-3, 3-4, 4-5	Laser acupuncture	Paresis, paralysis

Figure 1. Demonstration of located acupoints: *Liu-feng* and KID-1.

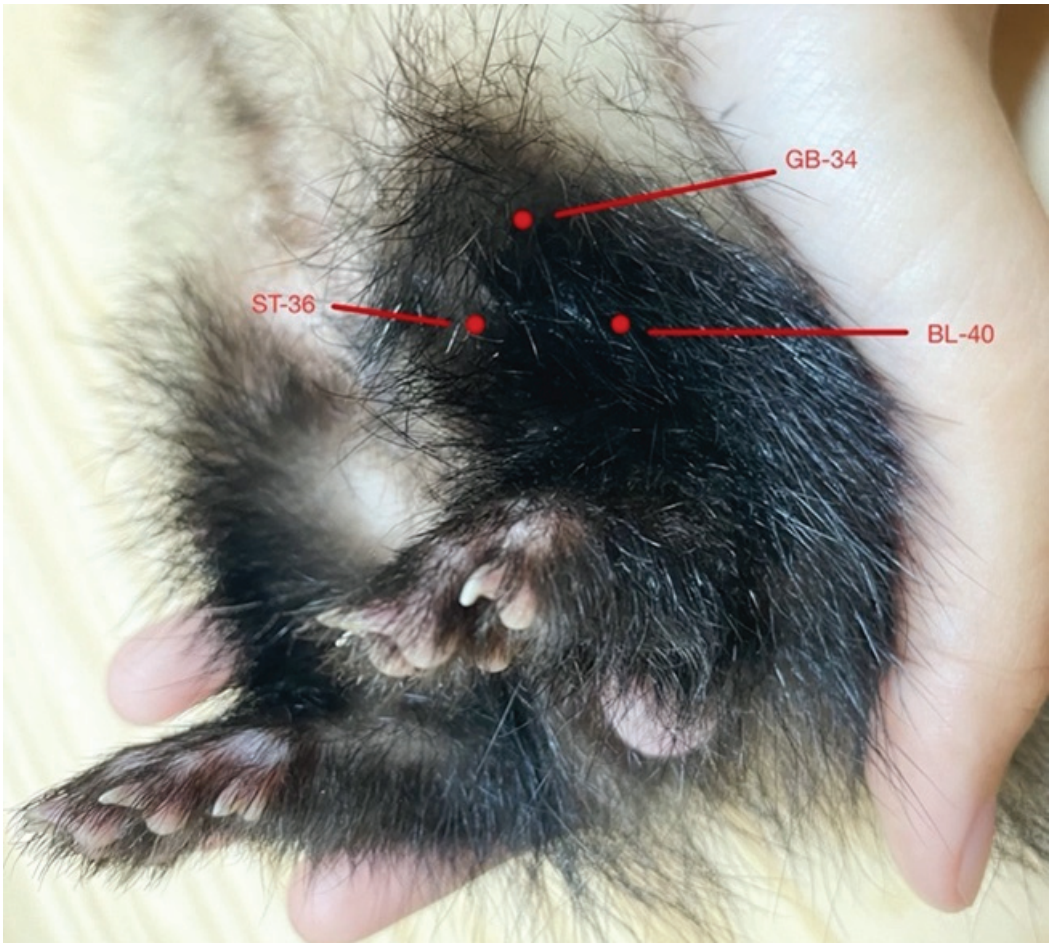


Figure 2. Demonstration of located acupoints: ST-36, GB-34, and BL-40.

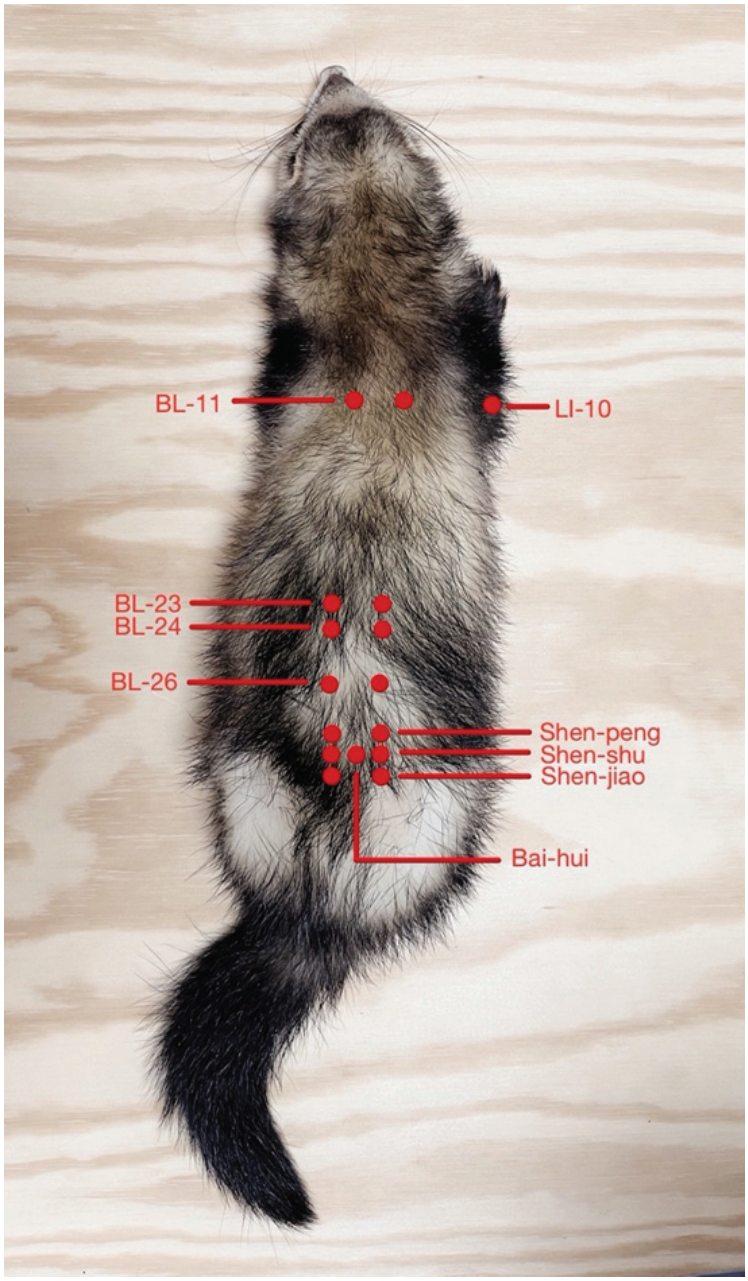


Figure 3. Demonstration of located acupoints: BL-11, BL-23, BL-24, BL-26, *Shen-shu*, *Shen-peng*, *Shen-jiao*, LI-10, and *Bai-hui*.

Figure 4. Gently holding the ferret during the laser acupuncture treatment.

Discussion

According to the ferret, a small zoological companion animal, acupuncture is a safe treatment with minimal side effects, but dry needle acupuncture or electroacupuncture can cause discomfort or pain in some acupoints. Generally, the duration of treatment for dry needle acupuncture is commonly around 10 to 30 minutes, less than 10 minutes for fatigued patients, or 1 to 15 minutes for sensitive points around the feet and eyes (Xie and Preast 2007). Electroacupuncture treatment duration is around 20 to 30 minutes, depending on the disease (Xie and Preast 2007). Even so, laser acupuncture is taken for a few seconds at each treatment point or acupoint (Xie and Preast 2007; Ruffoni and Pozzi 2020; Koh and Harrison 2023). From my perspective, laser acupuncture, one of the acupuncture techniques, gives great results for treating spinal cord injury disease in ferrets or animals that cannot accept needles being placed and stay still.

Acupuncture may serve as a primary or adjunctive therapy in the reduction of medication dosage or duration of treatment, as well as the potential reduction of some medications. This ferret received laser acupuncture as the primary treatment without any medication due to her limited conditions. According to other articles, ferret with hindlimb lameness and other illness conditions was received dry needle acupuncture and aquapuncture which had some similar acupoints that could resolve the hindlimb weakness in total 5 sessions but still occasional stumbling (Kordupel 2023). The utilization of laser acupuncture has been shown to have the potential to decrease the analgesic medication requirement of cats following an ovariohysterectomy procedure (Marques et al., 2015). Moreover, the laser acupuncture was used to treat the 10 dogs with behavioral problem related to phobia, anxiety, aggression or deficit of self-controls. The laser acupuncture parameters were frequency 1168 Hz, time for 10 seconds, energy was 2059J and dose was 16.39 J/cm2 (Ruffoni and Pozzi 2020). Laser acupuncture parameters which used in this case study were followed the set-up parameters in MLS® laser therapy which split into 3 times per acupoints due to ferret's intolerance. The resonance frequencies in the parameters are determine the therapeutic effect of laser treatment and currently used Nogier, Bahr or Reininger (Petermann 2017). It is recommended to consider using acupuncture as a treatment for animals with specific illnesses. This includes older patients who are cautious about using nonsteroidal anti-inflammatory drugs (NSAIDs) for a long time, as well as patients with liver or renal disease who need careful consideration of medication metabolism. Furthermore, laser acupuncture is a specific technique within the field of acupuncture that can provide the best possible results for individuals who are unable to withstand physical restraints or painful stimuli.

Conclusion

In this report, laser acupuncture is the effective treatment for posterior paresis, which could be the monotherapy for ferrets. However, it is imperative that future trials should standardize laser acupuncture parameters for ferrets and compare the results of laser acupuncture treatment and other acupuncture techniques.

Acknowledgements

This endeavor would not have been possible without the assistance of all personnel at the Panalai Veterinary Hospital, including the owner and patients who collaborate on the treatments. Special special thanks to Mr. Jeeraphat Janphon, a veterinary nurse, for restraining our case through the treatments.

References

Bockstahler B, Levine D, Maierl JW, Millis DL, Wittek K. Essential facts of physical medicine, rehabilitation and sports medicine in companion animals. Babenhausen: VBS GmbH; 2019.

Chon TY, Mallory MJ, Yang J, Bublitz SE, Do A, Dorsher PT. Laser acupuncture: A concise review. Med Acupunct. 2019;31(3):164-8.

Chrisman CL. Spinal cord disorders. Proceedings of the 13th Annual International TCVM Conference. Tianjin, China: Jing Tang Publishing; 2011. p. 225-322.

Harrison TM, Churgin SM. Acupuncture and traditional Chinese veterinary medicine in zoological and exotic animal medicine: A review and introduction of methods. Vet Sci. 2022;9(2):74.

Huynh M, Piazza S. Musculoskeletal and Neurologic Diseases. In: Quesenberry KE, Orcutt CJ, Mans C, Carpenter JW. Ferrets, rabbits, and rodents clinical medicine and surgery. 4th ed. St. Louis: Elsevier; 2021. p. 117-30.

Koh RB, Harrison TM. Acupuncture in zoological companion animals. Vet Clin Exot Anim Pract. 2023;26(1):257-80.

Kordupel M. Acupuncture for hindlimb lameness in a ferret. Am J Trad Chin Vet Med. 2023;18(1):49-53.

Lao L, Wong RH, Berman B, Wynn RL. Electroacupuncture reduces morphine-induced emesis in ferrets: a pilot study. J Altern Complement Med. 1995;1(3):257-61.

Marques VI, Cassu RN, Nascimento FF, Tavares RCP, Crociolli GC, Guilhen R, et al. Laser acupuncture for postoperative pain management in cats. Evid Based Complement Alternat Med. 2015;2015: 653270.

Mitchell MA, Tully Jr TN. Manual of exotic pet practice. St. Louis: Elsevier; 2009. p. 345-74.

Petermann U, Laser acupuncture and local laser therapy in veterinary medicine with overview of applied laser types and clinical uses. Am J Chinese Med. 2017;12(1):89-101.

Petermann U. Comparision of pre- and post- treatment pain scores of twentyone horse with laminitis treated with acupoint and topical low level impulse laser therapy. Am J Chinese Med. 2011;6(1):13-25.

Powers LV, Perpinan D. Basic anatomy, physiology, and husbandry of ferrets. In: Quesenberry KE, Orcutt CJ, Mans C, Carpenter JW. Ferrets, rabbits, and rodents clinical medicine and surgery. 4th ed. St. Louis: Elsevier; 2021. p. 1-12.

Ruffoni P, Pozzi R. Laser acupuncture in behavior problems of dog. Energy for health. 2020;20: 16-22.

Srionrod N. The use of traditional Chinese veterinary medicine; dry needle acupuncture integrated with conventional veterinary medicine for impaction colic treatment in horse: A clinical case report. J Appl Anim Sci. 2021;14(1):61-70.

Xie H, Preast V. Traditional Chinese veterinary medicine fundamental principles. Florida: Chi Institute of Chinese Medicine; 2013.

Xie H, Preast V. Xieís Veterinary acupuncture. Iowa: Blackwell publishing; 2007.

Yang Y, Litscher G, Sun Z, Sun W. The application of laser acupuncture in animal experiments: A narrative review of biological aspects. Evid Based Complement Alternat Med. 2021;2021:6646237.

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

มหาวิทยาลัยมหิดล
คณะสัตวแพทยศาสตร์

เปิดรับสมัคร นักศึกษา ประจำปี 2567

หลักสูตรประกาศนียบัตรบัณฑิต สาขาวิทยาศาสตร์ทางการสัตวแพทย์คลินิก
(Graduate Diploma Program in Clinical Veterinary Science)

เปิดรับสมัครผ่านทางระบบ ออนไลน์ บัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล

รับนักศึกษาจำนวน 6 คน

1 ธ.ค. 66 - 31 มี.ค. 67

กรณีมีผู้สละสิทธิ์ อาจเปิดรับเพิ่ม*
รอบที่ 2 : 25 เมษายน 2567 - 31 พฤษภาคม 2567

*ผู้สนใจสมัครรอบที่ 2
โปรดติดต่อประธานหลักสูตรก่อนทำการสมัครในระบบ
(เพื่อตรวจสอบจำนวนรับเพิ่ม ทั้งนี้เมื่อชำระค่าสมัครแล้ว
ทางหลักสูตรสงวนสิทธิ์ไม่คืนเงินในทุกกรณี)

- มุ่งเน้น Reskill & Upskill ความรู้และทักษะทางคลินิก
- รับผู้ที่จบสัตวแพทยศาสตร์บัณฑิต หรือเป็นนักศึกษาในชั้นปีสุดท้าย
- ใช้ระยะเวลาศึกษา 1 ปี (2 ภาคเรียน)

สอบถามข้อมูลเพิ่มเติม ติดต่อประธานหลักสูตร
Email: grisnarong.won@mahidol.edu

ศึกษารายละเอียดเพิ่มเติมได้ที่
www.grad.mahidol.ac.th

หรือ สแกนผ่าน QR Code

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