Academic Research of Graduate School of Veterinary Medicine

January – December
2025

Graduate School of Veterinary Medicine

Rakuno Gakuen University

2025 年（1 月～12 月）の獣医学研究科英文業績の発刊にあたって

酪農学園大学大学院 獣医学研究科
研究科長 山下 和人

獣医学研究科では、2002 年より、獣医学群教員が 1 年間に公表した英語論文を冊子体にまとめ、各教員の自己点検や研究の質保証、ならびに関係各位に獣医学群教員の研究状況を紹介する目的で業績集を刊行しています。

2025 年の筆頭著者（FA）および責任著者（CA）の論文合計は 81 報であり、2021 年の 83 報に続く英文業績数でした(2024 年 55 報、2023 年 51 報、2022 年 68 報、2021 年 83 報、2020 年 72 報、2019 年 54 報、2018 年 54 報、2017 年 48 報、2016 年 45 報、2015 年 44 報、2014 年 51 報)。本学獣医学群では、昨年までの 6 年間に獣医学教育の質の保証と国際化に取り組み、スキルスラボを整備して 3Rs（Replacement、Reduction、Refinement）に対応し、カリキュラムに Hands On 実習とクリニカルローテーションを導入して 2024 年 12 月にヨーロッパ獣医学教育機関協会（EAEVE: European Association of Establishments for Veterinary Education）による国際認証を取得しました。前研究科長が憂慮されていたように、「EAEVE 対応の教育負担増」によって個々の教員は研究活動エフォートを抑制せざるを得ない状況に直面し、英文業績の公表は影響を受けました。しかし、2026 年度にカリキュラムは完成年度を迎え、獣医学群の教員組織は 107 名体制（専任教員 81 名、嘱託教員 26 名）に整備され、教員組織の整備による「EAEVE 対応の教育負担増」の分散と教員個々の馴化で、研究活動を抑制せざるを得ない状況から切り抜けつつあります。

一方、本年度の本学全体の科学研究費助成事業（科研費）の採択件数は 40 件に減少し（2024 年度 52 件、2023 年度 56 件、2022 年度 48 件、2021 年度 37 件、2020 年度 31 件）、その配分額も 6,650 万円に減少して（2024 年度 7,732 万円、2023 年度 8,164 万円）、私立大学では 596 大学中 134 位でした（2024 年度 112 位、2023 年度 100 位、2022 年度 119 位、2021 年度 129 位、2020 年度 133 位、「教育学術新聞」より）。農食環境学群では、入学志望者・入学者の減少に対応すべく、教員組織のスリム化と学類再編を含む教育改革が進められており、このことが本学全体の科研費採択件数に影響しているのかもしれません。教員個々の研究活動の高まりを持続的に確保するためにも、酪農学研究科と獣医学研究科の両研究科を跨ぐリサーチセンターの設置などの研究活動の活性化を図る方策が必要と考えています。

最後に、関係各位には本業績集をご高覧頂き、教員個々の研究内容などから共同研究、さらには広範な研究体制の構築等へとご高配を頂けることを願っております。今後とも、獣医学研究科の教育・研究活動がさらに発展するよう、ご指導ご鞭撻をよろしくお願い申し上げます。

For the publication of the English publications of the Graduate School of Veterinary Medicine for 2024 (January to December)

Since 2002, the Graduate School of Veterinary Medicine has compiled the English papers published by veterinary medicine faculty members over the past year into a booklet, which allows each faculty member to conduct self-inspections and ensure the quality of their research, as well as inform everyone concerned about the research status of veterinary faculty members.

The total number of first author (FA) and corresponding author (CA) papers in the School of Veterinary Medicine in 2025 was 81 reports, in the past, 55 reports in 2024, 56 reports in 2023, 68 reports in 2022, 83 reports in 2021, 72 reports in 2020, 54 reports in 2019, 54 reports in 2018, 48 reports in 2017, 45 reports in 2016, 44 reports in 2015, and 51 reports in 2014. Over the past six years, our School of Veterinary Medicine has been working to ensure the quality of veterinary education and internationalize it. We have established a skills lab to address the 3Rs (Replacement, Reduction, Refinement), introduced hands-on training and clinical rotations into the curriculum, and received international accreditation from the European Association of Establishments for Veterinary Education (EAEVE) in December 2024. As the previous Dean had feared, the "increased teaching burden in response to EAEVE" forced individual faculty members to curtail their research efforts, which affected the publication of English-language academic achievements. However, the curriculum will reach completion in 2026, and the faculty organization in the School of Veterinary Medicine will be reorganized to 107 members (81 full-time faculty and 26 contract faculty). The reorganization of the faculty organization has helped to distribute the "increased teaching burden in response to EAEVE" and allow individual faculty members to become accustomed to it, allowing us to overcome the situation in which we were forced to curtail our research activities.

On the other hand, the number of Grants-in-Aid for Scientific Research (KAKENHI) selected for the Rakuno Gakuen University this year has decreased to 40 (52 in 2024, 56 in 2023, 48 in 2022, 37 in 2021, and 31 in 2020), and the amount allocated has also decreased to 66.5 million yen (77.32 million yen in 2024, and 81.64 million yen in 2023), ranking 134th out of 596 private universities (112th in 2024, 100th in 2023, 119th in 2022, 129th in 2021, and 133rd in 2020, according to the Education and Academic Newspaper). In response to a decline in applicants and enrollees, the School of Agriculture, Food and Environmental Sciences is undergoing educational reforms, including streamlining its faculty organization and restructuring its departments, which may be affecting the number of KAKENHI awarded to the university as a whole. In order to ensure a sustained increase in the research activities of individual faculty members, we believe that measures to revitalize research activities, such as the establishment of a research center that spans both the Graduate Schools of Dairy Science and Veterinary Medicine, are necessary.

Finally, we hope that all concerned parties will take a look at this collection of accomplishments, and that they will be interested in the research content of individual faculty members, joint research, and even the establishment of a broader research system. We look forward to your continued guidance and support so that the educational and research activities of the Graduate School of Veterinary Medicine can further develop.

Kazuto Yamashita DVM, MS, PhD, Charter Dipl. JCVS, Honorary Dipl. AiCVS. .
Dean, Rakuno Gakuen University Graduate School of Veterinary Medicine.

Contents（January –December 2025）

*: 大学院担当教員（Supervisor）

■ 獣医学類 **Department o Veterinary Medicine**

■ 獣医保健看護学類 Department of Veterinary Science

獣医細菌学（Veterinary Bacteriology）

Masato Akiba

Professor

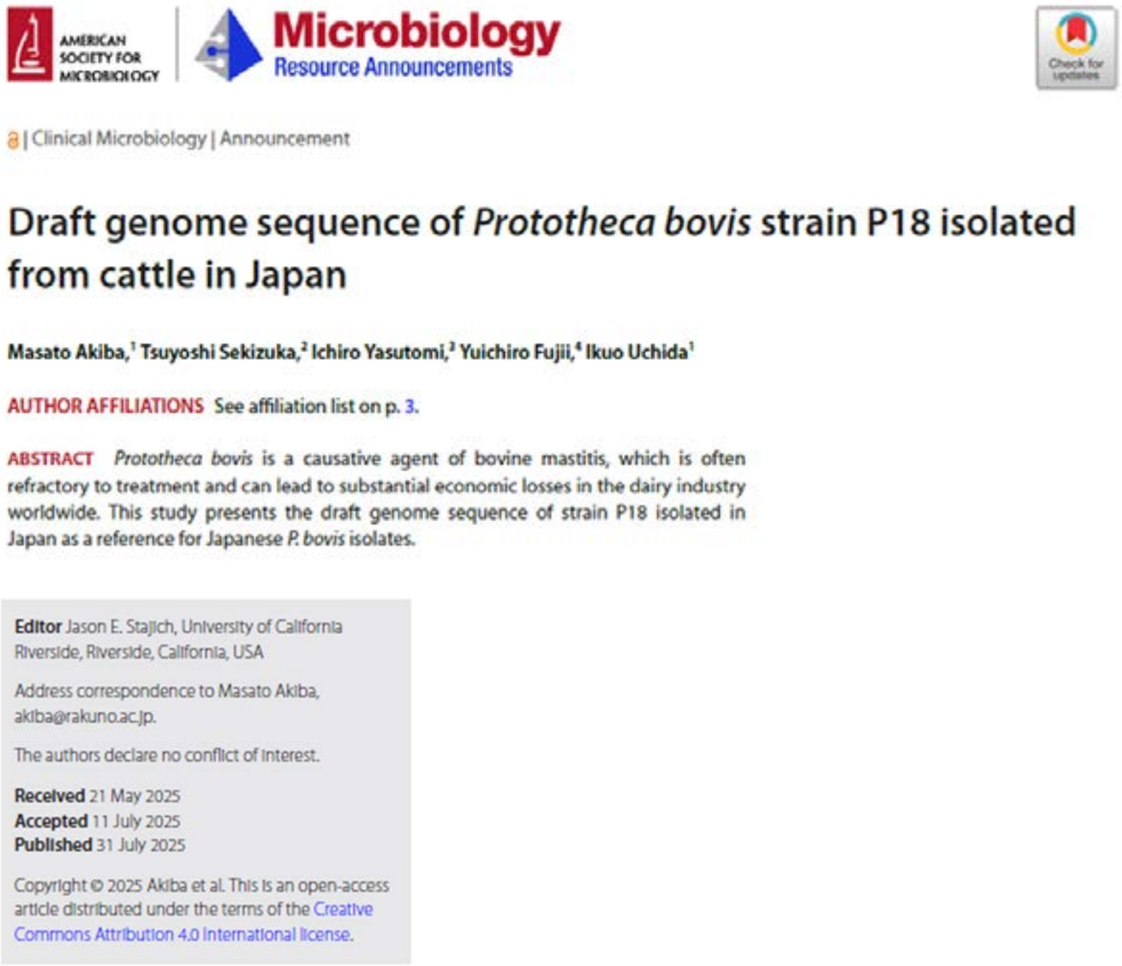
教授　秋庭　正人

Ⅰ. 筆頭または責任著者　＜First or Corresponding Author＞

1) Draft genome sequence of *Prototheca bovis* strain P18 isolated from cattle in Japan.
Akiba M, Sekizuka T, Yasutomi I, Fujii Y, Uchida I.
Microbiol Resour Announc. 14:e0054425. 2025. doi:10.1128/mra.00544-25.

Ⅱ. その他　＜Others＞

1) Association between farm-level antimicrobial usage and antimicrobial resistance in Japan.
Tamamura-Andoh Y, Kobayashi S, Yamane I, Watanabe M, Guruge KS, Uegaki R, Arai N, Watanabe-Yanai A, Iwata T, Katsuda K, **Akiba M**, Kusumoto M.
J Vet Med Sci. 87:1053-1059. 2025. doi: 10.1292/jvms.25-0221.



AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

Clinical Microbiology | Announcement

Draft genome sequence of *Prototheca bovis* strain P18 isolated from cattle in Japan

Masato Akiba,[1] **Tsuyoshi Sekizuka,**[2] **Ichiro Yasutomi,**[3] **Yuichiro Fujii,**[4] **Ikuo Uchida**[1]

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT *Prototheca bovis* is a causative agent of bovine mastitis, which is often refractory to treatment and can lead to substantial economic losses in the dairy industry worldwide. This study presents the draft genome sequence of strain P18 isolated in Japan as a reference for Japanese *P. bovis* isolates.

Editor Jason E. Stajich, University of California Riverside, Riverside, California, USA

Address correspondence to Masato Akiba, akiba@rakuno.ac.jp.

The authors declare no conflict of interest.

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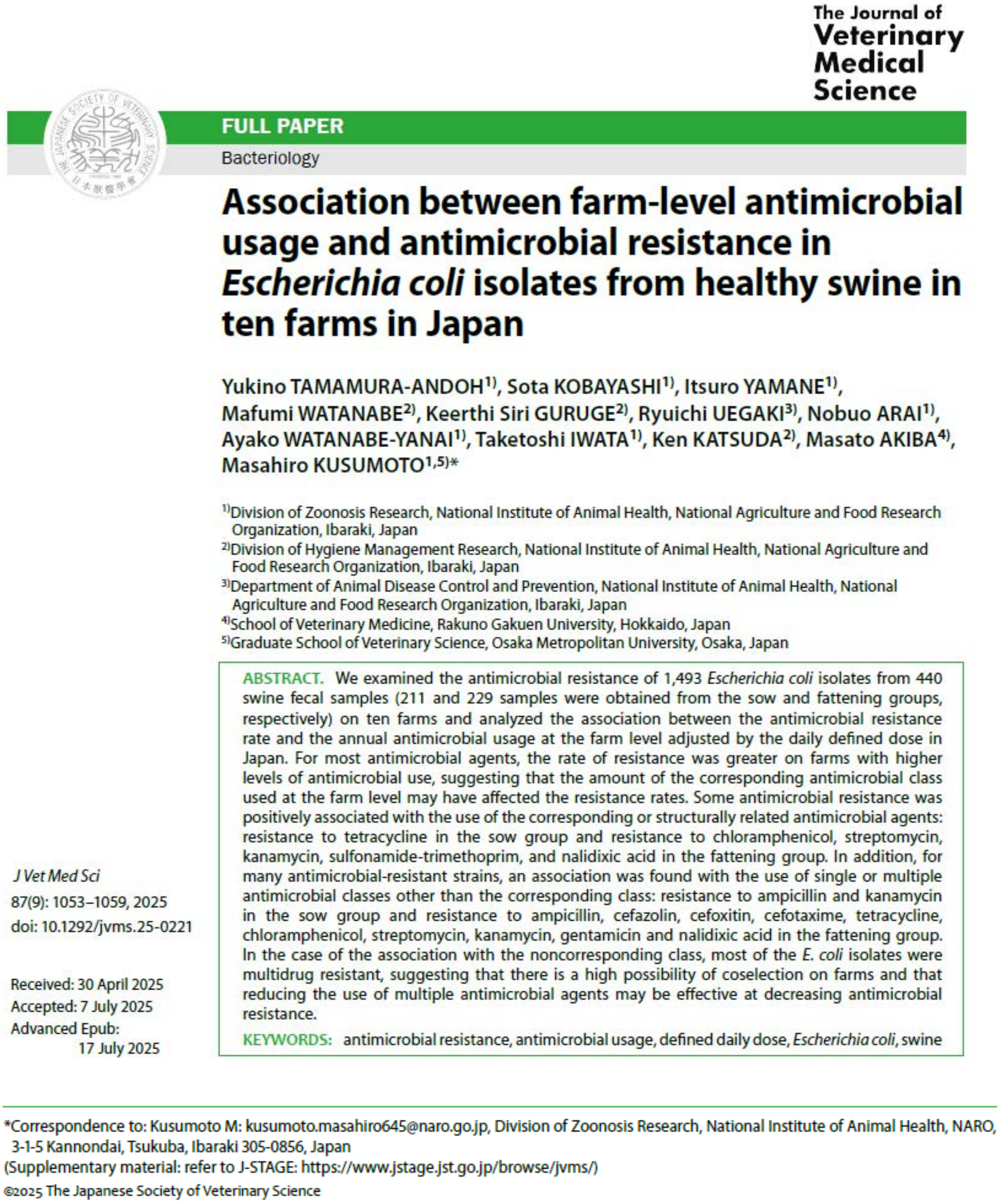
最終責任者　Masato Akiba (Corresponding Author)


The Journal of
Veterinary
Medical
Science

FULL PAPER
Bacteriology

Association between farm-level antimicrobial usage and antimicrobial resistance in *Escherichia coli* isolates from healthy swine in ten farms in Japan

Yukino TAMAMURA-ANDOH[1)], Sota KOBAYASHI[1)], Itsuro YAMANE[1)],
Mafumi WATANABE[2)], Keerthi Siri GURUGE[2)], Ryuichi UEGAKI[3)], Nobuo ARAI[1)],
Ayako WATANABE-YANAI[1)], Taketoshi IWATA[1)], Ken KATSUDA[2)], Masato AKIBA[4)],
Masahiro KUSUMOTO[1,5)]*

[1)]Division of Zoonosis Research, National Institute of Animal Health, National Agriculture and Food Research Organization, Ibaraki, Japan
[2)]Division of Hygiene Management Research, National Institute of Animal Health, National Agriculture and Food Research Organization, Ibaraki, Japan
[3)]Department of Animal Disease Control and Prevention, National Institute of Animal Health, National Agriculture and Food Research Organization, Ibaraki, Japan
[4)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[5)]Graduate School of Veterinary Science, Osaka Metropolitan University, Osaka, Japan

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ABSTRACT. We examined the antimicrobial resistance of 1,493 *Escherichia coli* isolates from 440 swine fecal samples (211 and 229 samples were obtained from the sow and fattening groups, respectively) on ten farms and analyzed the association between the antimicrobial resistance rate and the annual antimicrobial usage at the farm level adjusted by the daily defined dose in Japan. For most antimicrobial agents, the rate of resistance was greater on farms with higher levels of antimicrobial use, suggesting that the amount of the corresponding antimicrobial class used at the farm level may have affected the resistance rates. Some antimicrobial resistance was positively associated with the use of the corresponding or structurally related antimicrobial agents: resistance to tetracycline in the sow group and resistance to chloramphenicol, streptomycin, kanamycin, sulfonamide-trimethoprim, and nalidixic acid in the fattening group. In addition, for many antimicrobial-resistant strains, an association was found with the use of single or multiple antimicrobial classes other than the corresponding class: resistance to ampicillin and kanamycin in the sow group and resistance to ampicillin, cefazolin, cefoxitin, cefotaxime, tetracycline, chloramphenicol, streptomycin, kanamycin, gentamicin and nalidixic acid in the fattening group. In the case of the association with the noncorresponding class, most of the *E. coli* isolates were multidrug resistant, suggesting that there is a high possibility of coselection on farms and that reducing the use of multiple antimicrobial agents may be effective at decreasing antimicrobial resistance.

KEYWORDS: antimicrobial resistance, antimicrobial usage, defined daily dose, *Escherichia coli*, swine

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*Correspondence to: Kusumoto M: kusumoto.masahiro645@naro.go.jp, Division of Zoonosis Research, National Institute of Animal Health, NARO, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan
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最終責任者　Masahiro Kusumoto (Corresponding Author)

医動物学（**Medical Zoology**）

Shinya Fukumoto

Professor

教授　福本　晋也

Ⅰ. 筆頭または責任著者　＜First or Corresponding Author＞
該当なし

Ⅱ. その他　＜Others＞

1） Possibility of Vertical Transmission of *Sarcocystis* spp. in Sika Deer in Japan.
Yamazaki A, Yamaguchi Y, Hiroshima T, Urushibara Y, Shirafuji Y, **Fukumoto S**, Kamata Y.
Foodborne Pathog Dis. 22: 729-736. 2025. doi: 10.1089/fpd.2024.0090.

2） High pyrethroid resistance is associated with high frequencies of 1014F and 1014S kdr mutations in *Anopheles arabiensis* (Diptera: Culicidae) from Ouagadougou, Burkina Faso.
Somda Z, Zanre N, Wangrawa DW, Toe HK, Sombie A, Saiki E, **Fukumoto S**, Sakurai T, Sanon A, McCall PJ, Kanuka H, Weetman D, Badolo A.
J Med Entomol. 62:381-388. 2025. doi: 10.1093/jme/tjae135.

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Possibility of Vertical Transmission of *Sarcocystis* spp. in Sika Deer in Japan

Akiko Yamazaki,[1] Yoshitaka Yamaguchi,[2] Tatsuya Hiroshima,[3] Yui Urushibara,[4] Yukiko Shirafuji,[4]
Shinya Fukumoto,[5] and Yoichi Kamata[6]

Abstract

In recent years, the wild deer population in Japan has grown exponentially, causing severe feeding damage to the agricultural and forestry industries. Therefore, the game meat industry is being promoted for effective utilization of hunted animals. Wild animals are not hygienically controlled and can serve as reservoirs for pathogenic microorganisms. However, epidemiological information on wild animals in Japan remains insufficient. Recently, food poisoning-like cases have occurred because of raw venison infection with *Sarcocystis* spp. As the prevalence of *Sarcocystis* spp. in sika deer is very high in Japan and even fawns are infected, this study attempted to verify the vertical infection of *Sarcocystis* spp. in sika deer in Japan. Genetic detection of *Sarcocystis* 18S ribosomal RNA in fetal and maternal tissues from early to late gestation in sika deer revealed *Sarcocystis* Types 1–5 and *Sarcocystis fayeri* in the mother and fetus. Types 1, 2, 4, and 5 were detected in the maternal tissues of Ezo sika deer (*Cervus nippon yesoensis*) in Hokkaido, whereas Types 1 and 2 and *S. fayeri* were detected in fetuses. Types 1–5 were detected in Honshu sika deer (*Cervus nippon centrali*s) in Mie Prefecture but not in the fetuses. Types 1, 2, and 4 were detected in the udder and milk samples. This indicates that *Sarcocystis* Types 1 and 2 and *S. fayeri* have the ability to pass through the placenta of sika deer and invade fetal tissues and Types 1, 2, and 4 may be transmitted orally via milk. These findings suggest that there is transplacental and transmammary transmission of *Sarcocystis* spp. in sika deer.

Keywords: *Sarcocystis*, epidemiology, game meat, sika deer, vertical transmission, food poisoning

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[1] Department of Veterinary Sciences, University of Miyazaki, Miyazaki, Japan.
[2] Yamagata Prefecture Shonai Public Health Center, Yamagata, Japan.
[3] Aeon Animal Medical Center Makuhari-Shintoshin, Chiba, Japan.
[4] Cooperative Department of Veterinary Medicine, Iwate University, Morioka, Japan.
[5] National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.
[6] Department of Food and Nutrition, Senri Kinran Universit, Suita, Japan.

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最終責任者　Akiko Yamazaki (Corresponding Author)

High pyrethroid resistance is associated with high frequencies of 1014F and 1014S kdr mutations in *Anopheles arabiensis* (Diptera: Culicidae) from Ouagadougou, Burkina Faso

Zephirin Somda, Nicolas Zanré, Dimitri W Wangrawa, Hyacinthe K Toé, Aboubacar Sombié, Erisha Saiki, Shinya Fukumoto, Tatsuya Sakurai, Antoine Sanon, Philip J McCall, Hirotaka Kanuka, David Weetman, Athanase Badolo ✉

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Abstract

Malaria remains a major public health threat in Burkina Faso, as in most sub-Saharan Africa countries. Malaria control relies mainly on long-lasting insecticide-treated nets (LLINs) and indoor residual spraying. In Burkina Faso, an escalating of insecticide resistance has been observed over the last decades. This study aimed to investigate insecticide resistance and the underlying mechanisms in *Anopheles gambiae* complex in Ouagadougou. *Anopheles gambiae* s.l. larvae were collected from gutters and ponds, in Zogona, Tampouy and Tanghin, 3 localities in Ouagadougou from July to October 2018. The larvae were reared in the laboratory to adults stage and susceptibility profile to pyrethroid, carbamate, and organophosphate insecticides was assessed using WHO tube assays. Mosquito species and mutations linked with insecticide resistance, were identified through PCR. More than 95% of the collected *An. gambiae* s.l. were *An. arabiensis*. *An. arabiensis* displayed high resistance to permethrin and deltamethrin, with mortalities below 30%, but was fully susceptible to bendiocarb, fenitrothion, and malathion. A high-frequency of the pyrethroid resistance-associated *kdr* mutation 1014F (0.81) was recorded, while the frequency of 1014S mutation (0.18) was lower. However, the carbamate and organophosphate-associated *Ace-1* 119S mutation was not detected. Localities and breeding site type appear to influence pyrethroid resistance in the *An. arabiensis* population of Ouagadougou. The high resistance to pyrethroids in *An. arabiensis* of urban Ouagadougou is underpinned, at least in part by high-frequency kdr mutations. This result supports the switch to next-generation LLINs, in well-established pyrethroid resistance zones of Burkina Faso including Ouagadougout

Keywords: *Anopheles arabiensis*, insecticide, resistance, urban malaria, kdr mutations
Issue Section: VECTOR CONTROL, PEST Management, Resistance, Repellents

[1]Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo, Ouagadougou, Burkina Faso, [2]Unité de Formation et de Recherche en Sciences et Technologies, Université Norbert ZONGO, Koudougou, Burkina Faso, [3]Département des Sciences Biomédicales, Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [4]Laboratory Animal Facilities, The Jikei University School of Medicine, Tokyo, Japan, [5]Center for Medical Entomology, The Jikei University School of Medicine, Tokyo, Japan, [6]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, [7]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK, [8]Department of Tropical Medicine, The Jikei University School of Medicine, Tokyo, Japan *Corresponding author, e-mail: a.badolo@gmail.com

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最終責任者　Athanase Badolo(Corresponding Author)

獣医ウイルス学ユニット (Veterinary Virology)

Katsuro Hagiwara

Professor

教授　萩原　克郎

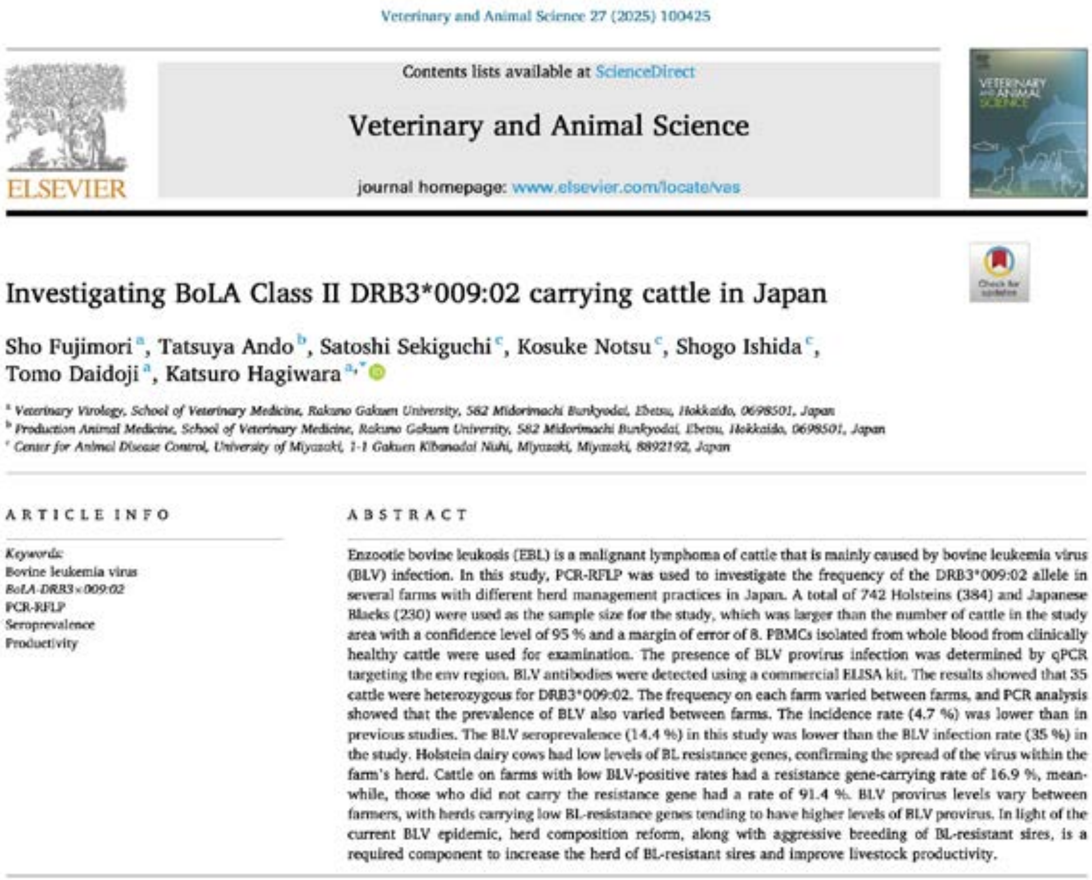
I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Investigating BoLA Class II DRB3* 009: 02 carrying cattle in Japan.
Fujimori S, Ando T, Sekiguchi S, Notsu K, Ishida S, Daidoji T, **Hagiwara K**.
Vet Anim Sci. 27:100425. 2025

2）Risk of hepatitis E virus infection associated with urban invasion of wild Deer (*Cervus nippon yesoensis*) in Hokkaido, Japan.
Kezuka Y, **Hagiwara K**.
Ger. J. Microbiol. 2024. vol. 4, Iss. 3: 36-44.

II. その他＜Others＞

該当なし



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Investigating BoLA Class II DRB3*009:02 carrying cattle in Japan

Sho Fujimori [a], Tatsuya Ando [b], Satoshi Sekiguchi [c], Kosuke Notsu [c], Shogo Ishida [c],
Tomo Daidoji [a], Katsuro Hagiwara [a,*]

[a] *Veterinary Virology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi Bunkyodai, Ebetsu, Hokkaido, 0698501, Japan*
[b] *Production Animal Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi Bunkyodai, Ebetsu, Hokkaido, 0698501, Japan*
[c] *Center for Animal Disease Control, University of Miyazaki, 1-1 Gakuen Kibanadai Nishi, Miyazaki, Miyazaki, 8892192, Japan*

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BoLA-DRB3×009:02
PCR-RFLP
Seroprevalence
Productivity

ABSTRACT

Enzootic bovine leukosis (EBL) is a malignant lymphoma of cattle that is mainly caused by bovine leukemia virus (BLV) infection. In this study, PCR-RFLP was used to investigate the frequency of the DRB3*009:02 allele in several farms with different herd management practices in Japan. A total of 742 Holsteins (384) and Japanese Blacks (230) were used as the sample size for the study, which was larger than the number of cattle in the study area with a confidence level of 95 % and a margin of error of 8. PBMCs isolated from whole blood from clinically healthy cattle were used for examination. The presence of BLV provirus infection was determined by qPCR targeting the env region. BLV antibodies were detected using a commercial ELISA kit. The results showed that 35 cattle were heterozygous for DRB3*009:02. The frequency on each farm varied between farms, and PCR analysis showed that the prevalence of BLV also varied between farms. The incidence rate (4.7 %) was lower than in previous studies. The BLV seroprevalence (14.4 %) in this study was lower than the BLV infection rate (35 %) in the study. Holstein dairy cows had low levels of BL resistance genes, confirming the spread of the virus within the farm's herd. Cattle on farms with low BLV-positive rates had a resistance gene-carrying rate of 16.9 %, meanwhile, those who did not carry the resistance gene had a rate of 91.4 %. BLV provirus levels vary between farmers, with herds carrying low BL-resistance genes tending to have higher levels of BLV provirus. In light of the current BLV epidemic, herd composition reform, along with aggressive breeding of BL-resistant sires, is a required component to increase the herd of BL-resistant sires and improve livestock productivity.

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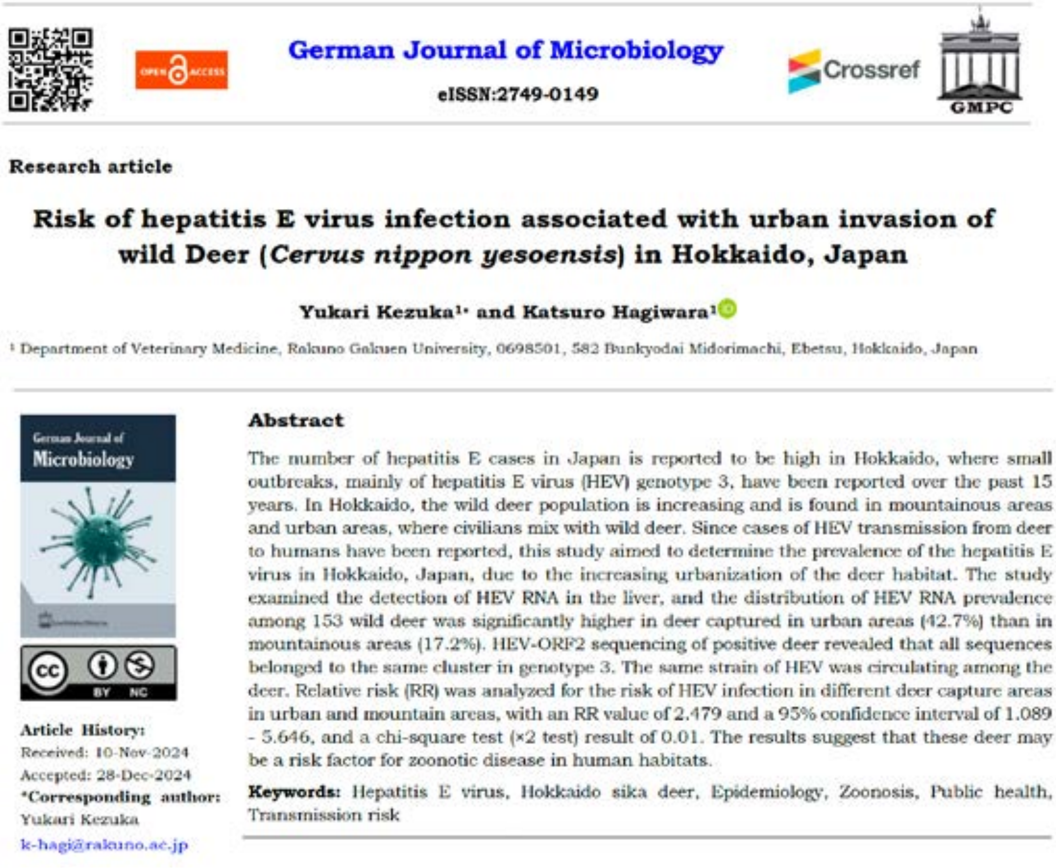
最終責任者　Katsuhiro Hagiwara (Corresponding Author)



German Journal of Microbiology
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Research article

Risk of hepatitis E virus infection associated with urban invasion of wild Deer (*Cervus nippon yesoensis*) in Hokkaido, Japan

Yukari Kezuka[1]* and **Katsuro Hagiwara**[1]

[1] Department of Veterinary Medicine, Rakuno Gakuen University, 0698501, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido, Japan

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***Corresponding author:**
Yukari Kezuka
k-hagi@rakuno.ac.jp

Abstract

The number of hepatitis E cases in Japan is reported to be high in Hokkaido, where small outbreaks, mainly of hepatitis E virus (HEV) genotype 3, have been reported over the past 15 years. In Hokkaido, the wild deer population is increasing and is found in mountainous areas and urban areas, where civilians mix with wild deer. Since cases of HEV transmission from deer to humans have been reported, this study aimed to determine the prevalence of the hepatitis E virus in Hokkaido, Japan, due to the increasing urbanization of the deer habitat. The study examined the detection of HEV RNA in the liver, and the distribution of HEV RNA prevalence among 153 wild deer was significantly higher in deer captured in urban areas (42.7%) than in mountainous areas (17.2%). HEV-ORF2 sequencing of positive deer revealed that all sequences belonged to the same cluster in genotype 3. The same strain of HEV was circulating among the deer. Relative risk (RR) was analyzed for the risk of HEV infection in different deer capture areas in urban and mountain areas, with an RR value of 2.479 and a 95% confidence interval of 1.089 - 5.646, and a chi-square test (×2 test) result of 0.01. The results suggest that these deer may be a risk factor for zoonotic disease in human habitats.

Keywords: Hepatitis E virus, Hokkaido sika deer, Epidemiology, Zoonosis, Public health, Transmission risk

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最終責任者　Katsuhiro Hagiwara (Corresponding Author)

動物生命科学(Animal Life Science)

Hideaki Hayashi

Professor

教授　林　英明

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他＜Others＞

1) Developmental changes in the capacity of mucosal immunoglobulin production and secretion in the intestines of growing calves.
Suzuki Y, Oishi M, Hirota S, **Hayashi H**, Haga S, Koike S, Kobayashi Y.
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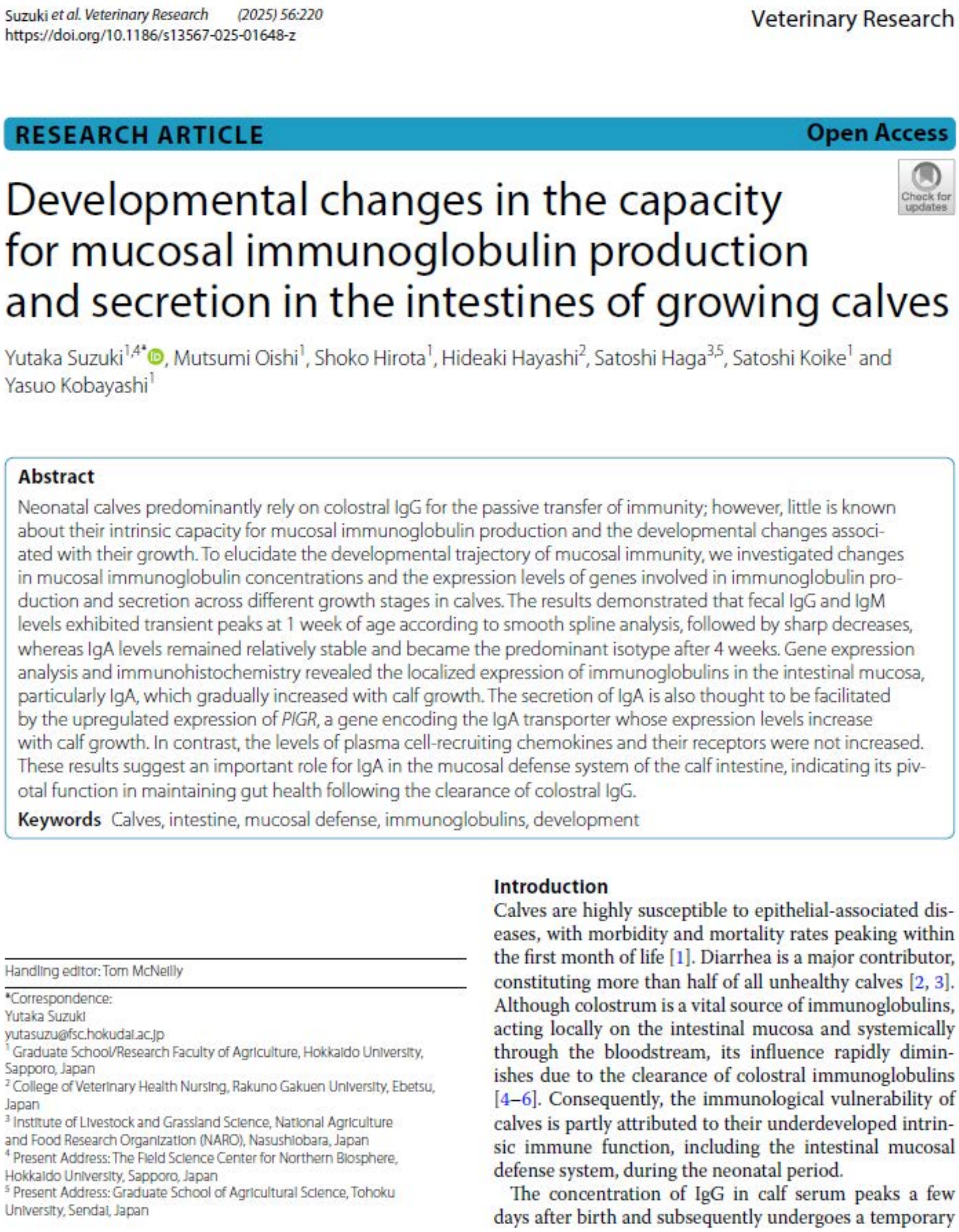

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Veterinary Research

RESEARCH ARTICLE **Open Access**

Developmental changes in the capacity for mucosal immunoglobulin production and secretion in the intestines of growing calves

Yutaka Suzuki[1,4*], Mutsumi Oishi[1], Shoko Hirota[1], Hideaki Hayashi[2], Satoshi Haga[3,5], Satoshi Koike[1] and Yasuo Kobayashi[1]

Abstract

Neonatal calves predominantly rely on colostral IgG for the passive transfer of immunity; however, little is known about their intrinsic capacity for mucosal immunoglobulin production and the developmental changes associated with their growth. To elucidate the developmental trajectory of mucosal immunity, we investigated changes in mucosal immunoglobulin concentrations and the expression levels of genes involved in immunoglobulin production and secretion across different growth stages in calves. The results demonstrated that fecal IgG and IgM levels exhibited transient peaks at 1 week of age according to smooth spline analysis, followed by sharp decreases, whereas IgA levels remained relatively stable and became the predominant isotype after 4 weeks. Gene expression analysis and immunohistochemistry revealed the localized expression of immunoglobulins in the intestinal mucosa, particularly IgA, which gradually increased with calf growth. The secretion of IgA is also thought to be facilitated by the upregulated expression of *PIGR*, a gene encoding the IgA transporter whose expression levels increase with calf growth. In contrast, the levels of plasma cell-recruiting chemokines and their receptors were not increased. These results suggest an important role for IgA in the mucosal defense system of the calf intestine, indicating its pivotal function in maintaining gut health following the clearance of colostral IgG.

Keywords Calves, intestine, mucosal defense, immunoglobulins, development

Introduction

Calves are highly susceptible to epithelial-associated diseases, with morbidity and mortality rates peaking within the first month of life [1]. Diarrhea is a major contributor, constituting more than half of all unhealthy calves [2, 3]. Although colostrum is a vital source of immunoglobulins, acting locally on the intestinal mucosa and systemically through the bloodstream, its influence rapidly diminishes due to the clearance of colostral immunoglobulins [4–6]. Consequently, the immunological vulnerability of calves is partly attributed to their underdeveloped intrinsic immune function, including the intestinal mucosal defense system, during the neonatal period.

The concentration of IgG in calf serum peaks a few days after birth and subsequently undergoes a temporary

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*Correspondence:
Yutaka Suzuki
yutasuzu@fsc.hokudai.ac.jp
[1] Graduate School/Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan
[2] College of Veterinary Health Nursing, Rakuno Gakuen University, Ebetsu, Japan
[3] Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization (NARO), Nasushiobara, Japan
[4] Present Address: The Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Japan
[5] Present Address: Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

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獣医衛生学（Animal Health）

Hidetoshi Higuchi

Professor

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Veterinary Research

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Biofilm characterisation of *Mycoplasma bovis* co-cultured with *Trueperella pyogenes*

Koji Nishi[1,2], Satoshi Gondaira[1*], Yuki Hirano[3], Masahide Ohashi[1], Ayano Sato[4], Kazuya Matsuda[5], Tomohito Iwasaki[6], Takuya Kanda[7], Ryoko Uemura[8] and Hidetoshi Higuchi[1*]

Abstract

Mycoplasma pneumonia, caused by *Mycoplasma bovis* (*Mycoplasmopsis bovis*; *M. bovis*), is linked with severe inflammatory reactions in the lungs and can be challenging to treat with antibiotics. Biofilms play a significant role in bacterial persistence and contribute to the development of chronic lesions. A recent study has shown that polymicrobial interactions between species are an important factor in biofilm formation, yet the precise mechanism of biofilm formation in *M. bovis* remains unknown. By assuming multiple pathogen infections in the bovine respiratory disease complex (BRDC), this study examined the characterisation of the polymicrobial relationship between *M. bovis* and *Trueperella pyogenes* (*T. pyogenes*) during biofilm formation. Autopsies were performed on four Holstein calves (two chronic *Mycoplasma* pneumonia calves and two control calves). Bacterium-like aggregation structures (> 10 µm), which were assumed to be biofilms of *M. bovis* in vivo, were observed adhering to the cilia in calves with *Mycoplasma* pneumonia. *M. bovis* released an extracellular matrix to connect with neighbouring bacteria and form a mature biofilm on the plate. Biofilm formation in the co-culture of *M. bovis* and *T. pyogenes* (strain T1: 1×10^5 and 1×10^6 CFU/well) significantly increased ($p < 0.05$ and $p < 0.01$; 64.1% and 64.8% increase) compared to that in a single culture of these bacteria. Furthermore, some large aggregates (> 40 µm), composed of *M. bovis* and *T. pyogenes*, were observed. The morphological characteristics of this biofilm were similar to those observed in vivo compared to a single culture. In conclusion, the polymicrobial interaction between *M. bovis* and *T. pyogenes* induces biofilm formation, which is associated with increased resistance to antimicrobial agents, and this exacerbates the progression of chronic *Mycoplasma* pneumonia.

Keywords Antibiotics, antimicrobial agents, bovine respiratory disease, extracellular matrix, trachea

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*Correspondence:
Satoshi Gondaira
s-gondaira@rakuno.ac.jp
Hidetoshi Higuchi
higuchi@rakuno.ac.jp

[1] Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[2] Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu, Hokkaido, Japan
[3] Animal Research Center, Agricultural Research Department, Hokkaido Research Organization, Shintoku, Hokkaido, Japan
[4] Large Animal Clinical Science, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[5] Department of Veterinary Pathology, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[6] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[7] Food Safety Laboratory, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan
[8] Animal Health, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

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Research Report
Microbiology

RNA sequencing of *Mycoplasma bovis* infecting bovine mammary epithelial cells and bovine mononuclear cells

Noriko Imaizumi[1], **Satoshi Gondaira**[1,*], **Tomochika Sugiura**[2], **Ayako Eguchi**[1], **Koji Nishi**[1,3], **Jumpei Fujiki**[4], **Hidetomo Iwano**[4], **Hidetoshi Higuchi**[1,*]

[1]Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[2]Theriogenology Unit, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[3]Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu 099-5171, Japan
[4]Veterinary Biochemistry Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan

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***Corresponding authors:**
Satoshi Gondaira
Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Japan.
Email: s-gondaira@rakuno.ac.jp
https://orcid.org/0000-0001-9745-7792

Hidetoshi Higuchi
Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Japan.
Email: higuchi@rakuno.ac.jp
https://orcid.org/0000-0002-7476-8544

ABSTRACT

Importance: *Mycoplasma bovis* is the major pathogen of bovine mycoplasmosis, such as mastitis, pneumonia, otitis media, and arthritis. Understanding the pathophysiology of mycoplasmal infections from the pathogen-side is as important as elucidating the host-side effects; however, few studies have focused on the variations in the expression of *M. bovis* genes.
Objective: In this study, we performed RNA sequencing of *M. bovis* infecting bovine mammary epithelial cells (bMECs) and mononuclear cells (MNCs) to elucidate pathogen-side factors involved in the pathophysiology of *M. bovis* mastitis.
Methods: *M. bovis* was cocultured with bMEC and MNC using a transwell system, and total RNA was extracted for RNA sequencing analysis.
Results: We identified 162 differentially expressed genes (DEGs), 104 upregulated and 58 downregulated, in *M. bovis* infecting bMEC, whereas 220 DEGs, 154 upregulated and 66 downregulated, in *M. bovis* infecting MNC. Enrichment analysis of upregulated DEGs in *M. bovis* infecting bMEC revealed multiple pathways with overlapping genes involved in lipid metabolism. The expression of six virulence genes were significantly upregulated in *M. bovis* infecting bMEC and MNC, whereas the expression of three virulence genes were significantly downregulated in *M. bovis* infecting MNC.
Conclusions and Relevance: Our findings indicate that *M. bovis* exhibits infecting cell-specific gene expression, and its virulence varies with the infecting cell type.

Keywords: Cattle; *Mycoplasma bovis*; bovine mastitis; virulence; gene expression profiling

INTRODUCTION

Mycoplasma bovis (also known as *Mycoplasmopsis bovis*) causes bovine mycoplasmosis, a group of severe bovine diseases that include mastitis, pneumonia, otitis media, and arthritis [1]. Mycoplasmal mastitis can be differentiated from mastitis caused by other bacteria because it is highly contagious, affects multiple quarters, is often resistant to antibiotics, and affected cows apparently appear normal even in severe cases [2]. *M. bovis* mastitis is sometimes

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FULL PAPER

Virology

Performance evaluation of an improved RAISING method for clonality analysis of bovine leukemia virus-infected cells: a collaborative study in Japan

Tomohiro OKAGAWA[1)#], Naomi NOJIRI[2)#], Hazuka YOSHIDA-FURIHATA[2)], Naganori NAO[3–5)], Misono TOMINAGA[6)], Junko KOHARA[7)], Satoshi GONDAIRA[8)], Hidetoshi HIGUCHI[8)], Yohei TAKEDA[9,10)], Haruko OGAWA[9)], Shinji YAMADA[11,12)], Kenji MURAKAMI[11,12)], Yasunori SUZUKI[13)], Shinji TAKAI[13)], Masaki MAEZAWA[14)], Hisashi INOKUMA[14,15)], Kaori SHIMIZU[16)], Yasuo INOSHIMA[16–18)], Tatsufumi USUI[19,20)], Michihito TAGAWA[21)], Mari YAMAMOTO[22)], Hirohisa MEKATA[22)], Mana ESAKI[23)], Makoto OZAWA[23,24)], Takahiro MATSUDAIRA[5)], Naoya MAEKAWA[1)], Shiro MURATA[1,6,25)], Kazuhiko OHASHI[1,6,25,26)], Masumichi SAITO[2,27)], Satoru KONNAI[1,6,25,28)]*

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[1)]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[2)]Center for Emergency Preparedness and Response, National Institute of Infectious Diseases, Tokyo, Japan
[3)]Division of International Research Promotion, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan
[4)]One Health Research Center, Hokkaido University, Hokkaido, Japan
[5)]Biotechnological Research Support Division, FASMAC Co., Ltd., Kanagawa, Japan
[6)]Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[7)]Animal Research Center, Agriculture Research Department, Hokkaido Research Organization, Hokkaido, Japan
[8)]Laboratory of Animal Health, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[9)]Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[10)]Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[11)]Graduate School of Veterinary Sciences, Iwate University, Iwate, Japan
[12)]Cooperative Department of Veterinary Medicine, Faculty of Veterinary Medicine, Iwate University, Iwate, Japan
[13)]Laboratory of Animal Hygiene, Kitasato University School of Veterinary Medicine, Aomori, Japan
[14)]Laboratory of OSG Veterinary Science for Global Disease Management, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[15)]Laboratory of Farm Animal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[16)]Laboratory of Food and Environmental Hygiene, Joint Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan
[17)]Education and Research Center for Food Animal Health, Gifu University (GeFAH), Gifu, Japan
[18)]Joint Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan
[19)]Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, Tottori, Japan
[20)]Laboratory of Veterinary Hygiene, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan
[21)]Department of Veterinary Science, Okayama University of Science, Ehime, Japan
[22)]Center for Animal Disease Control, University of Miyazaki, Miyazaki, Japan
[23)]Joint Graduate School of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[24)]Department of Pathogenetic and Preventive Veterinary Science, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[25)]Veterinary Research Unit, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan
[26)]International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[27)]Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan
[28)]Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Hokkaido, Japan

---

*Correspondence to: Konnai S: konnai@vetmed.hokudai.ac.jp, Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Nishi 9, Kita 18-jo, Kita-ku, Sapporo, Hokkaido 060-0818, Japan

#These authors contributed equally to this work.

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Original article

Anti-inflammatory effects of glycyrrhizin on lipoteichoic acid and lipopolysaccharide-induced bovine mastitis

T. Kurumisawa[1,2†], K. Kazama[1†], S. Gondaira[3], H. Higuchi[3], A. Eguchi[3], K. Onda[1], S-G. Roh[4], K. Kawai[1,2]

[1] School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe Chuo-ku, Sagamihara 252-5201, Japan
[2] Azabu University Mastitis Research Center, 1-17-71 Fuchinobe Chuo-ku, Sagamihara 252-5201, Japan
[3] Graduate School of Veterinary Medicine,
Rakuno Gakuen University, 852 Midorimachi Bunkyoudai, Ebetsu 069-8501, Japan
[4] Graduate School of Agricultural Science,
Tohoku University, 468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0842, Japan

Correspondence to: K. Kawai, e-mail: kawai@azabu-u.ac.jp
[†] These authors contributed equally to this work.

Abstract

Bovine mastitis is primarily treated with antimicrobial agents. Anti-inflammatory agents are also used to alleviate clinical symptoms or reduce antimicrobial use. Glycyrrhizin is an anti-inflammatory agent used in the treatment of bovine mastitis, but its effects are not fully understood. We therefore examined the anti-inflammatory effects of glycyrrhizin both in vivo and in vitro. We first tested whether glycyrrhizin exerts anti-inflammatory effects using MAC-T cells, an immortalized bovine mammary epithelial cell line. Glycyrrhizin decreased the expression of interleukin (IL)-1β mRNA in a concentration-dependent manner in MAC-T cells stimulated with lipoteichoic acid (LTA). We then investigated the effects of glycyrrhizin in bovine mammary epithelial cells (bMECs), which seem to retain more of the characteristics of actual mammary epithelial cells. Stimulation with LTA or lipopolysaccharide significantly increased cytokine mRNA expression in bMECs. Glycyrrhizin exhibited a slight inhibitory effect, but no significant difference was observed. The effect of glycyrrhizin on LTA-induced mastitis was examined in lactating cows. Quarters were divided into test and control areas (test quarter: n=8, control quarter: n=7). All quarters were stimulated with LTA at the start of the trial (0 h). In the test quarter group, glycyrrhizin was administered via intramammary infusion. The somatic cell count and relative gene expression of IL-1β and tumor necrosis factor–α were significantly lower in test quarters than control quarters. Both the in vitro and in vivo studies showed that glycyrrhizin reduces the expression of proinflammatory cytokine genes in response to LTA-induced inflammation and partially revealed the mechanism of the anti-inflammatory effect of glycyrrhizin on mastitis. Further investigations involving field cases of mastitis with bacterial infections are needed to demonstrate the anti-inflammatory effect of glycyrrhizin on bovine mastitis.

Keywords: anti-inflammatory effect, bovine mammary epithelial cells, bovine mastitis, glycyrrhizin, lipopolysaccharide, lipoteichoic acid

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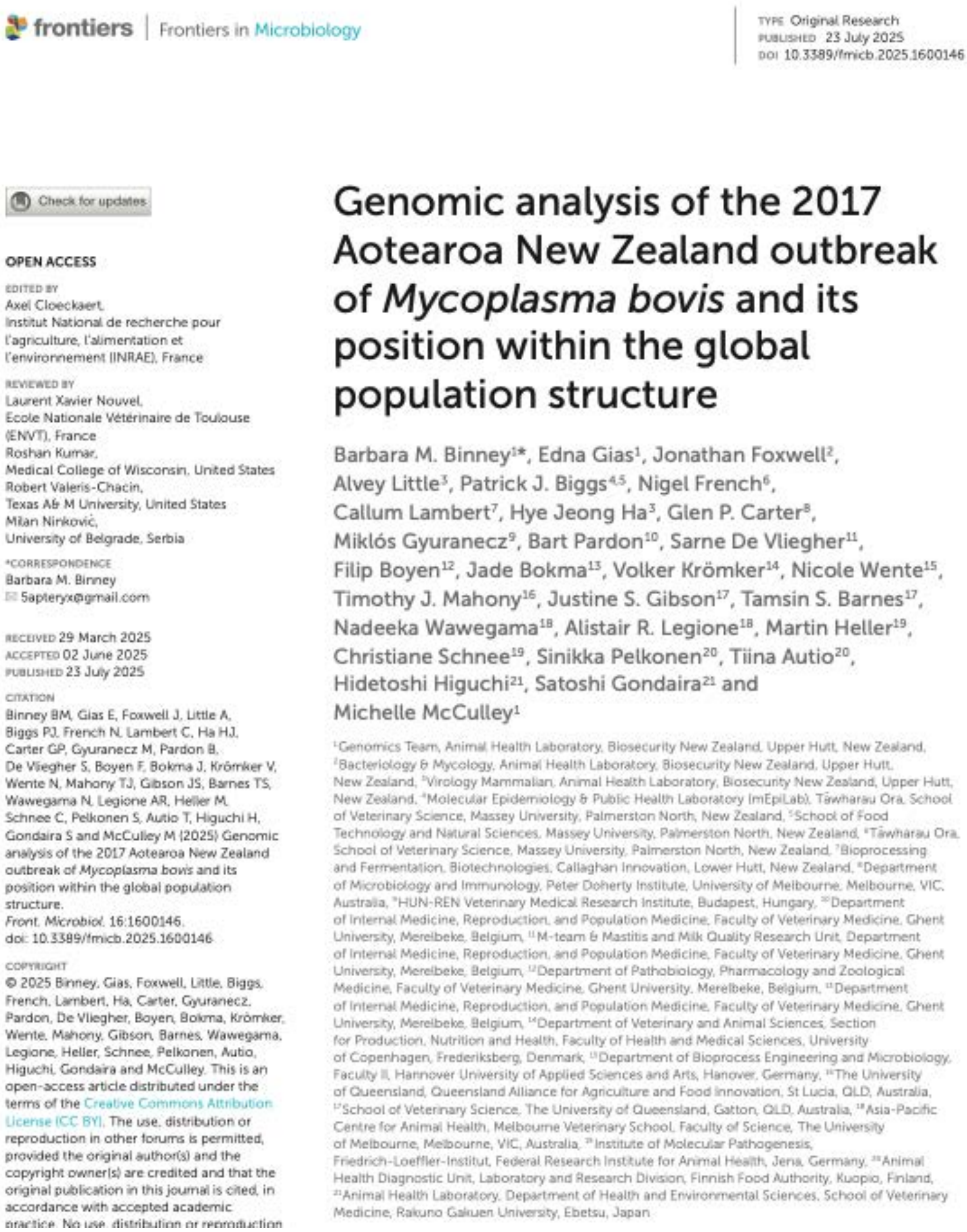

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Genomic analysis of the 2017 Aotearoa New Zealand outbreak of *Mycoplasma bovis* and its position within the global population structure

Barbara M. Binney[1]*, Edna Gias[1], Jonathan Foxwell[2], Alvey Little[3], Patrick J. Biggs[4,5], Nigel French[6], Callum Lambert[7], Hye Jeong Ha[3], Glen P. Carter[8], Miklós Gyuranecz[9], Bart Pardon[10], Sarne De Vliegher[11], Filip Boyen[12], Jade Bokma[13], Volker Krömker[14], Nicole Wente[15], Timothy J. Mahony[16], Justine S. Gibson[17], Tamsin S. Barnes[17], Nadeeka Wawegama[18], Alistair R. Legione[18], Martin Heller[19], Christiane Schnee[19], Sinikka Pelkonen[20], Tiina Autio[20], Hidetoshi Higuchi[21], Satoshi Gondaira[21] and Michelle McCulley[1]

[1]Genomics Team, Animal Health Laboratory, Biosecurity New Zealand, Upper Hutt, New Zealand, [2]Bacteriology & Mycology, Animal Health Laboratory, Biosecurity New Zealand, Upper Hutt, New Zealand, [3]Virology Mammalian, Animal Health Laboratory, Biosecurity New Zealand, Upper Hutt, New Zealand, [4]Molecular Epidemiology & Public Health Laboratory (mEpiLab), Tāwharau Ora, School of Veterinary Science, Massey University, Palmerston North, New Zealand, [5]School of Food Technology and Natural Sciences, Massey University, Palmerston North, New Zealand, [6]Tāwharau Ora, School of Veterinary Science, Massey University, Palmerston North, New Zealand, [7]Bioprocessing and Fermentation, Biotechnologies, Callaghan Innovation, Lower Hutt, New Zealand, [8]Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne, Melbourne, VIC, Australia, [9]HUN-REN Veterinary Medical Research Institute, Budapest, Hungary, [10]Department of Internal Medicine, Reproduction, and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [11]M-team & Mastitis and Milk Quality Research Unit, Department of Internal Medicine, Reproduction, and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [12]Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [13]Department of Internal Medicine, Reproduction, and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [14]Department of Veterinary and Animal Sciences, Section for Production, Nutrition and Health, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, [15]Department of Bioprocess Engineering and Microbiology, Faculty II, Hannover University of Applied Sciences and Arts, Hanover, Germany, [16]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, St Lucia, QLD, Australia, [17]School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia, [18]Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Melbourne, VIC, Australia, [19]Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Jena, Germany, [20]Animal Health Diagnostic Unit, Laboratory and Research Division, Finnish Food Authority, Kuopio, Finland, [21]Animal Health Laboratory, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

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EDITED BY
Axel Cloeckaert,
Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), France

REVIEWED BY
Laurent Xavier Nouvel,
Ecole Nationale Vétérinaire de Toulouse (ENVT), France
Roshan Kumar,
Medical College of Wisconsin, United States
Robert Valeris-Chacin,
Texas A& M University, United States
Milan Ninković,
University of Belgrade, Serbia

*CORRESPONDENCE
Barbara M. Binney
✉ 5apteryx@gmail.com

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Effects of amount of lactose in milk replacer on gastrointestinal function of dairy calves

R. Fukumori [a], T. Nakayama [a], M. Hirose [a], I. Norimura [b], K. Izumi [b], K. Shimada [c], H. Mineo [d], M.A. Steele [e], S. Gondaira [a], H. Higuchi [a], T. Watanabe [a], H. Ueda [a], T. Sano [a], K. Chisato [a], S. Oikawa [a,*]

[a] *Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[b] *Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[c] *The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Shinjuku, Tokyo 969-0223, Japan*
[d] *Department of Health and Nutrition, Hokkaido Bunkyo University, Eniwa 069-1449, Japan*
[e] *Department of Animal and Bioscience, University of Guelph, Guelph, Ontario N1G2W1, Canada*

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Keywords:
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ABSTRACT

The objective of this study was to evaluate the effects of feeding milk replacer (MR) at different lactose amount while keeping osmolality constant on gastrointestinal function, blood parameters, and inflammation-related mRNA expression in the livers of dairy calves. Fifteen Holstein bull calves were assigned to one of three dietary treatments differing in MR lactose content (L: 38 %, M: 41 %, and H: 46 %). Feeding of the test diets was started at 1 day of age and gradually increased to a maximum feeding rate at 20 days of age (L: 1.16 kg/d, M: 1.21 kg/d, and H: 1.26 kg/d DM). Under these conditions, the lactose dosages for the treatments were 441 g/d, 496 g/d, and 580 g/d, respectively. The MR were prepared to ensure isocaloric and iso-osmotic (451 mOsm/kg) conditions. Fecal scores were recorded daily, and at 14 and 28 days of age, blood and breath samples were collected before and after MR feeding. In addition, feces and urine were collected for 2 consecutive days. Then, the calves were slaughtered to evaluate intestinal permeability and liver mRNA expression. The permeability in the duodenum and ileum was lower in H and M than in L; the permeability in the jejunum was also lower in H than in L. The hepatic mRNA expressions of toll-like receptor-2, IL-1β, and tumor-necrosis factor-α were lower in H and M than L. Nitrogen retention was higher in H than in L, and linear and quadratic increasing trends were observed in tissue ratio of gastrointestinal tract with the increase in lactose amount. Postprandial increase in plasma glucose concentration was smaller and postprandial increase in TG concentration was higher in H than in L. Fecal properties, digestibility, and breath hydrogen concentrations were not affected by treatment. These results indicate that feeding high-lactose MR may increase gastrointestinal weight and decrease permeability in the small intestine of calves.

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* Corresponding author.
E-mail address: oishin@rakuno.ac.jp (S. Oikawa).

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Animal Science Journal

RESEARCH ARTICLE

Effects of Ruminal Administration of Propylene Glycol or Sucrose on Ruminal, Blood, and Hepatic Parameters in Nonlactating Cows With High Plasma Nonesterified Fatty Acid Concentrations

Kyoko Chisato[1] | Rika Fukumori[1] | Ryo Imaishi[1] | Satoshi Gondaira[1] | Hidetoshi Higuchi[1] | Kenichi Izumi[2] | Shin Oikawa[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu, Japan

Correspondence: Rika Fukumori (fukumori@rakuno.ac.jp) | Shin Oikawa (oishin@rakuno.ac.jp)

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Keywords: insulin | ketosis | metabolic diseases | NEFA | transition cows

ABSTRACT

Elevated blood nonesterified fatty acids (NEFA) concentration in prepartum increases the risk of postpartum diseases. This study evaluated whether intraruminal propylene glycol (PG) or sucrose (SC) administration could mitigate elevated blood NEFA induced by intravenous lipid infusion. Four nonlactating, nonpregnant Holstein cows fitted with rumen cannulas were used in a 4×4 Latin square design: intravenous saline infusion and ruminal administration of water (S+WT), lipid infusion and administration of water (L+WT), lipid infusion and PG administration (L+PG), and lipid infusion and SC administration (L+SC). The intravenous infusion lasted for 11 h, followed by ruminal administration 4 h after the start of the infusion. Blood NEFA concentration was increased by lipid infusion but was lower in L+PG compared to L+WT. In association with this, higher ruminal propionate and butyrate, blood glucose and insulin, and lower β-hydroxybutyrate (BHBA) concentrations were observed in L+PG. Hepatic carnitine palmitoyl transferase 1 mRNA expression was higher in L+PG and L+SC compared to L+WT. SC administration increased insulin concentrations associated with increased ruminal butyrate concentration but had a smaller NEFA-reducing effect than PG. To alleviate NEFA elevation, PG was more effective than SC, which may have involved sustained stimulation of insulin secretion of PG.

1 | Introduction

The 3 weeks before and after calving are called the “transition period,” (Grummer 1995; Drackley 1999) during which dramatic changes occur in dairy cows. This means that cows have an increased demand for energy for fetal growth and the production of colostrum and milk. If the energy supply from feed intake is insufficient, cows can experience a negative energy balance (NEB) (Bauman and Bruce Currie 1980; Baird 1982). The cow attempts to adapt to NEB by utilizing the carbohydrates, lipids, and proteins stored in the body. However, if these metabolic adaptations are not sufficiently successful, peripartum diseases, such as milk fever, ketosis, retained placenta, and displaced abomasum, may occur. The occurrence of peripartum diseases not only leads to reproductive disorders, such as decreased conception rates (Baird 1982), but also increases the risk of early removal due to death, culling, and sale, resulting in significant financial losses for dairy farms (Gröhn et al. 1998; Fetrow et al. 2006).

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獣医生化学（Veterinary Biochemistry）

Hidetomo Iwano

Professor

教授 岩野 英知

I. 筆頭または責任著者 ＜First or Corresponding Author＞

1） The first complete hand-rearing of two neonatal finless porpoises. Masahiko Kasamatsu M, Hasegawa K, Wakabayashi I, Furuta M, Inoue H, **Iwano H.**
PLoS One. 20:e0288891. 2025. doi: 10.1371/journal.pone.0288891.
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2） Viral whole-genome sequences of Pseudomonas jumbo phages, ΦNK1 and ΦBrmt, from sewage water in Japan.
Fujiki J, Maeda N, Nakamura T, Ichikawa N,Tamamura K, Yamamot H, Sakata Y, Nakamura K, **Iwano H.**
Microbiol Resour Announc. 14:e0104423. 2025. doi: 10.1128/mra.01044-23.

II. その他＜Others＞

1） Biocontrol of Phage Resistance in Pseudomonas Infections: Insights into Directed Breaking of Spontaneous Evolutionary Selection in Phage Therapy.
Fujiki J, Yokoyama D, Yamamot H, Kimura N, Shimizu M, Kobayashi H, Nakamura K, **Iwano H.**
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2） Enhanced expression of Cyp17a1 and production of DHEA-S in the liver of late-pregnant rats.
Ohtsuki Y, Fujiki J, Maeda N, **Iwano H.**
General and Comparative Endocrinology. 362:114661. 2025.
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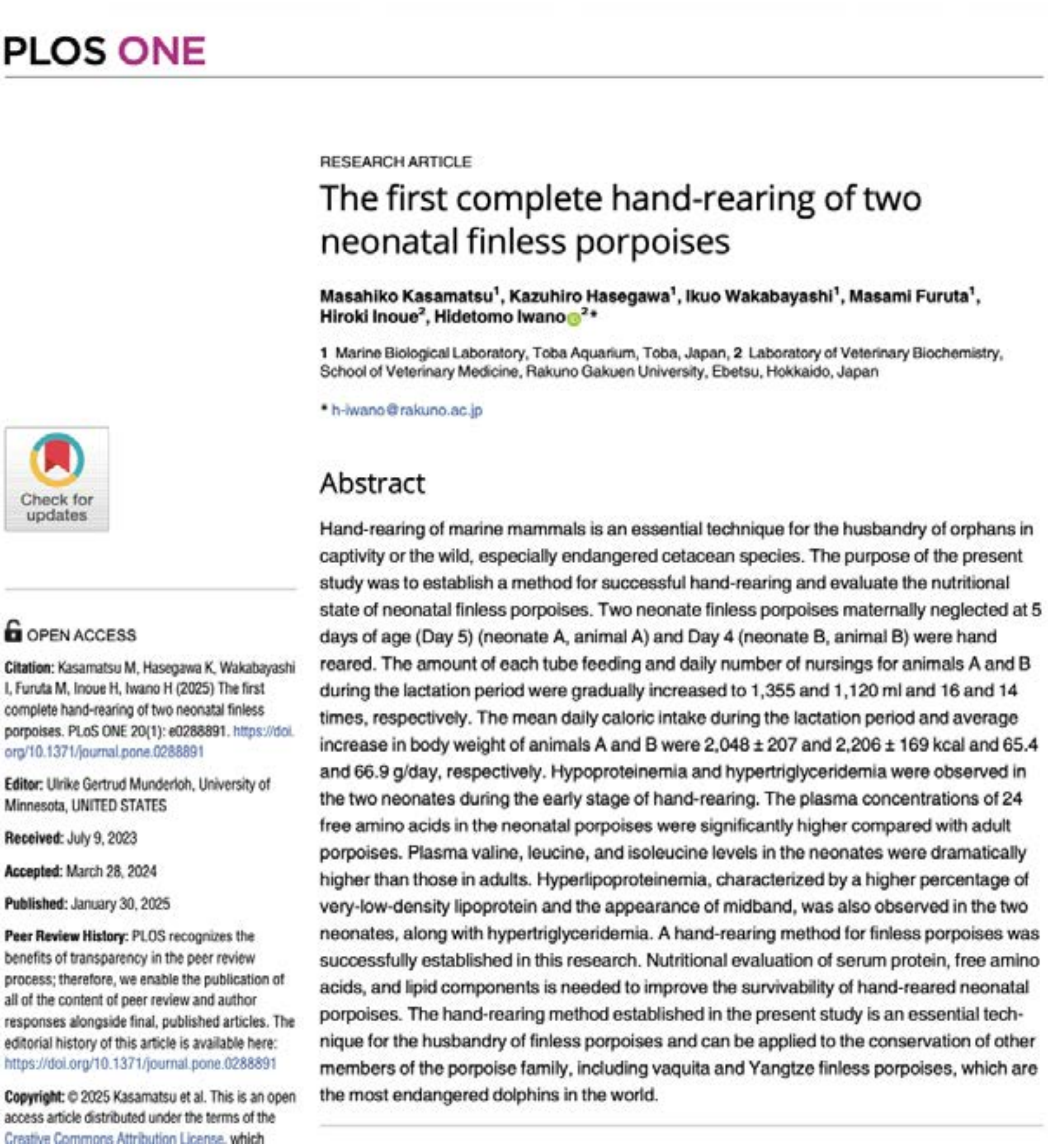
3） Blood amino acid changes associated with Lawsonia intracellularis infection in horses.
Mizuguchi Y, Niwa H, Inoue H, **Iwano H.**
Equine Vet J. 2025 May 22. doi: 10.1111/evj.14533.　Online ahead of print.

4） Isolation and characterization of novel bacteriophages targeting Stenotrophomonas maltophilia.
Yamashita W, Sato Y, Imanaka M, Kataoka M, Suzuki T, Azam H A, Ojima S, Hayakawa K, Saito S, Moriyama Y, Ohmagari N, Kurokawa M, Mezaki K, Tamura A, Cui L, Fujiki J, **Iwano H**, Takahashi Y, Watashi K, Tsuneda S & Kiga K.
Scientific Reports. 15:29743, 2025. https://doi.org/10.1038/s41598-025-14811-5

5） Protocol for end-design-free rebooting of terminally redundant Pseudomonas phages in clinical isolates of Pseudomonas aeruginosa.
Yokoyama D, Kimura N, Yamamot H, Sakata Y, Kobayashi H, Fujiki J, **Iwano H.**
STAR protocols. 6:104012. 2025. doi: 10.1016/j.xpro.2025.104012



PLOS ONE

RESEARCH ARTICLE

The first complete hand-rearing of two neonatal finless porpoises

Masahiko Kasamatsu[1], **Kazuhiro Hasegawa**[1], **Ikuo Wakabayashi**[1], **Masami Furuta**[1], **Hiroki Inoue**[2], **Hidetomo Iwano**[2]*

1 Marine Biological Laboratory, Toba Aquarium, Toba, Japan, 2 Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

* h-iwano@rakuno.ac.jp

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Abstract

Hand-rearing of marine mammals is an essential technique for the husbandry of orphans in captivity or the wild, especially endangered cetacean species. The purpose of the present study was to establish a method for successful hand-rearing and evaluate the nutritional state of neonatal finless porpoises. Two neonate finless porpoises maternally neglected at 5 days of age (Day 5) (neonate A, animal A) and Day 4 (neonate B, animal B) were hand reared. The amount of each tube feeding and daily number of nursings for animals A and B during the lactation period were gradually increased to 1,355 and 1,120 ml and 16 and 14 times, respectively. The mean daily caloric intake during the lactation period and average increase in body weight of animals A and B were 2,048 ± 207 and 2,206 ± 169 kcal and 65.4 and 66.9 g/day, respectively. Hypoproteinemia and hypertriglyceridemia were observed in the two neonates during the early stage of hand-rearing. The plasma concentrations of 24 free amino acids in the neonatal porpoises were significantly higher compared with adult porpoises. Plasma valine, leucine, and isoleucine levels in the neonates were dramatically higher than those in adults. Hyperlipoproteinemia, characterized by a higher percentage of very-low-density lipoprotein and the appearance of midband, was also observed in the two neonates, along with hypertriglyceridemia. A hand-rearing method for finless porpoises was successfully established in this research. Nutritional evaluation of serum protein, free amino acids, and lipid components is needed to improve the survivability of hand-reared neonatal porpoises. The hand-rearing method established in the present study is an essential technique for the husbandry of finless porpoises and can be applied to the conservation of other members of the porpoise family, including vaquita and Yangtze finless porpoises, which are the most endangered dolphins in the world.

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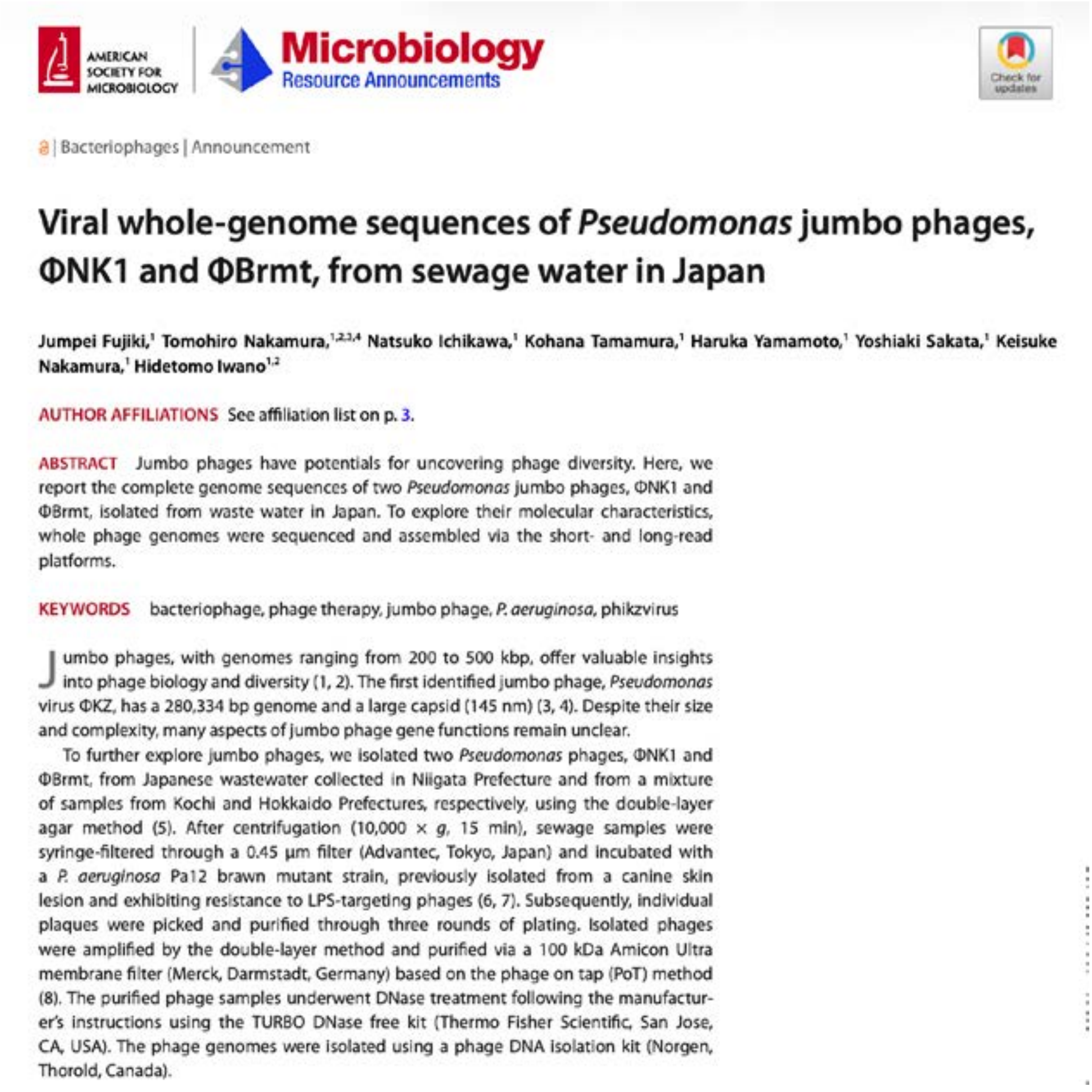

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

Bacteriophages | Announcement

Viral whole-genome sequences of *Pseudomonas* jumbo phages, ΦNK1 and ΦBrmt, from sewage water in Japan

Jumpei Fujiki,[1] Tomohiro Nakamura,[1,2,3,4] Natsuko Ichikawa,[1] Kohana Tamamura,[1] Haruka Yamamoto,[1] Yoshiaki Sakata,[1] Keisuke Nakamura,[1] Hidetomo Iwano[1,2]

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Jumbo phages have potentials for uncovering phage diversity. Here, we report the complete genome sequences of two *Pseudomonas* jumbo phages, ΦNK1 and ΦBrmt, isolated from waste water in Japan. To explore their molecular characteristics, whole phage genomes were sequenced and assembled via the short- and long-read platforms.

KEYWORDS bacteriophage, phage therapy, jumbo phage, *P. aeruginosa*, phikzvirus

Jumbo phages, with genomes ranging from 200 to 500 kbp, offer valuable insights into phage biology and diversity (1, 2). The first identified jumbo phage, *Pseudomonas* virus ΦKZ, has a 280,334 bp genome and a large capsid (145 nm) (3, 4). Despite their size and complexity, many aspects of jumbo phage gene functions remain unclear.

To further explore jumbo phages, we isolated two *Pseudomonas* phages, ΦNK1 and ΦBrmt, from Japanese wastewater collected in Niigata Prefecture and from a mixture of samples from Kochi and Hokkaido Prefectures, respectively, using the double-layer agar method (5). After centrifugation (10,000 × *g*, 15 min), sewage samples were syringe-filtered through a 0.45 µm filter (Advantec, Tokyo, Japan) and incubated with a *P. aeruginosa* Pa12 brawn mutant strain, previously isolated from a canine skin lesion and exhibiting resistance to LPS-targeting phages (6, 7). Subsequently, individual plaques were picked and purified through three rounds of plating. Isolated phages were amplified by the double-layer method and purified via a 100 kDa Amicon Ultra membrane filter (Merck, Darmstadt, Germany) based on the phage on tap (PoT) method (8). The purified phage samples underwent DNase treatment following the manufacturer's instructions using the TURBO DNase free kit (Thermo Fisher Scientific, San Jose, CA, USA). The phage genomes were isolated using a phage DNA isolation kit (Norgen, Thorold, Canada).

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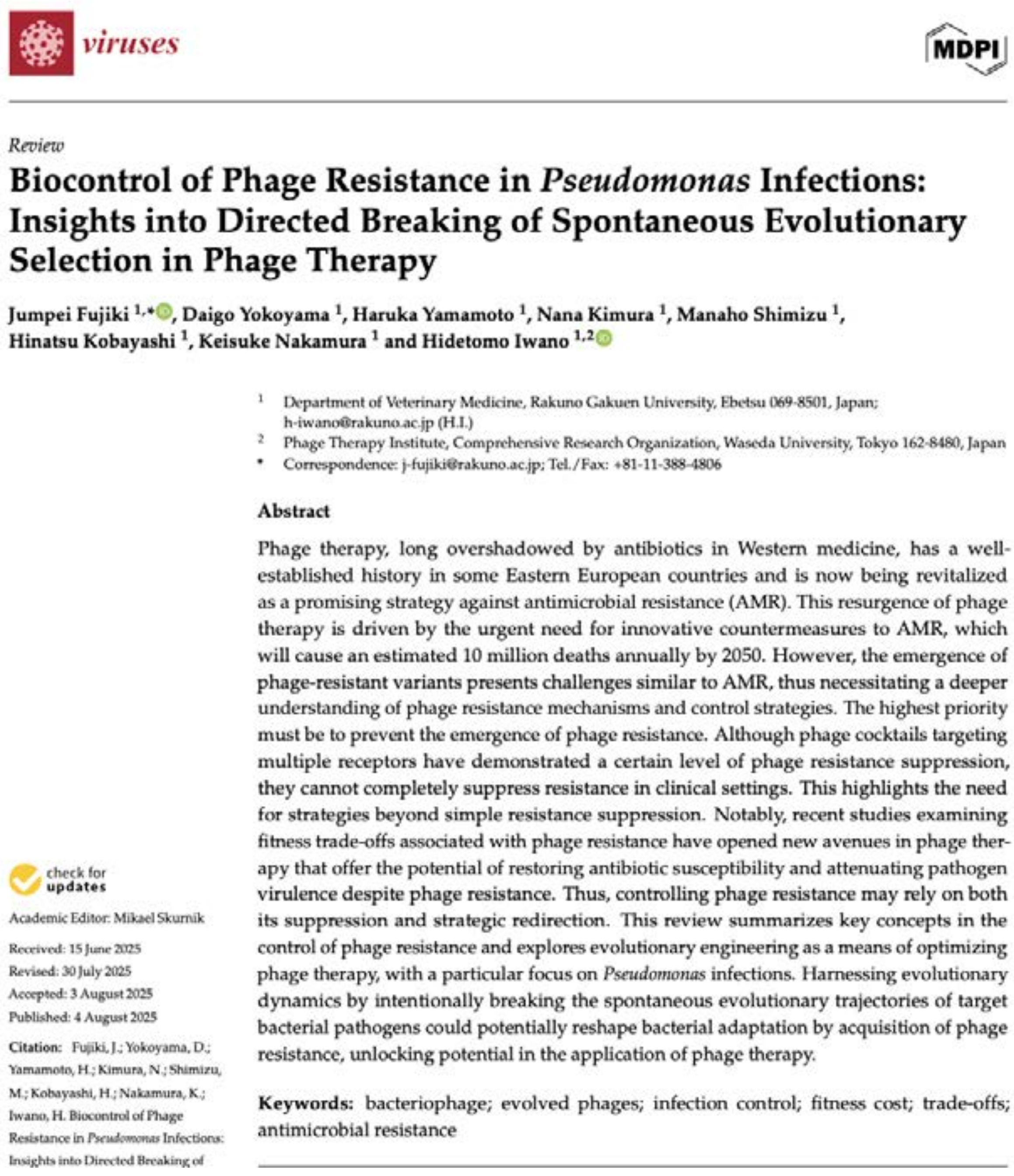
最終責任者　Hidetomo Iwano (Corresponding Author)



viruses

MDPI

Review

Biocontrol of Phage Resistance in *Pseudomonas* Infections: Insights into Directed Breaking of Spontaneous Evolutionary Selection in Phage Therapy

Jumpei Fujiki [1,*], **Daigo Yokoyama** [1], **Haruka Yamamoto** [1], **Nana Kimura** [1], **Manaho Shimizu** [1],
Hinatsu Kobayashi [1], **Keisuke Nakamura** [1] and **Hidetomo Iwano** [1,2]

[1] Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; h-iwano@rakuno.ac.jp (H.I.)
[2] Phage Therapy Institute, Comprehensive Research Organization, Waseda University, Tokyo 162-8480, Japan
* Correspondence: j-fujiki@rakuno.ac.jp; Tel./Fax: +81-11-388-4806

Abstract

Phage therapy, long overshadowed by antibiotics in Western medicine, has a well-established history in some Eastern European countries and is now being revitalized as a promising strategy against antimicrobial resistance (AMR). This resurgence of phage therapy is driven by the urgent need for innovative countermeasures to AMR, which will cause an estimated 10 million deaths annually by 2050. However, the emergence of phage-resistant variants presents challenges similar to AMR, thus necessitating a deeper understanding of phage resistance mechanisms and control strategies. The highest priority must be to prevent the emergence of phage resistance. Although phage cocktails targeting multiple receptors have demonstrated a certain level of phage resistance suppression, they cannot completely suppress resistance in clinical settings. This highlights the need for strategies beyond simple resistance suppression. Notably, recent studies examining fitness trade-offs associated with phage resistance have opened new avenues in phage therapy that offer the potential of restoring antibiotic susceptibility and attenuating pathogen virulence despite phage resistance. Thus, controlling phage resistance may rely on both its suppression and strategic redirection. This review summarizes key concepts in the control of phage resistance and explores evolutionary engineering as a means of optimizing phage therapy, with a particular focus on *Pseudomonas* infections. Harnessing evolutionary dynamics by intentionally breaking the spontaneous evolutionary trajectories of target bacterial pathogens could potentially reshape bacterial adaptation by acquisition of phage resistance, unlocking potential in the application of phage therapy.

Keywords: bacteriophage; evolved phages; infection control; fitness cost; trade-offs; antimicrobial resistance

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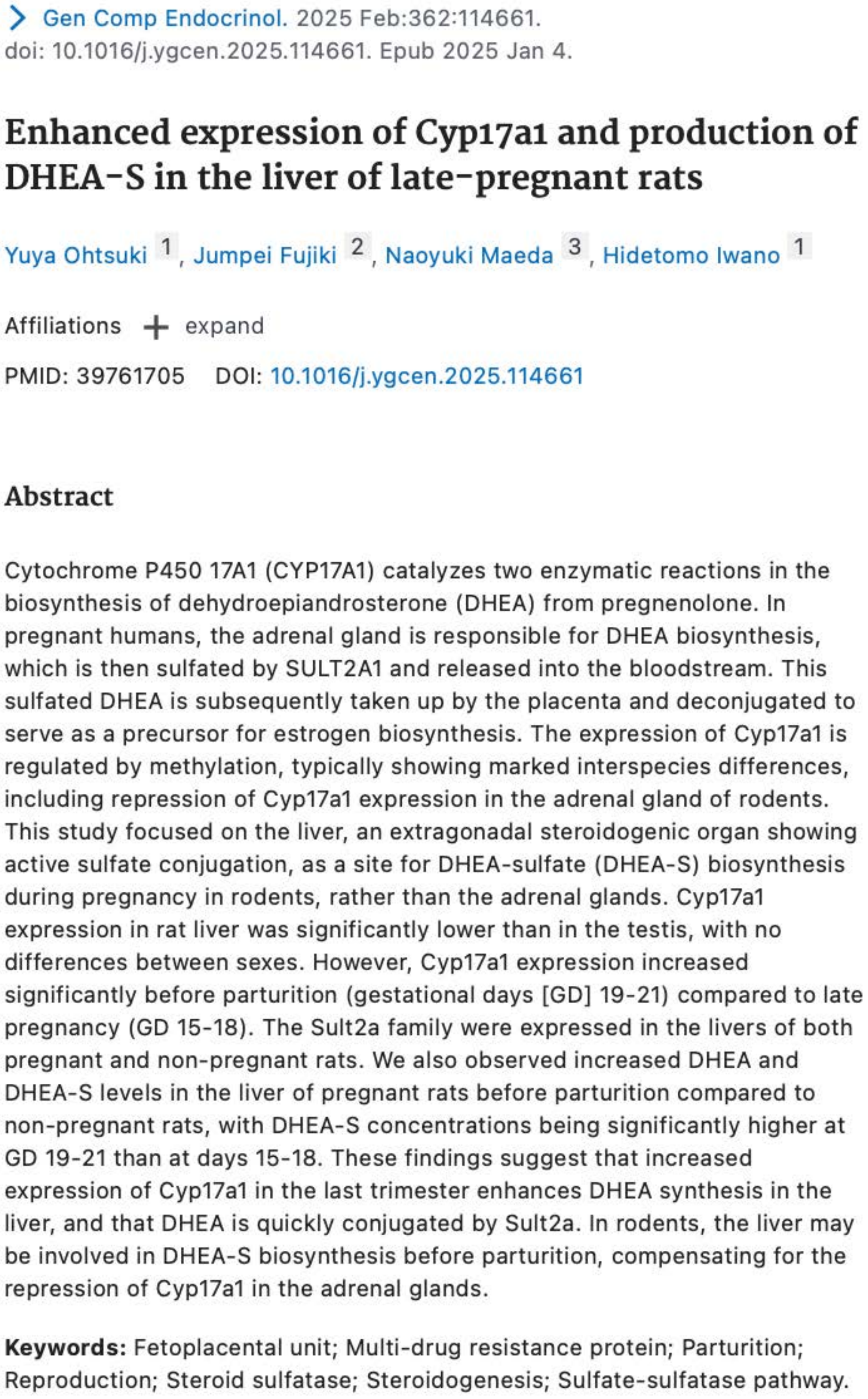

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Enhanced expression of Cyp17a1 and production of DHEA-S in the liver of late-pregnant rats

Yuya Ohtsuki [1], Jumpei Fujiki [2], Naoyuki Maeda [3], Hidetomo Iwano [1]

Affiliations + expand

PMID: 39761705 DOI: 10.1016/j.ygcen.2025.114661

Abstract

Cytochrome P450 17A1 (CYP17A1) catalyzes two enzymatic reactions in the biosynthesis of dehydroepiandrosterone (DHEA) from pregnenolone. In pregnant humans, the adrenal gland is responsible for DHEA biosynthesis, which is then sulfated by SULT2A1 and released into the bloodstream. This sulfated DHEA is subsequently taken up by the placenta and deconjugated to serve as a precursor for estrogen biosynthesis. The expression of Cyp17a1 is regulated by methylation, typically showing marked interspecies differences, including repression of Cyp17a1 expression in the adrenal gland of rodents. This study focused on the liver, an extragonadal steroidogenic organ showing active sulfate conjugation, as a site for DHEA-sulfate (DHEA-S) biosynthesis during pregnancy in rodents, rather than the adrenal glands. Cyp17a1 expression in rat liver was significantly lower than in the testis, with no differences between sexes. However, Cyp17a1 expression increased significantly before parturition (gestational days [GD] 19-21) compared to late pregnancy (GD 15-18). The Sult2a family were expressed in the livers of both pregnant and non-pregnant rats. We also observed increased DHEA and DHEA-S levels in the liver of pregnant rats before parturition compared to non-pregnant rats, with DHEA-S concentrations being significantly higher at GD 19-21 than at days 15-18. These findings suggest that increased expression of Cyp17a1 in the last trimester enhances DHEA synthesis in the liver, and that DHEA is quickly conjugated by Sult2a. In rodents, the liver may be involved in DHEA-S biosynthesis before parturition, compensating for the repression of Cyp17a1 in the adrenal glands.

Keywords: Fetoplacental unit; Multi-drug resistance protein; Parturition; Reproduction; Steroid sulfatase; Steroidogenesis; Sulfate-sulfatase pathway.

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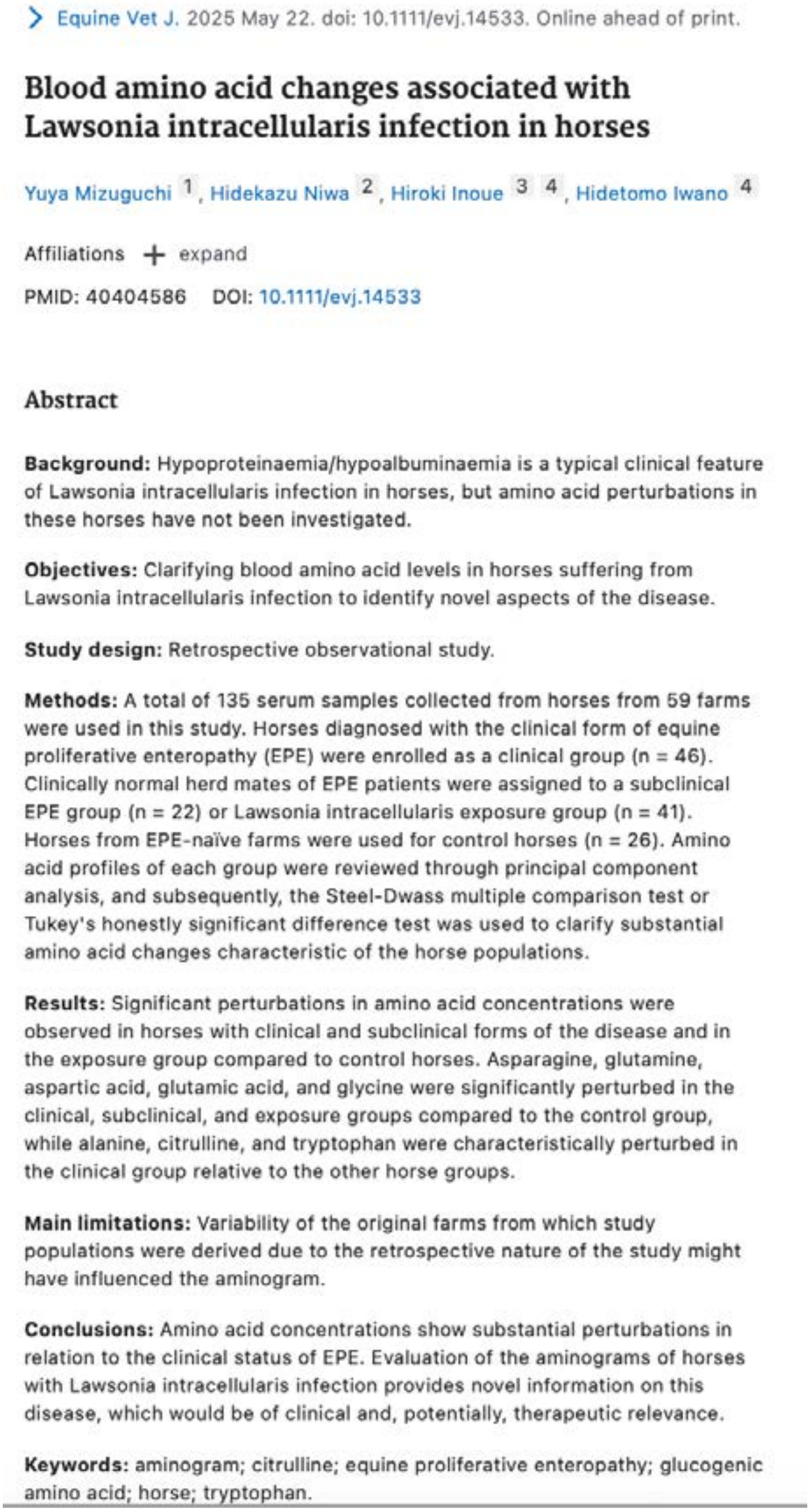
最終責任者　Jumpei Fujiki (Corresponding Author)



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Blood amino acid changes associated with Lawsonia intracellularis infection in horses

Yuya Mizuguchi[1], Hidekazu Niwa[2], Hiroki Inoue[3] [4], Hidetomo Iwano[4]

Affiliations + expand

PMID: 40404586 DOI: 10.1111/evj.14533

Abstract

Background: Hypoproteinaemia/hypoalbuminaemia is a typical clinical feature of Lawsonia intracellularis infection in horses, but amino acid perturbations in these horses have not been investigated.

Objectives: Clarifying blood amino acid levels in horses suffering from Lawsonia intracellularis infection to identify novel aspects of the disease.

Study design: Retrospective observational study.

Methods: A total of 135 serum samples collected from horses from 59 farms were used in this study. Horses diagnosed with the clinical form of equine proliferative enteropathy (EPE) were enrolled as a clinical group (n = 46). Clinically normal herd mates of EPE patients were assigned to a subclinical EPE group (n = 22) or Lawsonia intracellularis exposure group (n = 41). Horses from EPE-naïve farms were used for control horses (n = 26). Amino acid profiles of each group were reviewed through principal component analysis, and subsequently, the Steel-Dwass multiple comparison test or Tukey's honestly significant difference test was used to clarify substantial amino acid changes characteristic of the horse populations.

Results: Significant perturbations in amino acid concentrations were observed in horses with clinical and subclinical forms of the disease and in the exposure group compared to control horses. Asparagine, glutamine, aspartic acid, glutamic acid, and glycine were significantly perturbed in the clinical, subclinical, and exposure groups compared to the control group, while alanine, citrulline, and tryptophan were characteristically perturbed in the clinical group relative to the other horse groups.

Main limitations: Variability of the original farms from which study populations were derived due to the retrospective nature of the study might have influenced the aminogram.

Conclusions: Amino acid concentrations show substantial perturbations in relation to the clinical status of EPE. Evaluation of the aminograms of horses with Lawsonia intracellularis infection provides novel information on this disease, which would be of clinical and, potentially, therapeutic relevance.

Keywords: aminogram; citrulline; equine proliferative enteropathy; glucogenic amino acid; horse; tryptophan.

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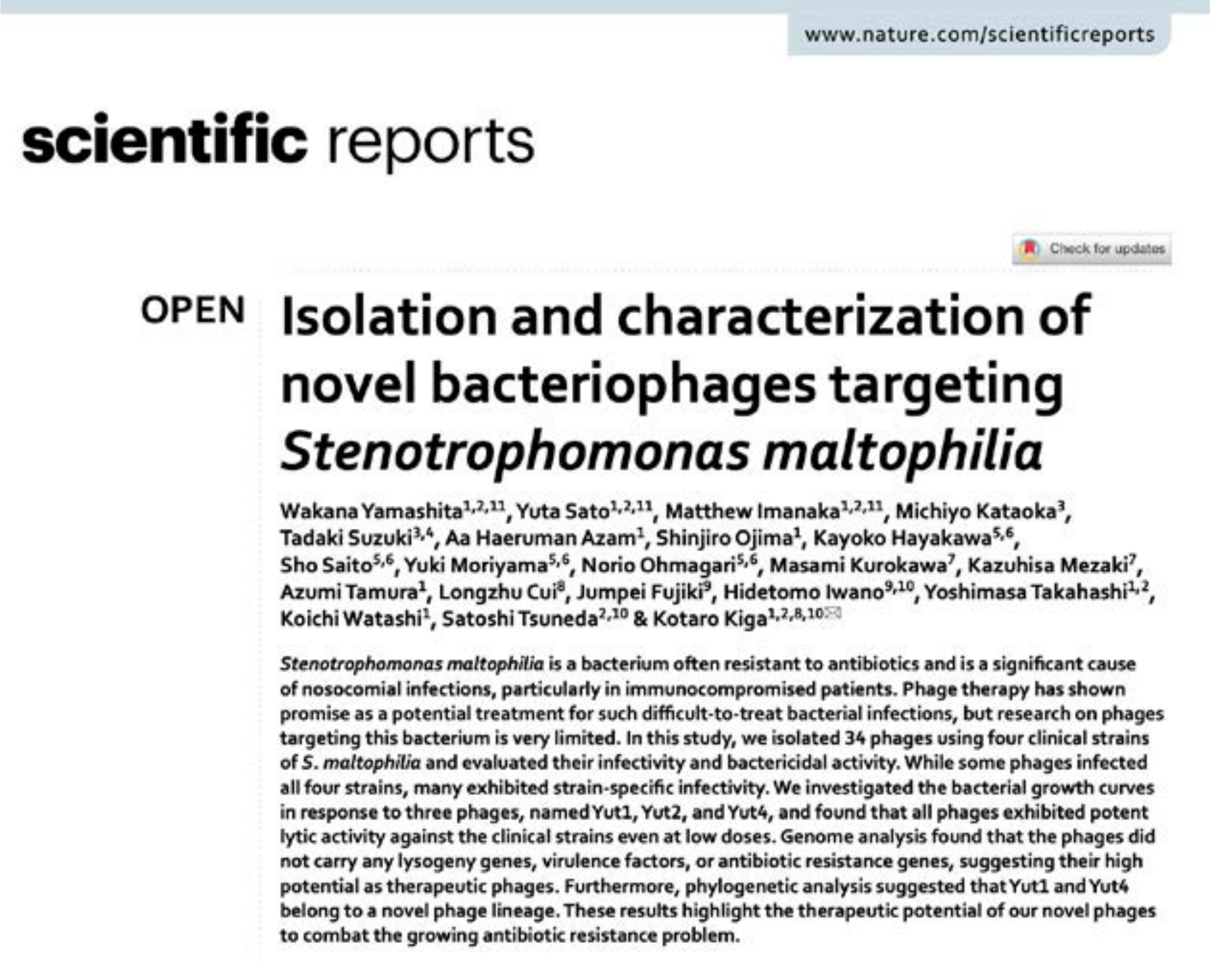
最終責任者　Yuya Mizuguchi (Corresponding Author)



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Isolation and characterization of novel bacteriophages targeting *Stenotrophomonas maltophilia*

Wakana Yamashita[1,2,11], Yuta Sato[1,2,11], Matthew Imanaka[1,2,11], Michiyo Kataoka[3], Tadaki Suzuki[3,4], Aa Haeruman Azam[1], Shinjiro Ojima[1], Kayoko Hayakawa[5,6], Sho Saito[5,6], Yuki Moriyama[5,6], Norio Ohmagari[5,6], Masami Kurokawa[7], Kazuhisa Mezaki[7], Azumi Tamura[1], Longzhu Cui[8], Jumpei Fujiki[9], Hidetomo Iwano[9,10], Yoshimasa Takahashi[1,2], Koichi Watashi[1], Satoshi Tsuneda[2,10] & Kotaro Kiga[1,2,8,10]✉

Stenotrophomonas maltophilia **is a bacterium often resistant to antibiotics and is a significant cause of nosocomial infections, particularly in immunocompromised patients. Phage therapy has shown promise as a potential treatment for such difficult-to-treat bacterial infections, but research on phages targeting this bacterium is very limited. In this study, we isolated 34 phages using four clinical strains of *S. maltophilia* and evaluated their infectivity and bactericidal activity. While some phages infected all four strains, many exhibited strain-specific infectivity. We investigated the bacterial growth curves in response to three phages, named Yut1, Yut2, and Yut4, and found that all phages exhibited potent lytic activity against the clinical strains even at low doses. Genome analysis found that the phages did not carry any lysogeny genes, virulence factors, or antibiotic resistance genes, suggesting their high potential as therapeutic phages. Furthermore, phylogenetic analysis suggested that Yut1 and Yut4 belong to a novel phage lineage. These results highlight the therapeutic potential of our novel phages to combat the growing antibiotic resistance problem.**

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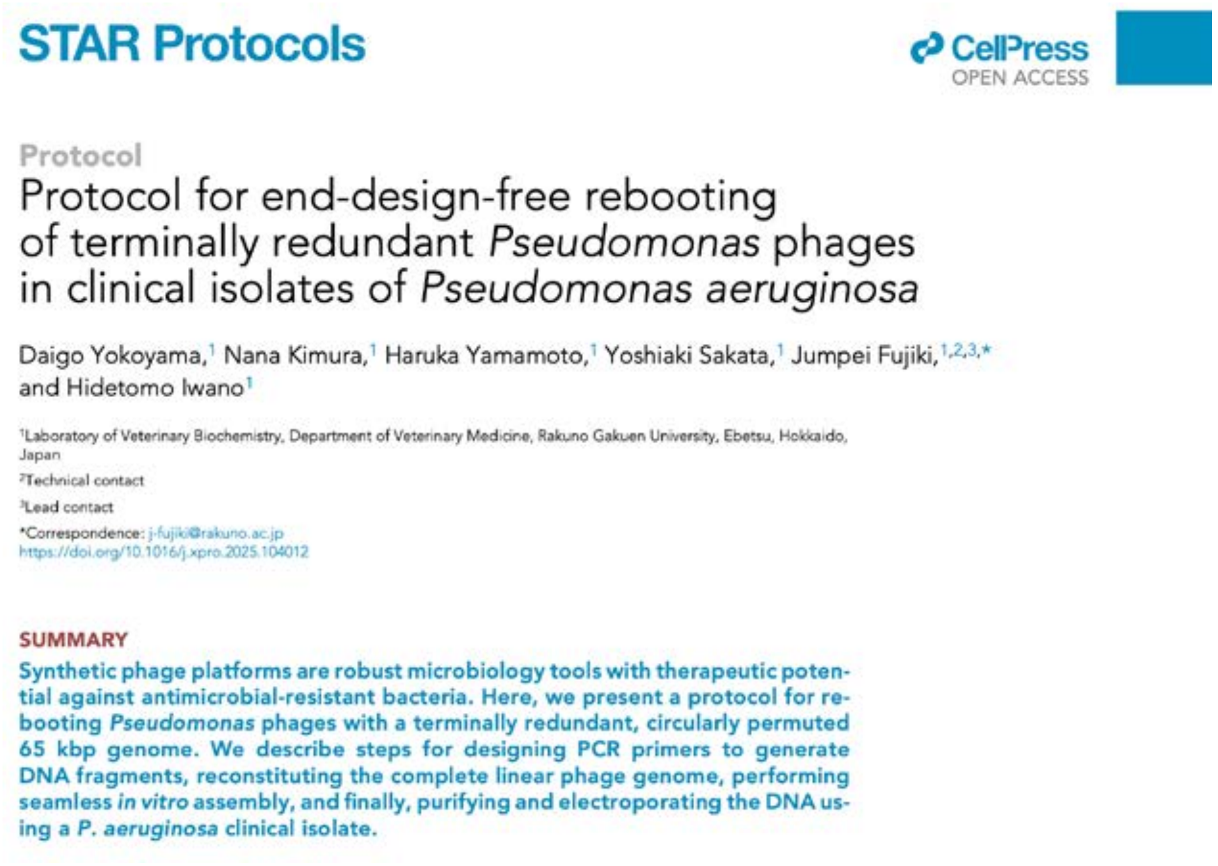

STAR Protocols

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Protocol

Protocol for end-design-free rebooting of terminally redundant *Pseudomonas* phages in clinical isolates of *Pseudomonas aeruginosa*

Daigo Yokoyama,[1] Nana Kimura,[1] Haruka Yamamoto,[1] Yoshiaki Sakata,[1] Jumpei Fujiki,[1,2,3,*] and Hidetomo Iwano[1]

[1]Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[2]Technical contact

[3]Lead contact

*Correspondence: j-fujiki@rakuno.ac.jp

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SUMMARY

Synthetic phage platforms are robust microbiology tools with therapeutic potential against antimicrobial-resistant bacteria. Here, we present a protocol for rebooting *Pseudomonas* phages with a terminally redundant, circularly permuted 65 kbp genome. We describe steps for designing PCR primers to generate DNA fragments, reconstituting the complete linear phage genome, performing seamless *in vitro* assembly, and finally, purifying and electroporating the DNA using a *P. aeruginosa* clinical isolate.

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最終責任者　Fujiki Jumpei (Corresponding Author)

動物と人の関係学（Animal Human Relations）

Takanori Kooriyama

Professor

教授　郡山　尚紀

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1）Exploring Factors of Dog Temperament Impacting Pass/Fail Outcomes in Therapy Dog Aptitude Tests.
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II. その他＜Others＞
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Article

Exploring Factors of Dog Temperament Impacting Pass/Fail Outcomes in Therapy Dog Aptitude Tests

Shiori Kawai, Saki Terashima, Natsuki Ebashi, Mayu Sakurama, Miki Ito, Yumiko Nakanowataru and Takanori Kooriyama *

Department of Veterinary Science, Rakuno Gakuen University, 582 Bunkyodai-Midori, Ebetsu, Hokkaido 069-8501, Japan
* Correspondence: kooriyam@rakuno.ac.jp; Tel.: +81-11-388-4755

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Correction Statement: This article has been republished with a minor change. The change does not affect the scientific content of the article and further details are available within the backmatter of the website version of this article.

Abstract

In recent years, requests for animal-assisted interventions (AAI) from medical institutions and welfare facilities have increased. Dogs are the most commonly used animals in AAI. Dogs that pass the "therapy dog" aptitude test can work in AAI. In previous research, we identified the Canine Behavior Assessment and Research Questionnaire (C-BARQ) factors common among dogs that passed the aptitude test. Using these factors, we developed the TC-BARQ, a screening questionnaire for therapy dogs that included 51 C-BARQ surveys. In this study, we conducted TC-BARQ screenings and compared the characteristics of dogs that passed and failed the aptitude test. We collected TC-BARQ data points from aptitude test examinees of the local AAI Dog Association. Each dog is identified by its breed, sex, neutering status, and whether it lives with another dog at home. For each question, we identified factors that differed between dogs that passed and those that failed. As a result, differences emerged in the presence of family dogs, particularly in behaviors related to aggression toward strangers and other dogs, as well as excitability toward people and situations. Continued surveillance is essential, but this study provides important information on selecting "therapy dogs".

Keywords: animal-assisted intervention; therapy dogs; C-BARQ; aptitude test; dog's personality

1. Introduction

Humans have long valued dogs, and interactions with them have been shown to have positive psychological and physical benefits for people [1]. Recently, this effect has been scientifically confirmed in various animals, increasing awareness of animal-assisted therapy [1]. Interactions with dogs, in particular, play a role in improving the quality of life (QOL) for individuals in medical and welfare settings, such as hospitals and nursing homes. Although different animals, including horses and dolphins, have been used in animal-assisted interventions, dogs have become the most common in recent years [2–4]. The reported benefits of animal-assisted intervention include reductions in depressive symptoms [2,5] and increased motivation to engage with society [4,6,7]. To support these benefits, many hospitals and facilities have introduced therapy dog visits. As demand increases, the number of therapy dogs now exceeds the available supply. Animal-assisted intervention can be divided into three types based on approach [8]. The first is animal-assisted therapy (AAT), the second is animal-assisted education (AAE), and the third is

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Kohei Makita

Professor

教授　蒔田　浩平

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1）Factors associated with the use of important human antimicrobials in Japanese small-animal clinics.
Makita K, Yoshida M, Ukita M, Matsuoka T, Sakai M, Tamura Y.
Front Vet Sci. 12:1496422. 2025. doi: 10.3389/fvets.2025.1496422

2) Direct economic losses in farms and government compensation costs due to highly pathogenic avian influenza outbreaks in Japan during the 2022-23 season.
Ukita M, Yasuda A, Kikuchi E, Hinata T, **Makita K**.
J Vet Med Sci. 87:976-985. 2025. doi: 10.1292/jvms.25-0088

3) Reduction in finisher pig death rate on a farm in Japan following use of a commercial killed vaccine against porcine reproductive and respiratory syndrome virus.
Yonezawa Y, Nakagami T, Tsutsumi N, Momma N, Taira O, Matsuyama R, **Makita K**.
J Vet Med Sci. 87:1060-1067. 2025. doi: 10.1292/jvms.24-0506

4) Genetic diversity and temporal shifts of porcine reproductive and respiratory syndrome virus type 2 (PRRSV-2) strains in Japan (2020–2023): Evidence of modified live vaccine influence on cluster distribution.
Yonezawa Y, Taira O, Kato A, Takai R, Nukui R, Tsutsumi N, Matsuyama R, **Makita K**.
Epidemiologia. 6:77. https://doi.org/10.3390/epidemiologia6040077

II. その他＜Others＞

1) Influence of pituitary pars intermedia dysfunction on reproductive performance in Thoroughbred mares.
   Murase H, Wachi S, Matsuyama R, **Makita K**, Sato F.
   J Equine Vet. Sci. 152:105651. 2025. doi: 10.1016/j.jevs.2025.105651
2) Ecology of pet birds and epidemiology of multidrug resistant *Escherichia coli* and *Staphylococcus* spp. and resistant patterns in Budgerigar.
   Hossain MS, Rahman MK, Nath C, Akter M, Uddin H, Islam A, **Makita K**, Hassan MM.
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*CORRESPONDENCE
Kohei Makita
kmakita@rakuno.ac.jp

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Factors associated with the use of important human antimicrobials in Japanese small-animal clinics

Kohei Makita[1]*, Mao Yoshida[1], Makoto Ukita[1],
Takeshi Matsuoka[2], Masato Sakai[2] and Yutaka Tamura[1]

[1]WOAH Collaborating Centre Consortium for Food Safety, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, [2]Japan Veterinary Medical Association, Tokyo, Japan

Introduction: In Japan, programs to monitor antimicrobial use in companion animals have not been established. To fill this gap, the Japan Veterinary Medical Association has conducted surveys of actual use. The aims of this paper are to clarify the frequency and factors associated with the use of human antimicrobials in Japanese small-animal clinics.

Methods: Antimicrobial usage and awareness surveys were conducted at 260 veterinary clinics between November 2021 and February 2022 using two questionnaires. The annual use of each antimicrobial drug was categorized by frequency, and the median value of each category, with a score of 50 for the choice ≥ 50, was used to quantify usage frequency. Important antimicrobial drugs for human use were defined as rank I antibiotics of the Food Safety Commission. Knowledge and awareness factors associated with the use of important antimicrobial drugs for human use were analyzed using three approaches. First, the use of important drugs was examined using a generalized linear model (GLM) with binomial errors. Second, a vector generalized linear and additive model with zero-inflated binomial errors was used to evaluate the proportion of important drugs among the annual frequency of use of human antimicrobial drugs. Third, at the drug level, selecting veterinary clinics using important human drugs, univariable GLMs with Poisson errors were used to evaluate the frequency of important human drug use, with the log number of employees as the offset term.

Results: The response rates were 71.2 and 72.3% for the antimicrobial usage and awareness surveys, respectively. All of the facilities used human antimicrobial drugs, and 57.1% (93/163) of facilities used important human antimicrobial drugs. Important human antimicrobial drugs accounted for 21.7% of the frequency of use of human antimicrobial drugs annually (7,342/33,896 times). In terms of the proportion of important human drugs and frequency of important human drug use, the use of important human antimicrobial drugs was low in cases of high awareness of prudent use of antimicrobial drugs and where tests for evidence-based judgment were introduced, but was high when price and ease of use were emphasized.

Discussion: Antibiotic stewardship should be further promoted in Japanese small-animal clinics through educational and information dissemination activities.

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FULL PAPER
Epidemiology

Direct economic losses in farms and government compensation costs due to highly pathogenic avian influenza outbreaks in Japan during the 2022–23 season

Makoto UKITA[1)], Ayaka YASUDA[1)], Eisaku KIKUCHI[2)], Takahisa HINATA[3)],
Kohei MAKITA[1)]*

[1)]Veterinary Epidemiology Unit, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]Agriculture & Livestock Industries Corporation, Tokyo, Japan
[3)]College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. Highly pathogenic avian influenza (HPAI) is a contagious disease of poultry characterized by high avian mortality and devastating economic losses. Between October 2022 and April 2023 (in the 2022–23 season), there were 84 cases of HPAI on poultry farms in 26 prefectures of Japan, resulting in the culling of approximately 17.7 million birds. This study examined the economic impact of HPAI outbreaks on poultry farms in Japan during the 2022–23 season. Farm direct economic losses and burden to the national and prefectural governments from compensation were estimated. Data were obtained from epidemiological survey reports prepared by the Ministry of Agriculture, Forestry and Fisheries, prefecture websites, and through direct contacts. Losses incurred by the national government as compensation granted to infected farms for birds culled and eggs discarded as contaminated goods were estimated. Economic losses for poultry farms located in movement and shipment restriction zones were assumed to be reduced sales and increased farm management costs and shared equally by the national government and infected prefectures. Economic losses on infected farms were estimated from the outbreak until recovery of production. Total economic losses incurred by the national government, infected prefectures, and infected poultry farms were estimated at approximately 16.9 billion, 183.5 million, and 14.2 billion Japanese yen (USD 126.6 million, 1.4 million, and 106.4 million), respectively. Although data are limited, the quantification of economic losses due to HPAI in this study could contribute to future evidence-based policy making.

KEYWORDS: compensation, economic loss, highly pathogenic avian influenza, net income, poultry farm

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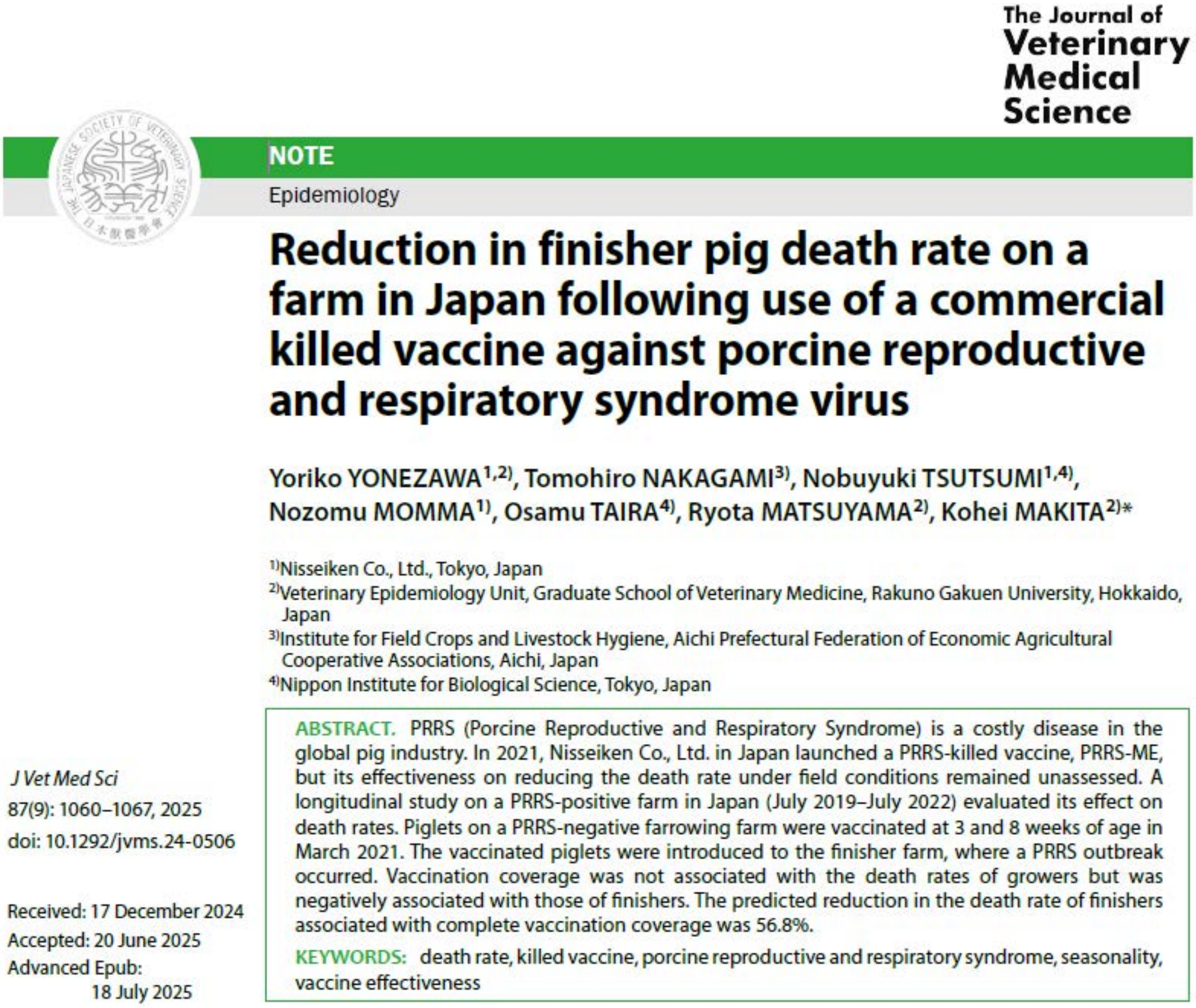

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NOTE

Epidemiology

Reduction in finisher pig death rate on a farm in Japan following use of a commercial killed vaccine against porcine reproductive and respiratory syndrome virus

Yoriko YONEZAWA[1,2], Tomohiro NAKAGAMI[3], Nobuyuki TSUTSUMI[1,4], Nozomu MOMMA[1], Osamu TAIRA[4], Ryota MATSUYAMA[2], Kohei MAKITA[2]*

[1]Nisseiken Co., Ltd., Tokyo, Japan
[2]Veterinary Epidemiology Unit, Graduate School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[3]Institute for Field Crops and Livestock Hygiene, Aichi Prefectural Federation of Economic Agricultural Cooperative Associations, Aichi, Japan
[4]Nippon Institute for Biological Science, Tokyo, Japan

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ABSTRACT. PRRS (Porcine Reproductive and Respiratory Syndrome) is a costly disease in the global pig industry. In 2021, Nisseiken Co., Ltd. in Japan launched a PRRS-killed vaccine, PRRS-ME, but its effectiveness on reducing the death rate under field conditions remained unassessed. A longitudinal study on a PRRS-positive farm in Japan (July 2019–July 2022) evaluated its effect on death rates. Piglets on a PRRS-negative farrowing farm were vaccinated at 3 and 8 weeks of age in March 2021. The vaccinated piglets were introduced to the finisher farm, where a PRRS outbreak occurred. Vaccination coverage was not associated with the death rates of growers but was negatively associated with those of finishers. The predicted reduction in the death rate of finishers associated with complete vaccination coverage was 56.8%.

KEYWORDS: death rate, killed vaccine, porcine reproductive and respiratory syndrome, seasonality, vaccine effectiveness

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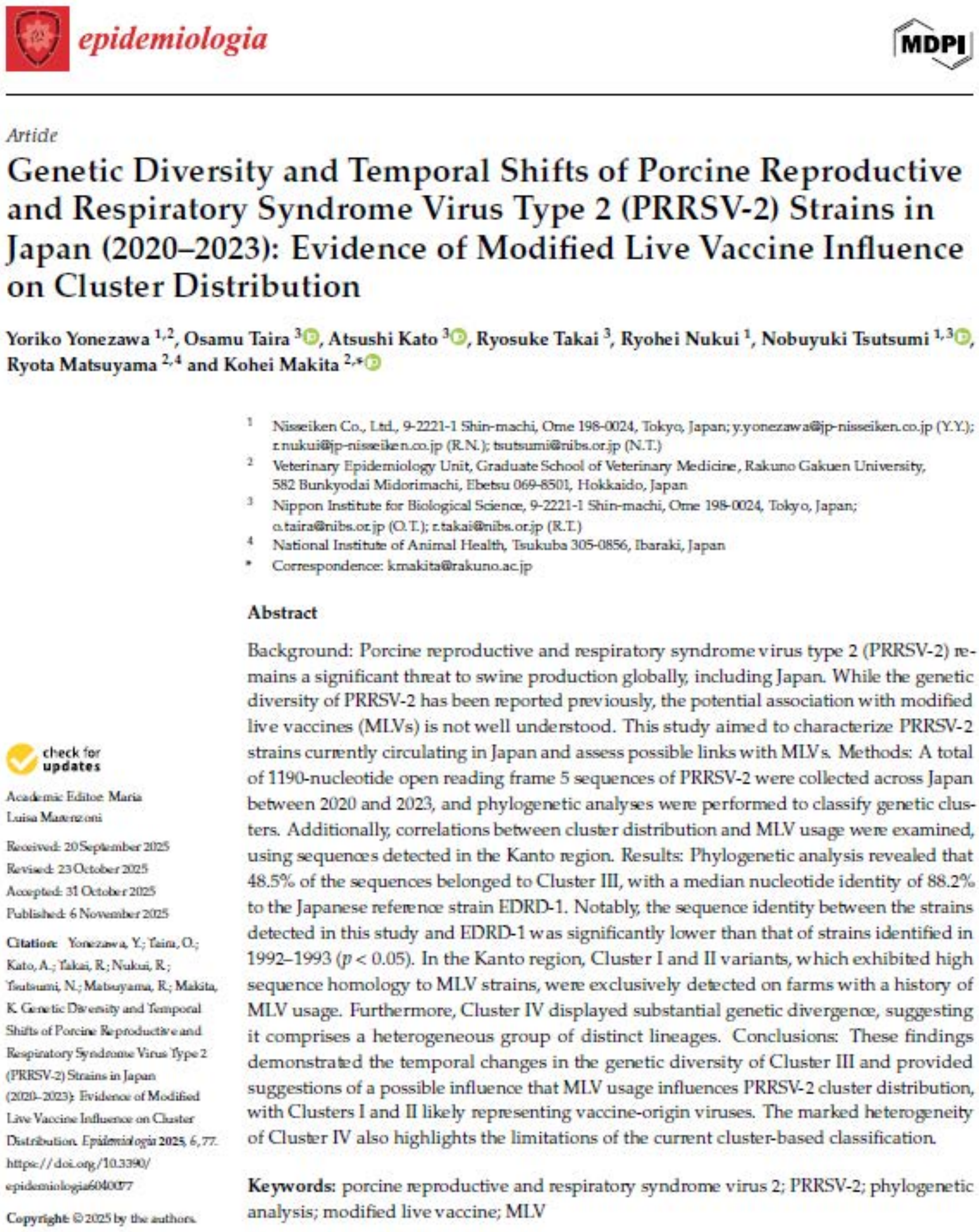

epidemiologia

Article

Genetic Diversity and Temporal Shifts of Porcine Reproductive and Respiratory Syndrome Virus Type 2 (PRRSV-2) Strains in Japan (2020–2023): Evidence of Modified Live Vaccine Influence on Cluster Distribution

Yoriko Yonezawa [1,2], Osamu Taira [3], Atsushi Kato [3], Ryosuke Takai [3], Ryohei Nukui [1], Nobuyuki Tsutsumi [1,3], Ryota Matsuyama [2,4] and Kohei Makita [2,*]

[1] Nisseiken Co., Ltd., 9-2221-1 Shin-machi, Ome 198-0024, Tokyo, Japan; y.yonezawa@jp-nisseiken.co.jp (Y.Y.); r.nukui@jp-nisseiken.co.jp (R.N.); tsutsumi@nibs.or.jp (N.T.)
[2] Veterinary Epidemiology Unit, Graduate School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu 069-8501, Hokkaido, Japan
[3] Nippon Institute for Biological Science, 9-2221-1 Shin-machi, Ome 198-0024, Tokyo, Japan; o.taira@nibs.or.jp (O.T.); r.takai@nibs.or.jp (R.T.)
[4] National Institute of Animal Health, Tsukuba 305-0856, Ibaraki, Japan
* Correspondence: kmakita@rakuno.ac.jp

Abstract

Background: Porcine reproductive and respiratory syndrome virus type 2 (PRRSV-2) remains a significant threat to swine production globally, including Japan. While the genetic diversity of PRRSV-2 has been reported previously, the potential association with modified live vaccines (MLVs) is not well understood. This study aimed to characterize PRRSV-2 strains currently circulating in Japan and assess possible links with MLVs. Methods: A total of 1190-nucleotide open reading frame 5 sequences of PRRSV-2 were collected across Japan between 2020 and 2023, and phylogenetic analyses were performed to classify genetic clusters. Additionally, correlations between cluster distribution and MLV usage were examined, using sequences detected in the Kanto region. Results: Phylogenetic analysis revealed that 48.5% of the sequences belonged to Cluster III, with a median nucleotide identity of 88.2% to the Japanese reference strain EDRD-1. Notably, the sequence identity between the strains detected in this study and EDRD-1 was significantly lower than that of strains identified in 1992–1993 ($p < 0.05$). In the Kanto region, Cluster I and II variants, which exhibited high sequence homology to MLV strains, were exclusively detected on farms with a history of MLV usage. Furthermore, Cluster IV displayed substantial genetic divergence, suggesting it comprises a heterogeneous group of distinct lineages. Conclusions: These findings demonstrated the temporal changes in the genetic diversity of Cluster III and provided suggestions of a possible influence that MLV usage influences PRRSV-2 cluster distribution, with Clusters I and II likely representing vaccine-origin viruses. The marked heterogeneity of Cluster IV also highlights the limitations of the current cluster-based classification.

Keywords: porcine reproductive and respiratory syndrome virus 2; PRRSV-2; phylogenetic analysis; modified live vaccine; MLV

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Influence of pituitary pars intermedia dysfunction on reproductive performance in Thoroughbred mares

H. Murase [a,*], S. Wachi [b], R. Matsuyama [c], K. Makita [c], F. Sato [a]

[a] *Hidaka Training and Research Center, Japan Racing Association, 535-13, Nishicha, Urakawa, Hokkaido, 057-0171 Japan*
[b] *Hidaka Horse Breeders Association, 2-1-1, Tsukiji, Urakawa, Hokkaido, 057-0024 Japan*
[c] *School of Veterinary Medicine, Rakuno Gakuen University, 582, Midorimachi, Bunkyodai, Ebetsu, Hokkaido, 069-8501 Japan*

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ABSTRACT

Background: Pituitary pars intermedia dysfunction (PPID) is an age-related disease considered to have a negative impact on fertility. To understand the true impact of PPID on fertility, the influence of age must be considered.
Aims/objectives: This study aimed to assess the impact of PPID on fertility while accounting for the effect of aging.
Methods: A total of 332 Thoroughbred mares aged 10–20 years and managed across 137 private farms in Hidaka, Hokkaido, Japan were classified as PPID, equivocal, or non-PPID based on plasma adrenocorticotropic hormone concentration in 2019 and 2020. The prevalence of PPID and its associations with age and blood biochemistry parameters, including prolactin, were investigated. Multivariable logistic regression analyses, adjustments for potentially confounders including mare's age, PPID status were conducted to evaluate their impact on fertility.
Results: The prevalence of PPID was 9.04 %. No significant differences in prolactin concentrations were observed among PPID status groups. The adjusted odds ratio (AOR) for the interaction between age and PPID status was 0.49 (95 %CI; 0.26-0.91, $p = 0.02$), indicating a greater age-related decline in pregnancy rate in PPID mares compared to non-PPID mares. Of the 30 PPID mares, 24 were assigned to either treated or untreated groups, and monitored for pregnancy outcomes in the following spring. Pregnancy rates were 80 % (12/15) in treated mares and 44.4 % (4/9) in untreated mares ($p = 0.10$). The AOR for pregnancy in treated mares, relative to untreated mares, was 2.98 (95 %CI; 0.26-24.61, $p = 0.31$).
Conclusion: PPID was suggested to negatively impact on fertility with aging.

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Ecology of pet birds and epidemiology of multidrug resistant *Escherichia coli* and *Staphylococcus* spp. and resistant patterns in Budgerigar

Md. Saddam Hossain [a], Md. Kaisar Rahman [b,*], Chandan Nath [a], Mahfuja Akter [c], Helal Uddin [a], Ariful Islam [d], Kohei Makita [e], Mohammad Mahmudul Hassan [a,f]

[a] *Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh*
[b] *School of Veterinary Medicine, Texas Tech University, TX, USA*
[c] *Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh*
[d] *Gulbali Research Institute, Charles Sturt University, Wagga Wagga, NSW 2678, Australia*
[e] *Faculty of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan*
[f] *Queensland Alliance for One Health Sciences, School of Veterinary Science, The University of Queensland, Gatton, Australia*

ARTICLE INFO

Keywords:
Antimicrobial resistance
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E. coli
Staphylococcus sp.

ABSTRACT

Pet birds are most popular as pets in Bangladesh, with budgerigars being the most common species. However, the emergence of antimicrobial resistance poses a growing threat to both pet birds and their owners. This study aims to investigate the ecology of pet birds and the epidemiology of multidrug resistant (MDR) *Escherichia coli* and *Staphylococcus* spp. in pet birds. Data were collected from interviews with 49 pet bird farms using a pretested questionnaire, and cloacal swab samples were collected from 220 budgerigars between December 2016 and June 2017. Standard microbiological procedures were employed for bacterial isolation and identification. Antimicrobial susceptibility was tested using micro-disc diffusion techniques for 9 antibiotics. Budgerigar birds were found to be 24.7 % of all pet species. The prevalences of *E. coli* and *Staphylococcus* spp. were 22.3 % and 18.2 %, respectively. All the *E. coli* ($n = 49$) isolates displayed resistance to amoxicillin, sulfamethoxazole, trimethoprim, and cefixime, with the lowest resistance observed against ciprofloxacin (6.1 %). For *Staphylococcus* spp., all the isolates ($n = 40$) were resistant against enrofloxacin and gentamycin, with the lowest resistance to ciprofloxacin and azithromycin (5 %). Significant factors associated with MDR included female budgerigars with poor body condition score (BCS), which was linked to a significantly higher prevalence of MDR *E. coli*. For MDR *Staphylococcus* spp., young age emerged as a significant factor ($p < 0.005$). To mitigate the risk of public health implications associated with multidrug resistant bacteria, there is a need to reduce the indiscriminate use of antibiotics in pet birds.

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最終責任者　Mohammad Mahmudul Hassan (Corresponding Author)

獣医病理学（Veterinary Pathology）

Kazuya Matsuda

Professor

教授　松田　一哉

I. 筆頭または責任著者　＜First or Corresponding Author＞

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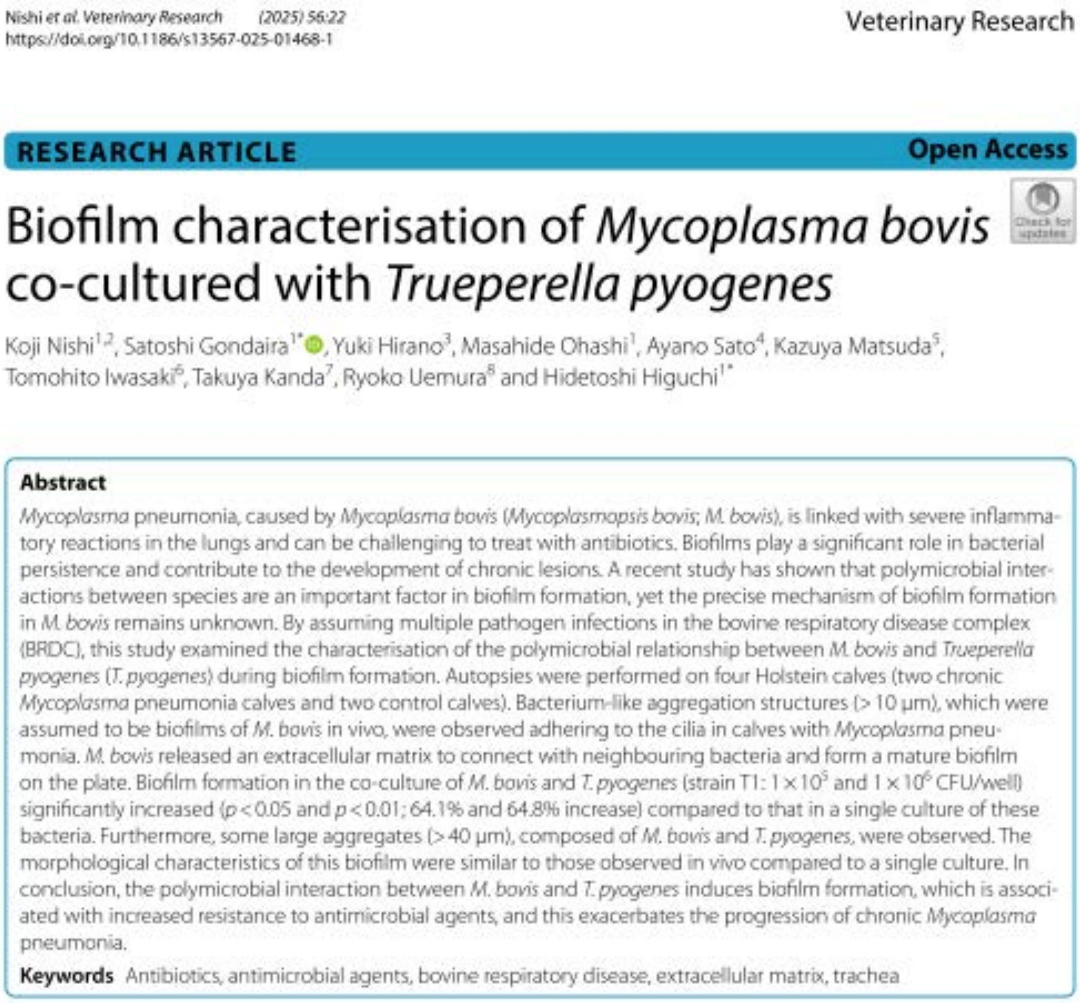
II. その他＜Others＞

1) Biofilm characterisation of Mycoplasma bovis co-cultured with *Trueperella pyogenes*.
Nishi K, Gondaira S, Hirano Y, Ohashi M, Sato A, **Matsuda K**, Iwasaki T, Kanda T, Uemura R, Higuchi H.
Vet Res. 56:22. 2025. doi: 10.1186/s13567-025-01468-1.

2) Bilateral congenital cystic eye with intraocular tissue differentiation in a horse.
Sano Y, Miura C, Kinoshita Y, Kakimoto M, Tsuda T, **Matsuda K**.
J Vet Med Sci. 87: 52-56. 2025. doi: 10.1292/jvms.24-0285.



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Veterinary Research

RESEARCH ARTICLE **Open Access**

Biofilm characterisation of *Mycoplasma bovis* co-cultured with *Trueperella pyogenes*

Koji Nishi[1,2], Satoshi Gondaira[1*], Yuki Hirano[3], Masahide Ohashi[1], Ayano Sato[4], Kazuya Matsuda[5], Tomohito Iwasaki[6], Takuya Kanda[7], Ryoko Uemura[8] and Hidetoshi Higuchi[1*]

Abstract

Mycoplasma pneumonia, caused by *Mycoplasma bovis* (*Mycoplasmopsis bovis*; *M. bovis*), is linked with severe inflammatory reactions in the lungs and can be challenging to treat with antibiotics. Biofilms play a significant role in bacterial persistence and contribute to the development of chronic lesions. A recent study has shown that polymicrobial interactions between species are an important factor in biofilm formation, yet the precise mechanism of biofilm formation in *M. bovis* remains unknown. By assuming multiple pathogen infections in the bovine respiratory disease complex (BRDC), this study examined the characterisation of the polymicrobial relationship between *M. bovis* and *Trueperella pyogenes* (*T. pyogenes*) during biofilm formation. Autopsies were performed on four Holstein calves (two chronic *Mycoplasma* pneumonia calves and two control calves). Bacterium-like aggregation structures (> 10 μm), which were assumed to be biofilms of *M. bovis* in vivo, were observed adhering to the cilia in calves with *Mycoplasma* pneumonia. *M. bovis* released an extracellular matrix to connect with neighbouring bacteria and form a mature biofilm on the plate. Biofilm formation in the co-culture of *M. bovis* and *T. pyogenes* (strain T1: 1×10^5 and 1×10^6 CFU/well) significantly increased ($p < 0.05$ and $p < 0.01$; 64.1% and 64.8% increase) compared to that in a single culture of these bacteria. Furthermore, some large aggregates (> 40 μm), composed of *M. bovis* and *T. pyogenes*, were observed. The morphological characteristics of this biofilm were similar to those observed in vivo compared to a single culture. In conclusion, the polymicrobial interaction between *M. bovis* and *T. pyogenes* induces biofilm formation, which is associated with increased resistance to antimicrobial agents, and this exacerbates the progression of chronic *Mycoplasma* pneumonia.

Keywords Antibiotics, antimicrobial agents, bovine respiratory disease, extracellular matrix, trachea

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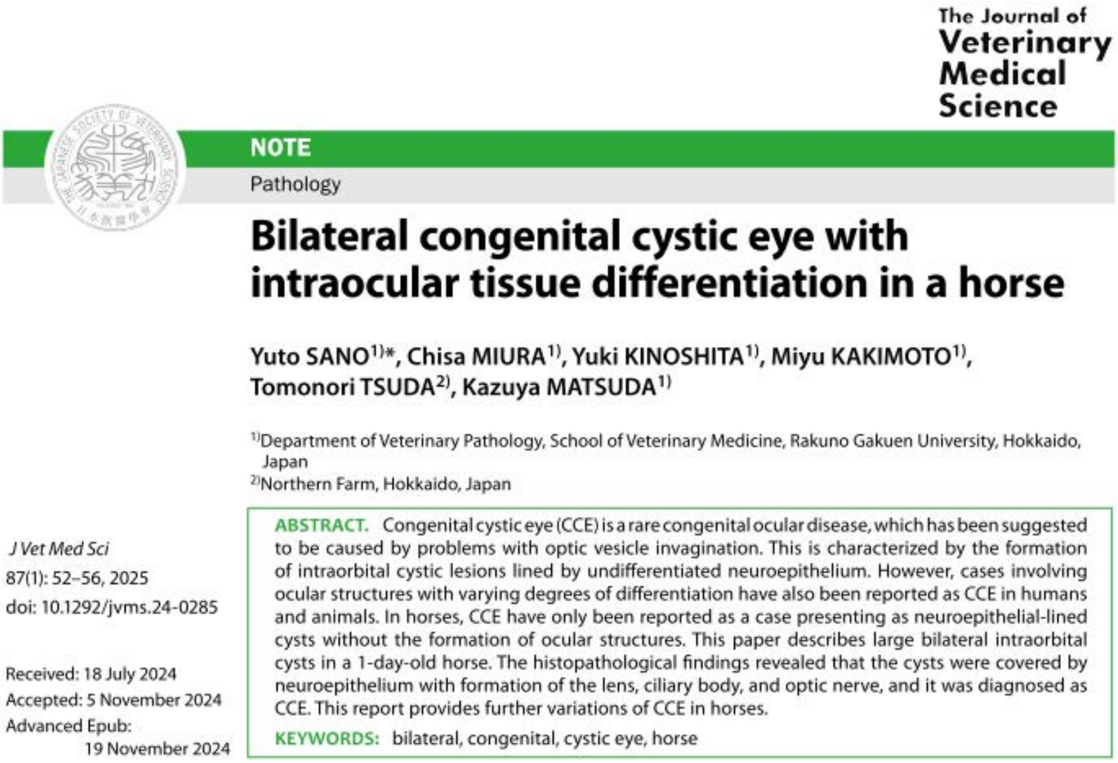

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NOTE

Pathology

Bilateral congenital cystic eye with intraocular tissue differentiation in a horse

Yuto SANO[1)]*, Chisa MIURA[1)], Yuki KINOSHITA[1)], Miyu KAKIMOTO[1)], Tomonori TSUDA[2)], Kazuya MATSUDA[1)]

[1)]Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]Northern Farm, Hokkaido, Japan

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ABSTRACT. Congenital cystic eye (CCE) is a rare congenital ocular disease, which has been suggested to be caused by problems with optic vesicle invagination. This is characterized by the formation of intraorbital cystic lesions lined by undifferentiated neuroepithelium. However, cases involving ocular structures with varying degrees of differentiation have also been reported as CCE in humans and animals. In horses, CCE have only been reported as a case presenting as neuroepithelial-lined cysts without the formation of ocular structures. This paper describes large bilateral intraorbital cysts in a 1-day-old horse. The histopathological findings revealed that the cysts were covered by neuroepithelium with formation of the lens, ciliary body, and optic nerve, and it was diagnosed as CCE. This report provides further variations of CCE in horses.

KEYWORDS: bilateral, congenital, cystic eye, horse

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最終責任者　Yuto Sano (Corresponding Author)

人獣共通感染症学（Zoonotic Diseases）

Yasukazu Muramatsu

Professor

教授　村松　康和

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1）Detection of haemosporidian parasite lineages from wild bird carcasses collected in eastern Japan.
Eki M, Sasaki M, Muramatsu Y, Kobayashi M, Ishikawa T, **Uchida L.**
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NOTE
Public Health

Detection of haemosporidian parasite lineages from wild bird carcasses collected in eastern Japan

Momoko EKI[1,3], Mizuki SASAKI[2], Yasukazu MURAMATSU[3], Mari KOBAYASHI[4], Takanori ISHIKAWA[5], Leo UCHIDA[3]*

[1]Department of Ecosystem Studies, School of Environmental Science, The University of Shiga Prefecture, Shiga, Japan
[2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[3]Laboratory of Zoonotic Diseases, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[4]Department of Ocean and Fisheries Science, Faculty of Bioindustry, Tokyo University of Agriculture, Hokkaido, Japan
[5]Tochigi Prefectural Fisheries Experiment Station, Tochigi, Japan

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ABSTRACT. Avian haemosporidia are a group of vector-borne hemoparasites that are non-pathogenic to native bird hosts; however, some species/lineages cause severe disease or death in poultry and non-native birds. We investigated haemosporidian parasites in wild bird carcasses collected from four prefectures in eastern Japan. DNA was extracted from the liver, and the haemosporidian cytochrome *b* gene was amplified by nested PCR followed by direct sequencing. Of the 40 bird samples belonging to 25 species, 12 haemosporidian lineages were detected: four from *Plasmodium*, one from *Haemoproteus*, and seven from *Leucocytozoon*. Of these 12, two *Leucocytozoon* lineages were suspected to be novel. *Leucocytozoon* lineage TUMER02 was first detected in Japan. Six lineages were detected among the six novel host bird species.

KEYWORDS: cytochrome *b* gene, haemosporidia, Japan, wild bird

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*Correspondence to: Uchida L: uchidaleo@rakuno.ac.jp, Laboratory of Zoonotic Diseases, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan

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最終責任者　Leo Uchida (Corresponding Author)

動物生殖学（Theriogenology）

Ken Nakada

Professor

教授　中田　健

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Association of somatic cell score at first calving with milk productivity and mortality in dairy herds: a cross-sectional study in eastern Hokkaido, Japan.
Goto A, Yokoi M, Yamamoto N, Inoue Y, Hisaeda K, Shinozuka Y, **Nakada K.**
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2）Association between hygienic milk quality and culling rate or mortality in dairy herds: a cross-sectional study in eastern Hokkaido, Japan.
Goto A, Yokoi M, Yamamoto N. Kutara K, Inoue Y, Hisaeda K, Shinozuka,Y, **Nakada K.**
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II. その他＜Others＞

1) Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow.
Ohsaki K, Sano Y, Murakami T, Ishiguro Y, Ohtake A, Sato A, **Nakada,K**, Sugiura T.
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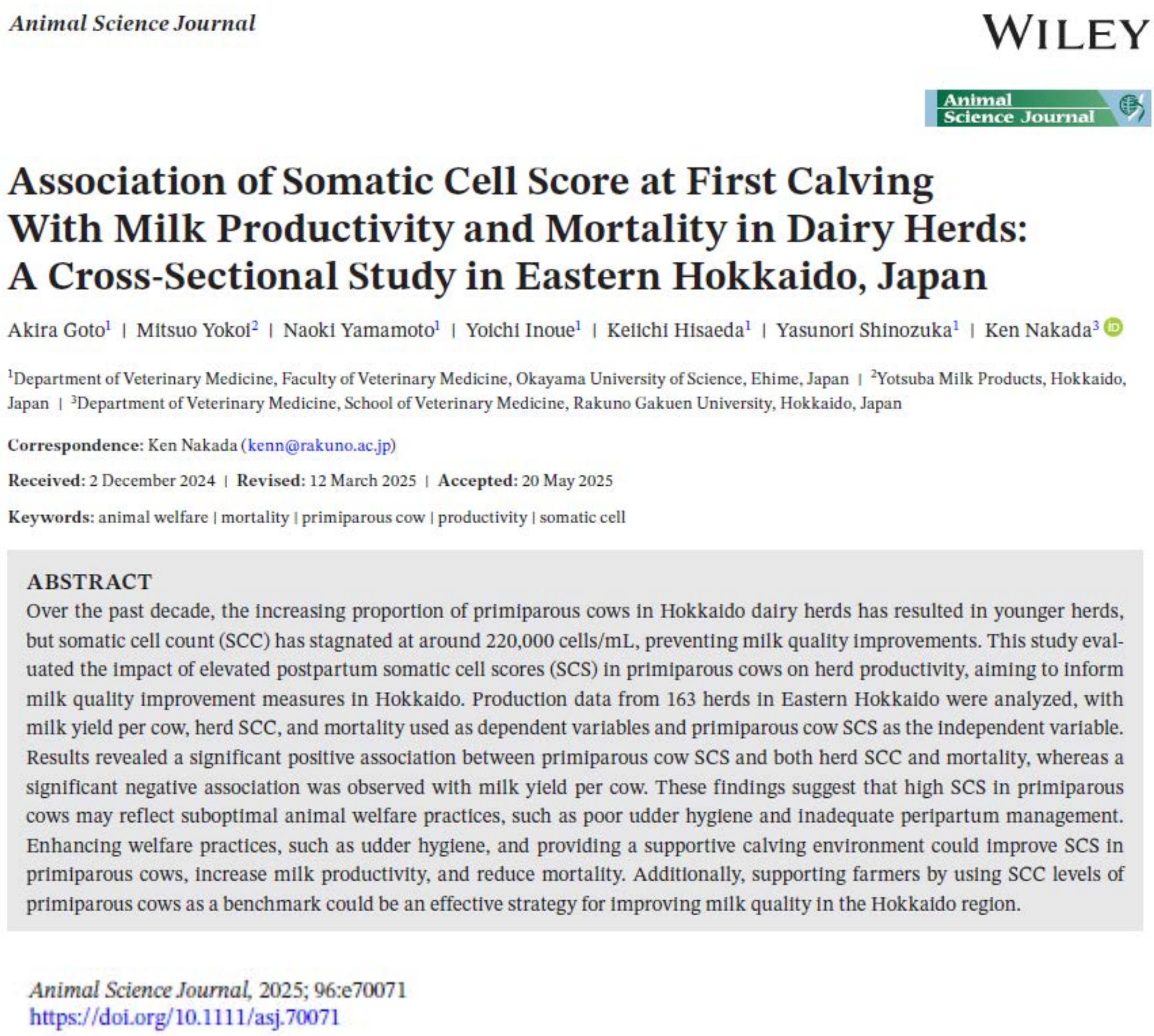

Animal Science Journal

Association of Somatic Cell Score at First Calving With Milk Productivity and Mortality in Dairy Herds: A Cross-Sectional Study in Eastern Hokkaido, Japan

Akira Goto[1] | Mitsuo Yokoi[2] | Naoki Yamamoto[1] | Yoichi Inoue[1] | Keiichi Hisaeda[1] | Yasunori Shinozuka[1] | Ken Nakada[3]

[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Ehime, Japan | [2]Yotsuba Milk Products, Hokkaido, Japan | [3]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

Correspondence: Ken Nakada (kenn@rakuno.ac.jp)

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Keywords: animal welfare | mortality | primiparous cow | productivity | somatic cell

ABSTRACT

Over the past decade, the increasing proportion of primiparous cows in Hokkaido dairy herds has resulted in younger herds, but somatic cell count (SCC) has stagnated at around 220,000 cells/mL, preventing milk quality improvements. This study evaluated the impact of elevated postpartum somatic cell scores (SCS) in primiparous cows on herd productivity, aiming to inform milk quality improvement measures in Hokkaido. Production data from 163 herds in Eastern Hokkaido were analyzed, with milk yield per cow, herd SCC, and mortality used as dependent variables and primiparous cow SCS as the independent variable. Results revealed a significant positive association between primiparous cow SCS and both herd SCC and mortality, whereas a significant negative association was observed with milk yield per cow. These findings suggest that high SCS in primiparous cows may reflect suboptimal animal welfare practices, such as poor udder hygiene and inadequate peripartum management. Enhancing welfare practices, such as udder hygiene, and providing a supportive calving environment could improve SCS in primiparous cows, increase milk productivity, and reduce mortality. Additionally, supporting farmers by using SCC levels of primiparous cows as a benchmark could be an effective strategy for improving milk quality in the Hokkaido region.

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最終責任者　Ken Nakada (Corresponding Author)

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Association between Hygienic Milk Quality and Culling Rate or Mortality in Dairy Herds: A Cross-Sectional Study in Eastern Hokkaido, Japan

Akira GOTO[1,2], Mitsuo YOKOI[3], Naoki YAMAMOTO[1], Kenji KUTARA[1], Yoichi INOUE[1], Keiichi HISAEDA[1], Yasunori SHINOZUKA[1] and Ken NAKADA[4]*

[1] Department of Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Japan
[2] Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan
[3] Yotsuba Milk Products, Otofuke, Hokkaido, Japan
[4] Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Abstract

This study analyzed production records from Hokkaido, Japan to develop a plan for farmers to improve milk quality. Over three years, 164 dairy herds were evaluated based on two milk quality indices: herd-level somatic cell count and chronic subclinical mastitis morbidity. Farms were ranked annually for each index, and the rankings were combined to determine an overall evaluation. After a comprehensive assessment, the herds were categorized into three groups: excellent (36 herds), good (90 herds), and poor (38 herds). A comparative analysis of nine production indices, including milk quality, was conducted between the excellent and poor groups. Our study revealed that poor herds exhibited significantly smaller herd sizes, higher culling rates at specific periods, higher mortality, and a greater proportion of dead cows among culled cows than excellent herds. The increased mortality in poor herds suggests lower animal welfare standards. Furthermore, the high rate of involuntary culling indicates suboptimal peripartum management. These findings underscore the strong link between poor milk quality and compromised animal welfare. The results suggest that improving animal welfare practices can lead to higher milk quality in dairy herds.

Discipline: Animal Science
Additional key words: animal welfare, somatic cell count

*Corresponding author: kenn@rakuno.ac.jp

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EDITED BY
Amal M. Aboelmaaty,
National Research Centre, Egypt

REVIEWED BY
Carolina Smok,
Universidad de La Frontera, Chile
Jagmohan Rajput,
Nanaji Deshmukh Veterinary Science
University, India

*CORRESPONDENCE
Tomochika Sugiura
✉ t-sugiura@rakuno.ac.jp

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Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow

Koto Ohsaki[1], Yuto Sano[2], Takashi Murakami[3], Yuki Ishiguro[2], Ayaka Ohtake[3], Ayano Sato[3], Ken Nakada[1] and Tomochika Sugiura[1]*

[1]Laboratory of Theriogenology, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [2]Laboratory of Veterinary Pathology, Pathobiology, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [3]Laboratory of Farm Animal Surgery, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan

This case report describes a rare instance of a bovine fetus with concurrent umbilical cord torsion and entanglement, which resulted in fetal growth restriction (FGR) and prolonged gestation. A 36-month-old primiparous Holstein cow, 285 days pregnant, was examined after failing to show signs of parturition. Fetal heartbeat was confirmed via abdominal ultrasonography until 295 days post-artificial insemination (AI) but was not detected thereafter, leading to a presumptive diagnosis of fetal death at 313 days post-AI. Following induction, the dead fetus was delivered at 316 days post-AI. Despite a gestational age of approximately 10.5 months, the fetus exhibited severe growth restriction that is equivalent to that of a normal 7-month-old fetus. The umbilical cord measured 40 cm—abnormally long for a 7-month-old fetus—and was tightly wrapped around the right hind limb with more than 360 degrees of torsion along its long axis. Maternal serum analysis revealed persistently high progesterone and markedly low estradiol concentrations before parturition, suggesting that the physiological process of parturition had not been initiated. It is speculated that the FGR resulted from chronic blood flow obstruction likely associated with the umbilical cord abnormalities, which were hypothesized to have occurred during the second trimester (approximately 4 months). The consequent absence of normal fetal signals to initiate parturition and the lack of periparturient endocrine changes contributed to prolonged gestation and underdeveloped mammary glands in the dam. This report represents the first detailed description of intrauterine umbilical cord abnormalities in cattle, demonstrating their potential to cause fetal developmental delay, prolonged gestation, and impaired mammary gland development.

KEYWORDS

bovine fetus, umbilical cord torsion, umbilical cord entanglement, fetal growth restriction, maternal serum analysis, parturition

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最終責任者　Tomochika Sugiura (Corresponding Author)

伴侶動物内科学（Companion Animal Internal Medicine）

Hiroshi Ohta

Professor

教授　大田　寛

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1）Prospective estimation of the prevalence of thromboembolism in dogs with inflammatory protein-losing enteropathy.
Oishi N, **Ohta H,** Tamura M, Hanazono K, Miyoshi K, Yokoyama N, Shinbo G.
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II. その他＜Others＞

1) Evaluating the influence of hepatic triglyceride and lipoprotein lipases on lipase assays used to diagnose pancreatitis in dogs and cats.
Harada H, Tamura M, Ishii K, Osuga T, **Ohta H**.
Vet Clin Pathol. 54:132-141. 2025. doi: 10.1111/vcp.70002.
2) Development of caninized anti-CTLA-4 antibody as salvage combination therapy for anti-PD-L1 refractory tumors in dogs.
Maekawa N, Konnai S, Watari K, Takeuchi H, Nakanishi T, Tachibana T, Hosoya K, Kim S, Kinoshita R, Owaki R, Yokokawa M, Kagawa Y, Takagi S, Deguchi T, **Ohta H,** Kato Y, Yamamoto S, Yamamoto K, Suzuki Y, Okagawa T, Murata S, Ohashi K.
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3) Factors affecting the quality of histopathologic specimens obtained via small intestinal endoscopic biopsy in dogs and cats.
Nakashima K, Kojima K, Takeuchi Y, Ito M, Matsumoto I, Ushigusa T, **Ohta H**, Uchida K.
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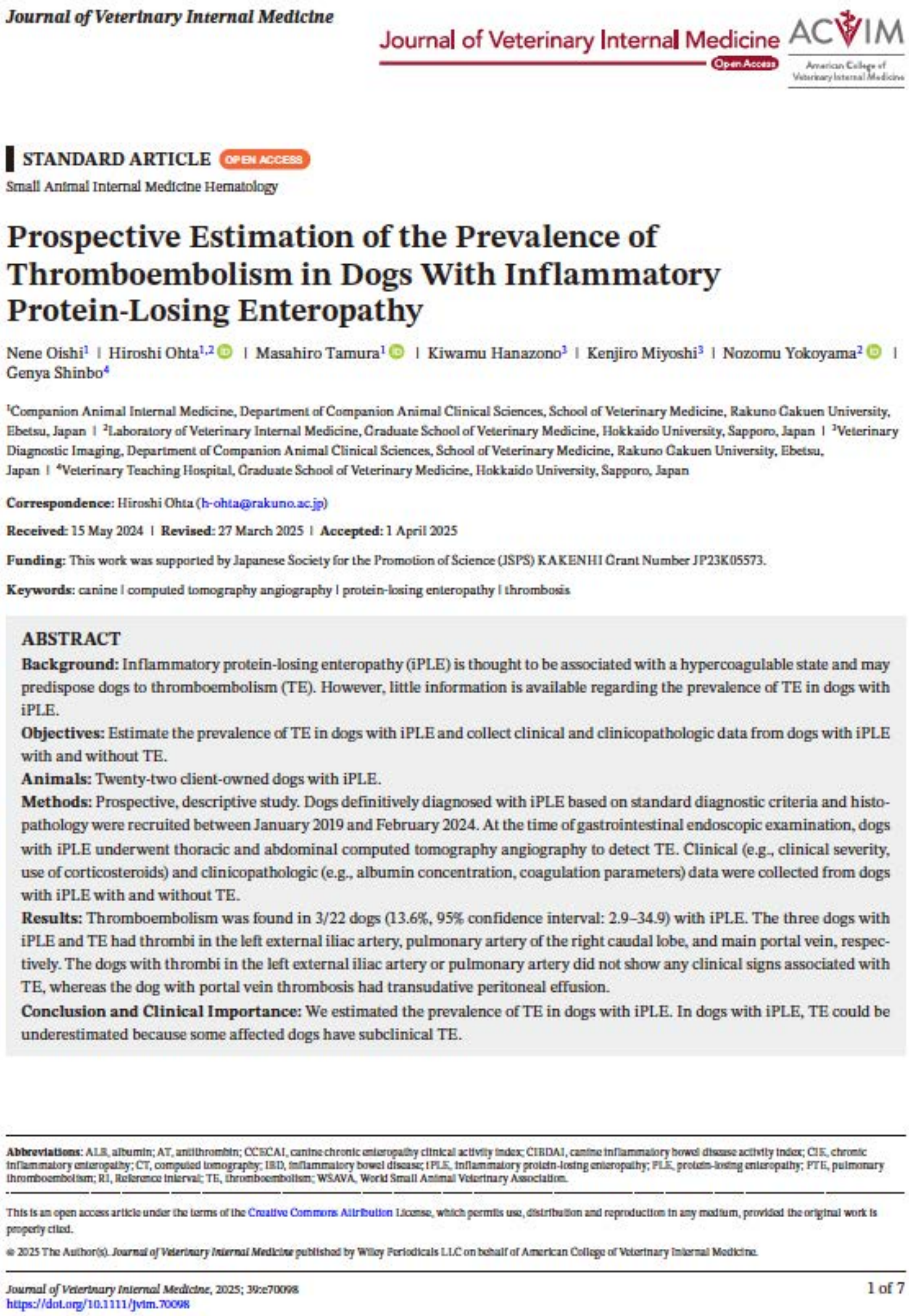

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STANDARD ARTICLE OPEN ACCESS

Small Animal Internal Medicine Hematology

Prospective Estimation of the Prevalence of Thromboembolism in Dogs With Inflammatory Protein-Losing Enteropathy

Nene Oishi[1] | Hiroshi Ohta[1,2] | Masahiro Tamura[1] | Kiwamu Hanazono[3] | Kenjiro Miyoshi[3] | Nozomu Yokoyama[2] | Genya Shinbo[4]

[1]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Laboratory of Veterinary Internal Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan | [3]Veterinary Diagnostic Imaging, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [4]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Correspondence: Hiroshi Ohta (h-ohta@rakuno.ac.jp)

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Keywords: canine | computed tomography angiography | protein-losing enteropathy | thrombosis

ABSTRACT

Background: Inflammatory protein-losing enteropathy (iPLE) is thought to be associated with a hypercoagulable state and may predispose dogs to thromboembolism (TE). However, little information is available regarding the prevalence of TE in dogs with iPLE.

Objectives: Estimate the prevalence of TE in dogs with iPLE and collect clinical and clinicopathologic data from dogs with iPLE with and without TE.

Animals: Twenty-two client-owned dogs with iPLE.

Methods: Prospective, descriptive study. Dogs definitively diagnosed with iPLE based on standard diagnostic criteria and histopathology were recruited between January 2019 and February 2024. At the time of gastrointestinal endoscopic examination, dogs with iPLE underwent thoracic and abdominal computed tomography angiography to detect TE. Clinical (e.g., clinical severity, use of corticosteroids) and clinicopathologic (e.g., albumin concentration, coagulation parameters) data were collected from dogs with iPLE with and without TE.

Results: Thromboembolism was found in 3/22 dogs (13.6%, 95% confidence interval: 2.9–34.9) with iPLE. The three dogs with iPLE and TE had thrombi in the left external iliac artery, pulmonary artery of the right caudal lobe, and main portal vein, respectively. The dogs with thrombi in the left external iliac artery or pulmonary artery did not show any clinical signs associated with TE, whereas the dog with portal vein thrombosis had transudative peritoneal effusion.

Conclusion and Clinical Importance: We estimated the prevalence of TE in dogs with iPLE. In dogs with iPLE, TE could be underestimated because some affected dogs have subclinical TE.

Abbreviations: ALB, albumin; AT, antithrombin; CCECAI, canine chronic enteropathy clinical activity index; CIBDAI, canine inflammatory bowel disease activity index; CIE, chronic inflammatory enteropathy; CT, computed tomography; IBD, inflammatory bowel disease; iPLE, inflammatory protein-losing enteropathy; PLE, protein-losing enteropathy; PTE, pulmonary thromboembolism; RI, Reference interval; TE, thromboembolism; WSAVA, World Small Animal Veterinary Association.

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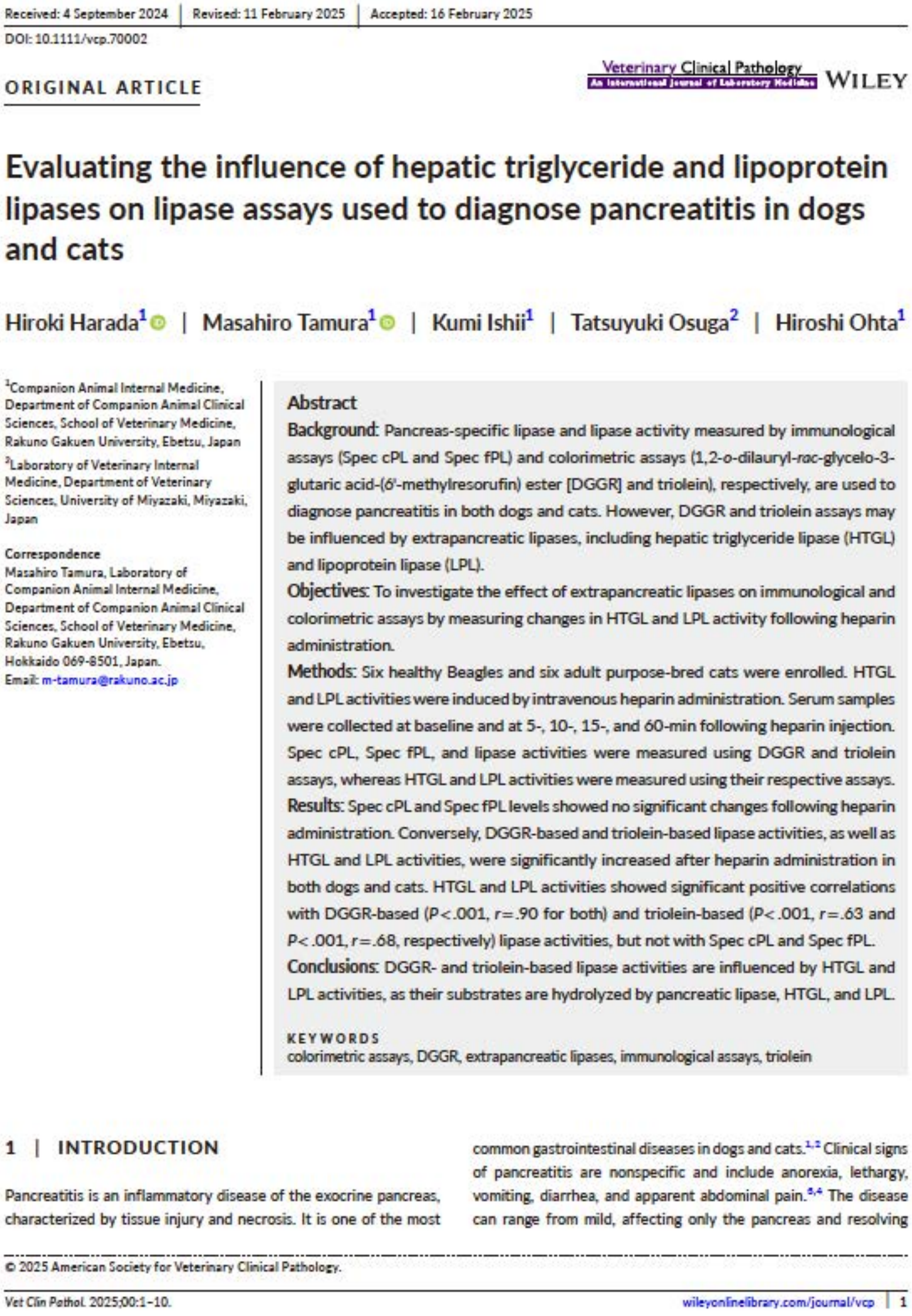

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Evaluating the influence of hepatic triglyceride and lipoprotein lipases on lipase assays used to diagnose pancreatitis in dogs and cats

Hiroki Harada[1] | Masahiro Tamura[1] | Kumi Ishii[1] | Tatsuyuki Osuga[2] | Hiroshi Ohta[1]

[1]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan
[2]Laboratory of Veterinary Internal Medicine, Department of Veterinary Sciences, University of Miyazaki, Miyazaki, Japan

Correspondence
Masahiro Tamura, Laboratory of Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan.
Email: m-tamura@rakuno.ac.jp

Abstract

Background: Pancreas-specific lipase and lipase activity measured by immunological assays (Spec cPL and Spec fPL) and colorimetric assays (1,2-*o*-dilauryl-*rac*-glycelo-3-glutaric acid-(6'-methylresorufin) ester [DGGR] and triolein), respectively, are used to diagnose pancreatitis in both dogs and cats. However, DGGR and triolein assays may be influenced by extrapancreatic lipases, including hepatic triglyceride lipase (HTGL) and lipoprotein lipase (LPL).

Objectives: To investigate the effect of extrapancreatic lipases on immunological and colorimetric assays by measuring changes in HTGL and LPL activity following heparin administration.

Methods: Six healthy Beagles and six adult purpose-bred cats were enrolled. HTGL and LPL activities were induced by intravenous heparin administration. Serum samples were collected at baseline and at 5-, 10-, 15-, and 60-min following heparin injection. Spec cPL, Spec fPL, and lipase activities were measured using DGGR and triolein assays, whereas HTGL and LPL activities were measured using their respective assays.

Results: Spec cPL and Spec fPL levels showed no significant changes following heparin administration. Conversely, DGGR-based and triolein-based lipase activities, as well as HTGL and LPL activities, were significantly increased after heparin administration in both dogs and cats. HTGL and LPL activities showed significant positive correlations with DGGR-based ($P < .001$, $r = .90$ for both) and triolein-based ($P < .001$, $r = .63$ and $P < .001$, $r = .68$, respectively) lipase activities, but not with Spec cPL and Spec fPL.

Conclusions: DGGR- and triolein-based lipase activities are influenced by HTGL and LPL activities, as their substrates are hydrolyzed by pancreatic lipase, HTGL, and LPL.

KEYWORDS
colorimetric assays, DGGR, extrapancreatic lipases, immunological assays, triolein

1 | INTRODUCTION

Pancreatitis is an inflammatory disease of the exocrine pancreas, characterized by tissue injury and necrosis. It is one of the most common gastrointestinal diseases in dogs and cats.[1,2] Clinical signs of pancreatitis are nonspecific and include anorexia, lethargy, vomiting, diarrhea, and apparent abdominal pain.[3,4] The disease can range from mild, affecting only the pancreas and resolving

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EDITED BY
Jiong Chen,
Ningbo University, China

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Marilia Takada,
University of Florida, United States
Marcos Daniel Mendes Padilha,
Federal University of Pará, Brazil

*CORRESPONDENCE
Satoru Konnai
✉ konnai@vetmed.hokudai.ac.jp

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Development of caninized anti-CTLA-4 antibody as salvage combination therapy for anti-PD-L1 refractory tumors in dogs

Naoya Maekawa[1,2], Satoru Konnai[1,2,3,4,5*], Kei Watari[3], Hiroto Takeuchi[3,4], Takeshi Nakanishi[6], Taro Tachibana[6], Kenji Hosoya[2,7], Sangho Kim[2,7], Ryohei Kinoshita[2,7], Ryo Owaki[7], Madoka Yokokawa[7], Yumiko Kagawa[8], Satoshi Takagi[9], Tatsuya Deguchi[10], Hiroshi Ohta[10], Yukinari Kato[11], Satoshi Yamamoto[1,12], Keiichi Yamamoto[1,4,12], Yasuhiko Suzuki[1,4,13,14], Tomohiro Okagawa[1], Shiro Murata[1,3,5] and Kazuhiko Ohashi[1,3,5,15]

[1]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [2]Cancer Research Unit, One Health Research Center, Hokkaido University, Sapporo, Japan, [3]Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [4]Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Sapporo, Japan, [5]Veterinary Research Unit, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, [6]Department of Chemistry and Bioengineering, Division of Science and Engineering for Materials, Chemistry and Biology, Graduate School of Engineering, Osaka Metropolitan University, Osaka, Japan, [7]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [8]North Lab, Sapporo, Japan, [9]Department of Veterinary Surgery 1, School of Veterinary Medicine, Azabu University, Sagamihara, Japan, [10]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, [11]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan, [12]Fuso Pharmaceutical Industries, Ltd., Osaka, Japan, [13]Division of Bioresources, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, [14]Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo, Japan, [15]International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Immune checkpoint inhibitors (ICIs) are widely used for cancer immunotherapy; however, the clinical efficacy of anti-PD-1/PD-L1 monotherapy is generally limited, highlighting the need to develop combination therapies. Dogs develop spontaneous tumors in immunocompetent settings, and anti-PD-1/PD-L1 antibodies exert similar clinical benefits. However, no clinically relevant anti-CTLA-4 antibody has been reported, limiting the value of canine tumors as comparative models for human ICI research. Here, canine CTLA-4 was molecularly characterized, and a caninized anti-CTLA-4 antibody (ca1C5) that blocks CTLA-4/ligand binding was developed. Treatment with ca1C5 increased cytokine production in canine immune cell cultures, and the immunostimulatory effect was enhanced when used in combination with the anti-PD-L1 antibody c4G12. As a proof-of-concept, a veterinary clinical study was conducted to demonstrate the safety and clinical efficacy of anti-CTLA-4 antibody as salvage combination therapy in dogs with advanced tumors refractory to prior c4G12 monotherapy. The combination treatment (c4G12 plus ca1C5) was well-

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Small Animal Internal Medicine Gastroenterology

Factors Affecting the Quality of Histopathologic Specimens Obtained via Small Intestinal Endoscopic Biopsy in Dogs and Cats

Ko Nakashima[1] | Kazuhiro Kojima[2] | Yoshinori Takeuchi[3] | Manami Ito[1] | Isao Matsumoto[1] | Takahiro Ushigusa[4] | Hiroshi Ohta[5] | Kazuyuki Uchida[2]

[1]Japan Small Animal Medical Center, Saitama, Japan | [2]Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan | [3]Department of Data Science, School of Data Science, Yokohama City University, Yokohama, Kanagawa, Japan | [4]Yokohama Animal Medical Center Kannai Animal Clinic, Kanagawa, Japan | [5]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

Correspondence: Ko Nakashima (endoscopist@jsamc.jp)

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Keywords: duodenum | filter paper-fixation | generalized estimating equations | histopathologic adequacy | ileum

ABSTRACT

Background: The factors affecting the quality of histopathologic specimens obtained via small intestinal endoscopic biopsy (SIEB) remain unclear.

Hypothesis/Objectives: To identify factors related to the quality of histopathologic specimens obtained via SIEB.

Animals: Histopathologic duodenal and ileal specimens were obtained from 116 dogs and 38 cats that underwent SIEB for diagnostic purposes.

Methods: This retrospective study analyzed 3354 individual histopathologic specimens scored using the grading system of histopathologic adequacy (GSHA). A lower GSHA score indicates lower quality specimens. Univariate and multivariate ordinal logistic models were used to assess the relationship between the GSHA score of the specimens and various explanatory factors, including fixation method, biopsy forceps size, biopsy site, and histopathologic diagnosis. The generalized estimating equation method was used to account for the clustering of specimens among animals.

Results: Multivariate models using the specimens showed that filter paper fixation of endoscopic samples resulted in a higher GSHA score than floating fixation in both dogs (ordinal odds ratio [OR]: 0.19; 95% confidence interval [CI]: 0.15–0.25) and cats (ordinal OR: 0.19; 95% CI: 0.13–0.29). In dogs, the scores were lower for duodenal specimens than for ileal specimens and for specimens obtained using smaller forceps. In cats, the scores were lower for ileal specimens than for duodenal specimens and for older animals.

Conclusion and Clinical Importance: The quality of histopathologic specimens obtained via SIEB is influenced by the fixation method. Additionally, other factors differ between dogs and cats. These results contribute to improved SIEB practices in veterinary medicine.

Abbreviations: CI, confidence interval; GEE, generalized estimating equations; GSHA, grading system of histopathologic adequacy; OR, odds ratio; SIEB, small intestinal endoscopic biopsy.

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最終責任者　Ko Nakashima (Corresponding Author)

ハードヘルス学（Veterinary Herd Health）

Shin Oikawa

Professor

教授　及川　伸

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1）Effects of amount of lactose in milk replacer on gastrointestinal function of dairy calves.
Fukumori R, Nakayama T, Hirose M, Norimura I, Izumi K, Shimada K, Mineo H, Steele MA, Gondaira S, Higuchi H, Watanabe T, Ueda H, Sano T, Chisato K, **Oikawa S.**
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2）Lipoprotein composition of calves before and after weaning and comparison with adult cows.
Satoh H, Kumano R, Fukumori R, **Oikawa S.**
J Vet Med Sci. 87:167-170. 2025. doi.org/10.1292/jvms.24-0172

3）Effects of ruminal administration of propylene glycol or sucrose on ruminal, blood, and hepatic parameters in nonlactating cows with high plasma nonesterified fatty acid concentrations.
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6）Significance of serum pepsinogen value in close-up dairy cattle as a displaced abomasum predictive marker: a case-control study.
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1）Production diseases in farm animals: a comprehensive and illustrated clinical, laboratory, and pathological overview.
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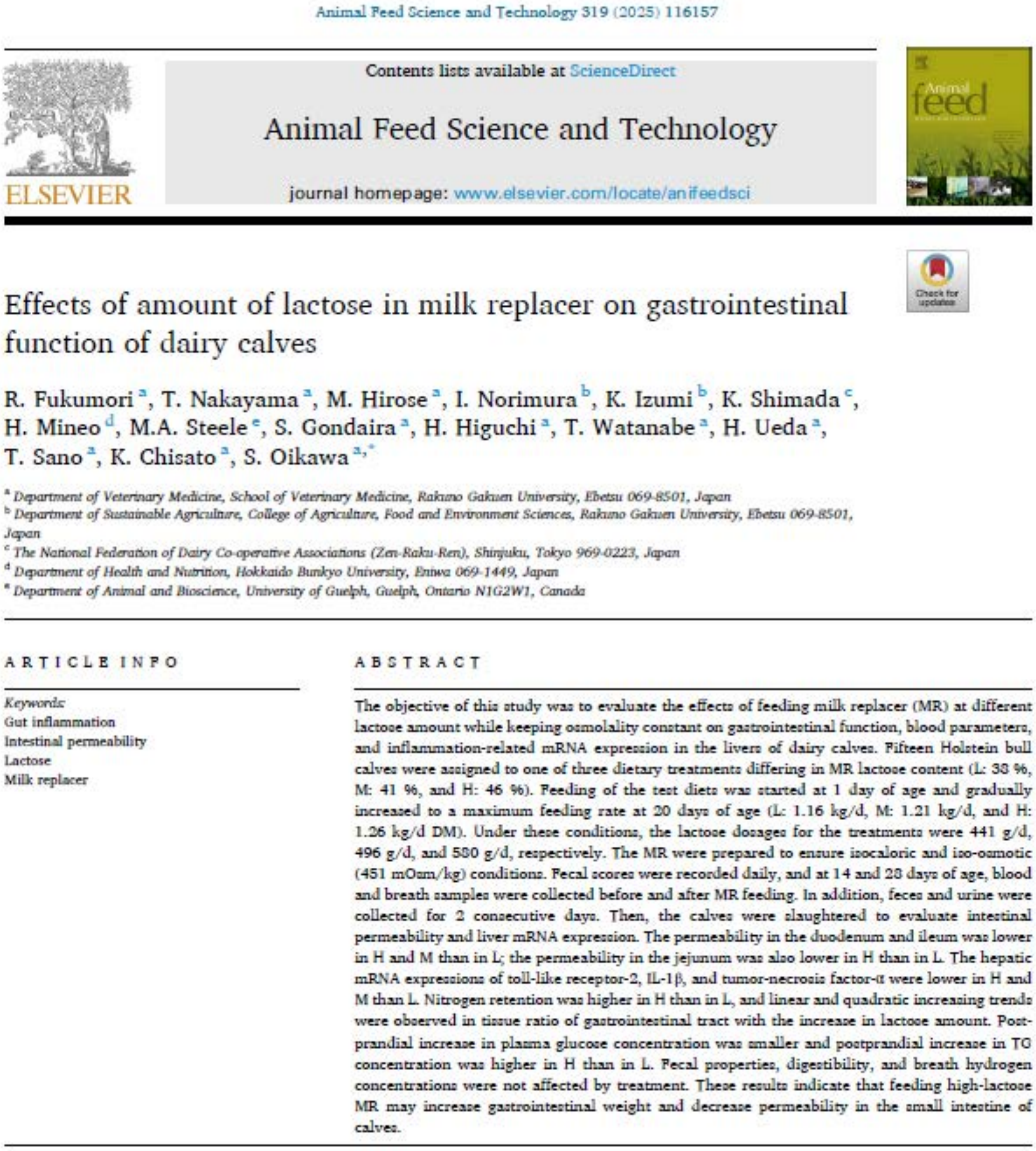

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Effects of amount of lactose in milk replacer on gastrointestinal function of dairy calves

R. Fukumori [a], T. Nakayama [a], M. Hirose [a], I. Norimura [b], K. Izumi [b], K. Shimada [c], H. Mineo [d], M.A. Steele [e], S. Gondaira [a], H. Higuchi [a], T. Watanabe [a], H. Ueda [a], T. Sano [a], K. Chisato [a], S. Oikawa [a,*]

[a] *Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[b] *Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[c] *The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Shinjuku, Tokyo 969-0223, Japan*
[d] *Department of Health and Nutrition, Hokkaido Bunkyo University, Eniwa 069-1449, Japan*
[e] *Department of Animal and Bioscience, University of Guelph, Guelph, Ontario N1G2W1, Canada*

ARTICLE INFO

Keywords:
Gut inflammation
Intestinal permeability
Lactose
Milk replacer

ABSTRACT

The objective of this study was to evaluate the effects of feeding milk replacer (MR) at different lactose amount while keeping osmolality constant on gastrointestinal function, blood parameters, and inflammation-related mRNA expression in the livers of dairy calves. Fifteen Holstein bull calves were assigned to one of three dietary treatments differing in MR lactose content (L: 38 %, M: 41 %, and H: 46 %). Feeding of the test diets was started at 1 day of age and gradually increased to a maximum feeding rate at 20 days of age (L: 1.16 kg/d, M: 1.21 kg/d, and H: 1.26 kg/d DM). Under these conditions, the lactose dosages for the treatments were 441 g/d, 496 g/d, and 580 g/d, respectively. The MR were prepared to ensure isocaloric and iso-osmotic (451 mOsm/kg) conditions. Fecal scores were recorded daily, and at 14 and 28 days of age, blood and breath samples were collected before and after MR feeding. In addition, feces and urine were collected for 2 consecutive days. Then, the calves were slaughtered to evaluate intestinal permeability and liver mRNA expression. The permeability in the duodenum and ileum was lower in H and M than in L; the permeability in the jejunum was also lower in H than in L. The hepatic mRNA expressions of toll-like receptor-2, IL-1β, and tumor-necrosis factor-α were lower in H and M than L. Nitrogen retention was higher in H than in L, and linear and quadratic increasing trends were observed in tissue ratio of gastrointestinal tract with the increase in lactose amount. Postprandial increase in plasma glucose concentration was smaller and postprandial increase in TG concentration was higher in H than in L. Fecal properties, digestibility, and breath hydrogen concentrations were not affected by treatment. These results indicate that feeding high-lactose MR may increase gastrointestinal weight and decrease permeability in the small intestine of calves.

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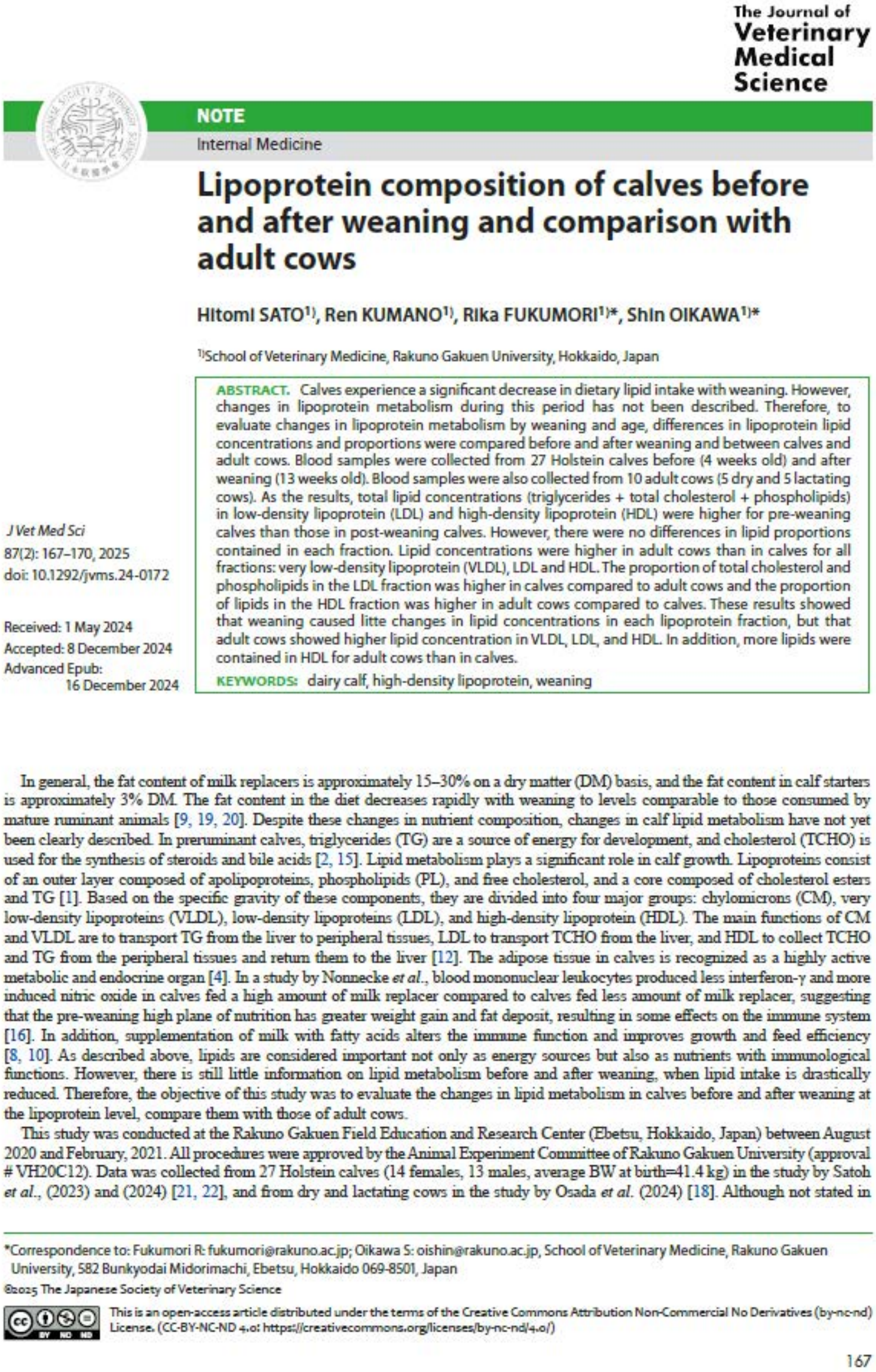

The Journal of
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NOTE
Internal Medicine

Lipoprotein composition of calves before and after weaning and comparison with adult cows

Hitomi SATO[1)], Ren KUMANO[1)], Rika FUKUMORI[1)]*, Shin OIKAWA[1)]*

[1)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. Calves experience a significant decrease in dietary lipid intake with weaning. However, changes in lipoprotein metabolism during this period has not been described. Therefore, to evaluate changes in lipoprotein metabolism by weaning and age, differences in lipoprotein lipid concentrations and proportions were compared before and after weaning and between calves and adult cows. Blood samples were collected from 27 Holstein calves before (4 weeks old) and after weaning (13 weeks old). Blood samples were also collected from 10 adult cows (5 dry and 5 lactating cows). As the results, total lipid concentrations (triglycerides + total cholesterol + phospholipids) in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were higher for pre-weaning calves than those in post-weaning calves. However, there were no differences in lipid proportions contained in each fraction. Lipid concentrations were higher in adult cows than in calves for all fractions: very low-density lipoprotein (VLDL), LDL and HDL. The proportion of total cholesterol and phospholipids in the LDL fraction was higher in calves compared to adult cows and the proportion of lipids in the HDL fraction was higher in adult cows compared to calves. These results showed that weaning caused litte changes in lipid concentrations in each lipoprotein fraction, but that adult cows showed higher lipid concentration in VLDL, LDL, and HDL. In addition, more lipids were contained in HDL for adult cows than in calves.

KEYWORDS: dairy calf, high-density lipoprotein, weaning

In general, the fat content of milk replacers is approximately 15–30% on a dry matter (DM) basis, and the fat content in calf starters is approximately 3% DM. The fat content in the diet decreases rapidly with weaning to levels comparable to those consumed by mature ruminant animals [9, 19, 20]. Despite these changes in nutrient composition, changes in calf lipid metabolism have not yet been clearly described. In preruminant calves, triglycerides (TG) are a source of energy for development, and cholesterol (TCHO) is used for the synthesis of steroids and bile acids [2, 15]. Lipid metabolism plays a significant role in calf growth. Lipoproteins consist of an outer layer composed of apolipoproteins, phospholipids (PL), and free cholesterol, and a core composed of cholesterol esters and TG [1]. Based on the specific gravity of these components, they are divided into four major groups: chylomicrons (CM), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoprotein (HDL). The main functions of CM and VLDL are to transport TG from the liver to peripheral tissues, LDL to transport TCHO from the liver, and HDL to collect TCHO and TG from the peripheral tissues and return them to the liver [12]. The adipose tissue in calves is recognized as a highly active metabolic and endocrine organ [4]. In a study by Nonnecke *et al.*, blood mononuclear leukocytes produced less interferon-γ and more induced nitric oxide in calves fed a high amount of milk replacer compared to calves fed less amount of milk replacer, suggesting that the pre-weaning high plane of nutrition has greater weight gain and fat deposit, resulting in some effects on the immune system [16]. In addition, supplementation of milk with fatty acids alters the immune function and improves growth and feed efficiency [8, 10]. As described above, lipids are considered important not only as energy sources but also as nutrients with immunological functions. However, there is still little information on lipid metabolism before and after weaning, when lipid intake is drastically reduced. Therefore, the objective of this study was to evaluate the changes in lipid metabolism in calves before and after weaning at the lipoprotein level, compare them with those of adult cows.

This study was conducted at the Rakuno Gakuen Field Education and Research Center (Ebetsu, Hokkaido, Japan) between August 2020 and February, 2021. All procedures were approved by the Animal Experiment Committee of Rakuno Gakuen University (approval # VH20C12). Data was collected from 27 Holstein calves (14 females, 13 males, average BW at birth=41.4 kg) in the study by Satoh *et al.*, (2023) and (2024) [21, 22], and from dry and lactating cows in the study by Osada *et al.* (2024) [18]. Although not stated in

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*Correspondence to: Fukumori R: fukumori@rakuno.ac.jp; Oikawa S: oishin@rakuno.ac.jp, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

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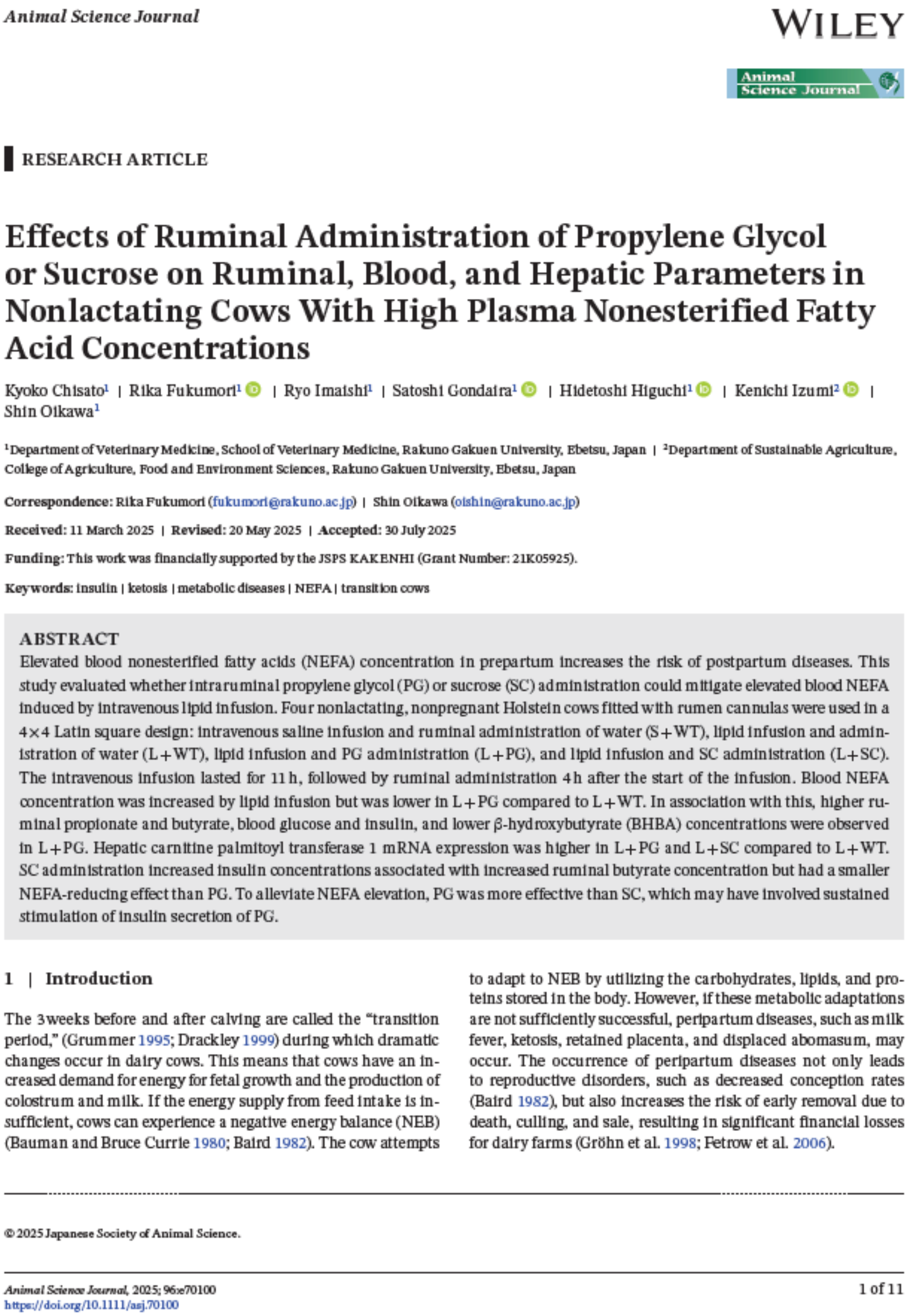

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RESEARCH ARTICLE

Effects of Ruminal Administration of Propylene Glycol or Sucrose on Ruminal, Blood, and Hepatic Parameters in Nonlactating Cows With High Plasma Nonesterified Fatty Acid Concentrations

Kyoko Chisato[1] | Rika Fukumori[1] | Ryo Imaishi[1] | Satoshi Gondaira[1] | Hidetoshi Higuchi[1] | Kenichi Izumi[2] | Shin Oikawa[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu, Japan

Correspondence: Rika Fukumori (fukumori@rakuno.ac.jp) | Shin Oikawa (oishin@rakuno.ac.jp)

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Keywords: insulin | ketosis | metabolic diseases | NEFA | transition cows

ABSTRACT

Elevated blood nonesterified fatty acids (NEFA) concentration in prepartum increases the risk of postpartum diseases. This study evaluated whether intraruminal propylene glycol (PG) or sucrose (SC) administration could mitigate elevated blood NEFA induced by intravenous lipid infusion. Four nonlactating, nonpregnant Holstein cows fitted with rumen cannulas were used in a 4×4 Latin square design: intravenous saline infusion and ruminal administration of water (S+WT), lipid infusion and administration of water (L+WT), lipid infusion and PG administration (L+PG), and lipid infusion and SC administration (L+SC). The intravenous infusion lasted for 11 h, followed by ruminal administration 4 h after the start of the infusion. Blood NEFA concentration was increased by lipid infusion but was lower in L+PG compared to L+WT. In association with this, higher ruminal propionate and butyrate, blood glucose and insulin, and lower β-hydroxybutyrate (BHBA) concentrations were observed in L+PG. Hepatic carnitine palmitoyl transferase 1 mRNA expression was higher in L+PG and L+SC compared to L+WT. SC administration increased insulin concentrations associated with increased ruminal butyrate concentration but had a smaller NEFA-reducing effect than PG. To alleviate NEFA elevation, PG was more effective than SC, which may have involved sustained stimulation of insulin secretion of PG.

1 | Introduction

The 3 weeks before and after calving are called the "transition period," (Grummer 1995; Drackley 1999) during which dramatic changes occur in dairy cows. This means that cows have an increased demand for energy for fetal growth and the production of colostrum and milk. If the energy supply from feed intake is insufficient, cows can experience a negative energy balance (NEB) (Bauman and Bruce Currie 1980; Baird 1982). The cow attempts to adapt to NEB by utilizing the carbohydrates, lipids, and proteins stored in the body. However, if these metabolic adaptations are not sufficiently successful, peripartum diseases, such as milk fever, ketosis, retained placenta, and displaced abomasum, may occur. The occurrence of peripartum diseases not only leads to reproductive disorders, such as decreased conception rates (Baird 1982), but also increases the risk of early removal due to death, culling, and sale, resulting in significant financial losses for dairy farms (Gröhn et al. 1998; Fetrow et al. 2006).

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Article

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Effects of bypass sugar supplementation from the close-up period through 5 days after calving on milk production, blood profiles, and health conditions in dairy cows

Hitomi Satoh[1], Rika Fukumori[1,*], Moeri Kondo[2], Junna Morimoto[2], Kentaro Abe[2], Shuichi Iki[2], Hideaki Hayashi[1], Hidetoshi Hayashi[1], Kyoko Chisato[1], Shin Oikawa[1]

[1]Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[2]Snow Brand Seed Co., LTD., Naganuma, Hokkaido, Japan

Correspondence: Rika Fukumori, Tel: +81-11-388-4814, Fax: +81-11-388-4814, Email: fukumori@rakuno.ac.jp

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Abstract

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animals

MDPI

Article

Evaluation of Colostrum Components and Milking Status Affecting Colostrum IgG Concentration

Shuji Kayasaki [1,2], Hitomi Satoh [2], Keitaro Oguchi [2], Kyoko Chisato [2], Rika Fukumori [2] and Shin Oikawa [2,*]

[1] Nijibetsu Livestock Clinic, Kushiro Central Branch, East Regional Center, Hokkaido Agricultural Mutual Aid Association, 103-1, Line 67, Nijibetsu Genya, Shibecha-cho 088-2461, Hokkaido, Japan; s22041006@g.rakuno.ac.jp
[2] Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu-shi 069-8501, Hokkaido, Japan; s22241004@stu.rakuno.ac.jp (H.S.); s21761048@g.rakuno.ac.jp (K.O.); k-chisato@rakuno.ac.jp (K.C.); fukumori@rakuno.ac.jp (R.F.)
* Correspondence: oishin@rakuno.ac.jp; Tel.: +81-11-388-4825

Simple Summary: The objective of this study was to examine the factors that contribute to high-quality colostrum in dairy farms in eastern Hokkaido, Japan. The percentage of samples with a high colostrum IgG concentration, an indicator of passive transfer ($\geq$50 g/L), was low, at 48.9%, but the percentage of those with a low total plate count, an indicator of bacterial contamination (<100,000 CFU/mL), was high, at 86.5%. Measurement of colostrum Brix value, a measure of solid component concentration, provided a practical estimate of colostrum IgG concentration. The criteria for obtaining colostrum with high IgG concentration were high parity, low milking volume, and a short time from calving to milking. The estimation of colostrum IgG concentration by Brix meter and the approaching of ideal milking status were thought to lead to the obtaining of high-IgG colostrum.

Abstract: This study investigated IgG concentrations, bacterial contamination, and nutrient components of colostrum on dairy farms in eastern Hokkaido, Japan, to examine the factors making it possible to obtain high-quality colostrum. Colostrum samples were collected from 266 Holstein cows. The general criteria for high-quality colostrum are an IgG concentration of 50 g/L or higher and a total plate count of less than 100,000 CFU/mL. The percentage of samples with the criteria for IgG concentration was low, at 48.9%, but the percentage with the criteria for total plate count was high, at 86.5%. The colostrum Brix value was significantly correlated with the colostrum IgG concentration ($r^2 = 0.233$, $p < 0.001$) and provided a rough estimate of that concentration. There was no association between the colostrum IgG concentration and milk components other than protein. For colostrum milking conditions, the time from calving to milking tended to be shorter for high-IgG colostrum than for low-IgG colostrum ($p < 0.01$). The likelihood of high-IgG colostrum increased 1.28-fold with first parity higher and decreased 0.92-fold with a 1 L higher milking volume ($p < 0.01$). The results suggest that, in addition to estimating the colostrum IgG concentration via the Brix value, the likelihood of obtaining high-IgG colostrum is increased by taking into account parity, milking volume, and time from calving to milking.

Keywords: cow; colostrum; immunoglobulin G (IgG); bacteria; Brix; milking status

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1. Introduction

In calf management, the health implications of colostrum feeding are highlighted. Due to their anatomical structure, newborn calves have no transfer of antibodies via

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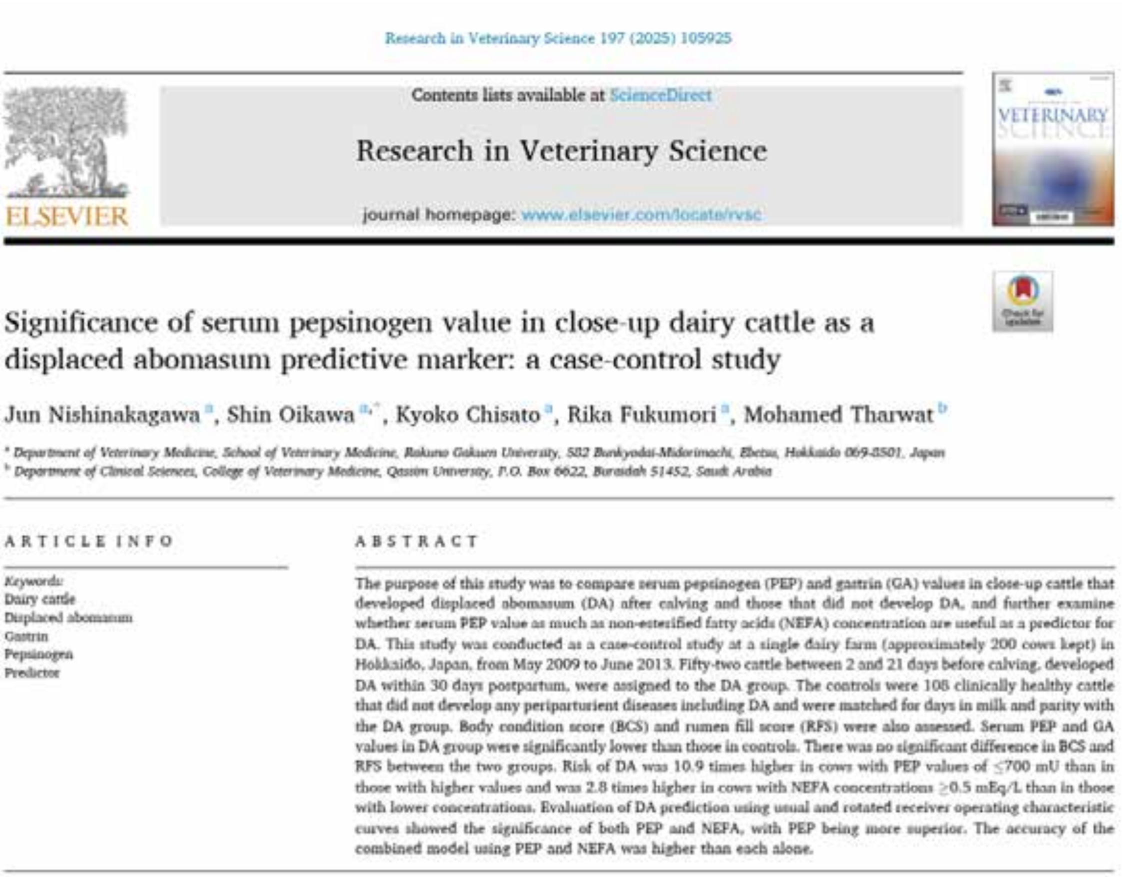

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Significance of serum pepsinogen value in close-up dairy cattle as a displaced abomasum predictive marker: a case-control study

Jun Nishinakagawa[a], Shin Oikawa[a,*], Kyoko Chisato[a], Rika Fukumori[a], Mohamed Tharwat[b]

[a] *Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan*
[b] *Department of Clinical Sciences, College of Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah 51452, Saudi Arabia*

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Keywords:
Dairy cattle
Displaced abomasum
Gastrin
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Predictor

ABSTRACT

The purpose of this study was to compare serum pepsinogen (PEP) and gastrin (GA) values in close-up cattle that developed displaced abomasum (DA) after calving and those that did not develop DA, and further examine whether serum PEP value as much as non-esterified fatty acids (NEFA) concentration are useful as a predictor for DA. This study was conducted as a case-control study at a single dairy farm (approximately 200 cows kept) in Hokkaido, Japan, from May 2009 to June 2013. Fifty-two cattle between 2 and 21 days before calving, developed DA within 30 days postpartum, were assigned to the DA group. The controls were 108 clinically healthy cattle that did not develop any periparturient diseases including DA and were matched for days in milk and parity with the DA group. Body condition score (BCS) and rumen fill score (RFS) were also assessed. Serum PEP and GA values in DA group were significantly lower than those in controls. There was no significant difference in BCS and RFS between the two groups. Risk of DA was 10.9 times higher in cows with PEP values of ≤700 mU than in those with higher values and was 2.8 times higher in cows with NEFA concentrations ≥0.5 mEq/L than in those with lower concentrations. Evaluation of DA prediction using usual and rotated receiver operating characteristic curves showed the significance of both PEP and NEFA, with PEP being more superior. The accuracy of the combined model using PEP and NEFA was higher than each alone.

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animals

MDPI

Article

Effects of Preventive Administration of Propylene Glycol or Sucrose in Dairy Cows with Elevated Blood Non-Esterified Fatty Acids During the Close-Up Period

Kyoko Chisato , Miki Ishizaka, Takumi Honjo, Yuta Watanabe, Rika Fukumori and Shin Oikawa *

Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan; k-chisato@rakuno.ac.jp (K.C.); fukumori@rakuno.ac.jp (R.F.)
* Correspondence: oishin@rakuno.ac.jp; Tel.: +81-11-388-4825

Simple Summary

The objective of this study was to evaluate the effects of administration of propylene glycol (PG) or sucrose (SC) on health and production outcomes in dairy cows with elevated non-esterified fatty acids (NEFA) levels of 0.3 mEq/L or higher during the close-up period. Thirty-five cows from two farms in Hokkaido were assigned to PG, SC, or untreated control groups, with treatments administered for 5 days starting from the blood testing. In PG and SC cows, blood profiles related to energy metabolism, including NEFA and β-hydroxybutyrate concentrations, improved after calving compared with controls, and liver function was maintained as well. Cows in both treatment groups exhibited significant decreases in postpartum culling rates. These findings suggest that prophylactic administration of PG or SC may contribute to postpartum productivity.

Abstract

The purpose of this study was to evaluate the preventive effects of propylene glycol (PG) or sucrose (SC) in dairy cows with high levels of non-esterified fatty acids (NEFAs) during the close-up period. From July 2021 to August 2022, blood samples were collected from 193 cows between 14 and 7 days prior to the expected calving date in two farms, and 35 multiparous cows with serum NEFA ≥ 0.3 mEq/L were randomly assigned to PG (500 mL/day, $n = 11$), SC (1000 mL/day of 50% solution, $n = 11$), and untreated control (HC; $n = 13$) groups. Treatments were administered orally for 5 consecutive days. Compared with HC cows, the serum NEFA concentration tended to be lower in SC cows at 3 days in milk (DIM) and was significantly lower in PG cows at 14 DIM. Serum β-hydroxybutyrate concentrations tended to be lower in SC cows at 21 DIM. Blood glucose concentrations were higher in both treatment groups at 3 DIM, and the serum total bilirubin concentration remained lower until 14 DIM in PG cows and until 7 DIM in SC cows. At 7 DIM, PG cows showed significantly higher total very low-density lipoprotein levels and PG and SC cows had significantly or tendentially higher low-density lipoprotein triglyceride concentrations. Cows in both treatment groups had significantly reduced culling after calving. These results suggest that prophylactic administration of PG or SC improves energy metabolism by supporting liver function, thereby reducing postpartum culling, with the PG group showing a more consistent effect.

Keywords: dairy cow; close-up period; propylene glycol; sucrose; prophylactic administration

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Review Article
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Production diseases in farm animals: A comprehensive and illustrated clinical, laboratory, and pathological overview

Mohamed Tharwat[1]*, Abdulrahman A. Alkheraif[2] and Shin Oikawa[3]

[1]*Department of Clinical Sciences, College of Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia*
[2]*Department of Pathology and Laboratory Diagnosis, College of Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia*
[3]*Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*

ABSTRACT

The rising demand of the rapidly growing global population for animal-derived foods and other products requires intense animal farming. However, the husbandry and breeding of livestock are associated with a conflict between the economic requirements of producers and the biological needs of the animals. This conflict is rapidly gaining recognition not only by veterinarians, animal scientists, and producers but also by the general public. Any defect in animal feeding, housing, or breeding strategy may trigger the development of production diseases (PDs), leading to decreased producer income, reduced consumer product quality, and impaired animal welfare. Because of the need for high production during the past decades, several animals have been subjected to intense genetic selection, thus increasing animal productivity to a standard where the requirement for nutrients from the ration and internal tissue stores has greatly increased. Therefore, the inability to address the high metabolic needs of increased production is also elevated. In farm animals, high production is greatly challenged by various risk factors, such as improper nutrition, infection, stress, and housing systems. Thus, new data concerning understanding the physiology, detection, treatment, and prevention of PD are urgently required. This review highlights the most important PDs that may influence different livestock species, including cows, sheep, camels, and goats. Recently, PD has been widened to include other conditions, such as fatty infiltration of the liver, sub-acute ruminal acidosis, hepatic abscess, caudal vena cava thrombosis, endocarditis, abomasal ulcerations, displacement of the abomasum, pregnancy toxemia, and mastitis. Because many PDs emerge during the transition period, the first section of this review focuses on a series of physiological events that occur during this period. Following, different partial discharges including will be discussed.

Keywords: Animals, Diagnosis, Diseases, Pathology, Production.

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獣医臨床病理学(Veterinary Clinicopathology)

Kazuyuki Suzuki

Professor

教授　鈴木　一由

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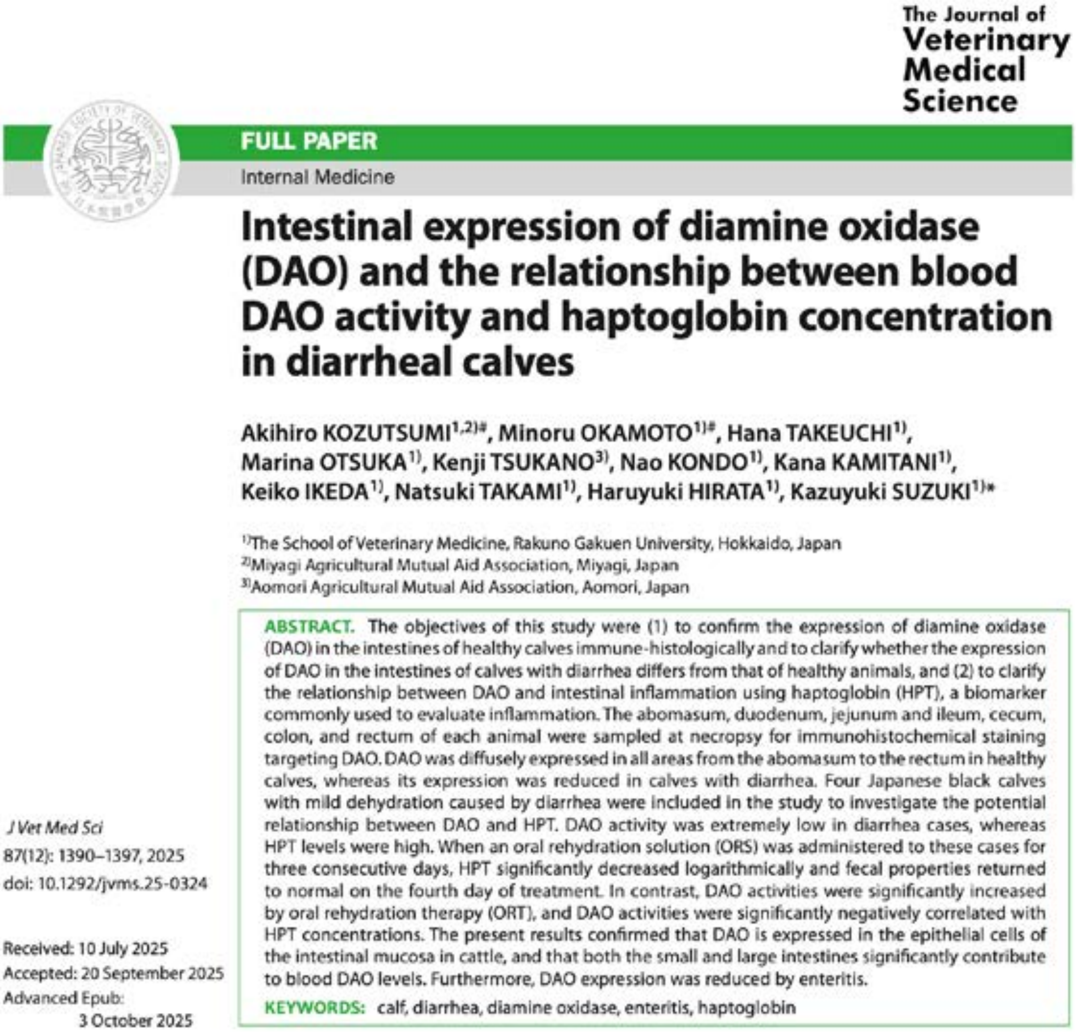
3) Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (Choloepus didactylus).
Murakami M, Iwasa A, Okamoto M, Ihizaki T, **Suzuki K**, Hirata H.
J Vet Med Sci. 87:972-975. 2025doi: 10.1292/jvms.25-0063.



The Journal of
Veterinary
Medical
Science

FULL PAPER
Internal Medicine

Intestinal expression of diamine oxidase (DAO) and the relationship between blood DAO activity and haptoglobin concentration in diarrheal calves

Akihiro KOZUTSUMI[1,2)#], Minoru OKAMOTO[1)#], Hana TAKEUCHI[1)], Marina OTSUKA[1)], Kenji TSUKANO[3)], Nao KONDO[1)], Kana KAMITANI[1)], Keiko IKEDA[1)], Natsuki TAKAMI[1)], Haruyuki HIRATA[1)], Kazuyuki SUZUKI[1)*]

[1)]The School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]Miyagi Agricultural Mutual Aid Association, Miyagi, Japan
[3)]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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ABSTRACT. The objectives of this study were (1) to confirm the expression of diamine oxidase (DAO) in the intestines of healthy calves immune-histologically and to clarify whether the expression of DAO in the intestines of calves with diarrhea differs from that of healthy animals, and (2) to clarify the relationship between DAO and intestinal inflammation using haptoglobin (HPT), a biomarker commonly used to evaluate inflammation. The abomasum, duodenum, jejunum and ileum, cecum, colon, and rectum of each animal were sampled at necropsy for immunohistochemical staining targeting DAO. DAO was diffusely expressed in all areas from the abomasum to the rectum in healthy calves, whereas its expression was reduced in calves with diarrhea. Four Japanese black calves with mild dehydration caused by diarrhea were included in the study to investigate the potential relationship between DAO and HPT. DAO activity was extremely low in diarrhea cases, whereas HPT levels were high. When an oral rehydration solution (ORS) was administered to these cases for three consecutive days, HPT significantly decreased logarithmically and fecal properties returned to normal on the fourth day of treatment. In contrast, DAO activities were significantly increased by oral rehydration therapy (ORT), and DAO activities were significantly negatively correlated with HPT concentrations. The present results confirmed that DAO is expressed in the epithelial cells of the intestinal mucosa in cattle, and that both the small and large intestines significantly contribute to blood DAO levels. Furthermore, DAO expression was reduced by enteritis.

KEYWORDS: calf, diarrhea, diamine oxidase, enteritis, haptoglobin

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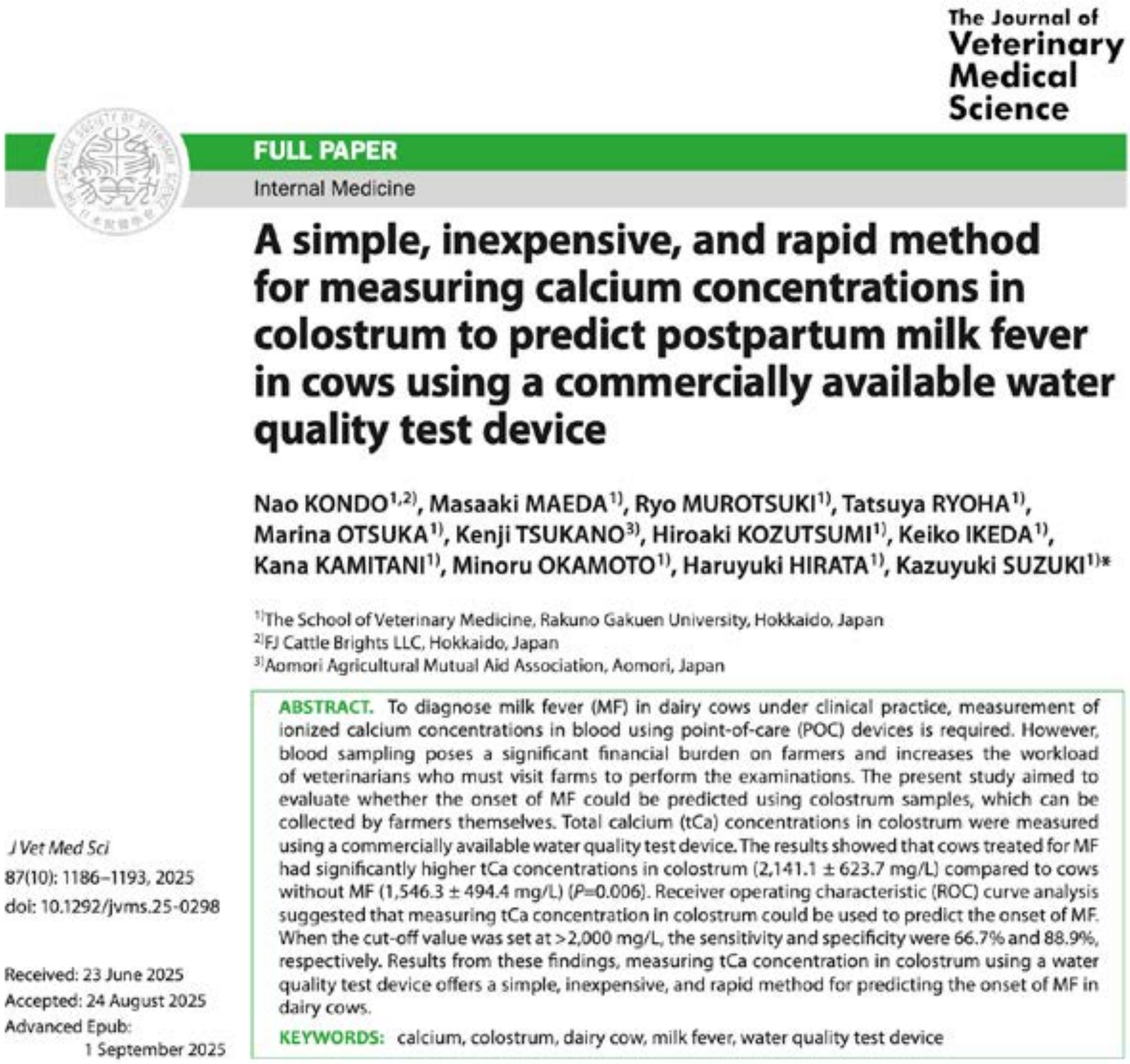
最終責任者　Kazuyuki Suzuki (Corresponding Author)



The Journal of
Veterinary
Medical
Science

FULL PAPER

Internal Medicine

A simple, inexpensive, and rapid method for measuring calcium concentrations in colostrum to predict postpartum milk fever in cows using a commercially available water quality test device

Nao KONDO[1,2], Masaaki MAEDA[1], Ryo MUROTSUKI[1], Tatsuya RYOHA[1], Marina OTSUKA[1], Kenji TSUKANO[3], Hiroaki KOZUTSUMI[1], Keiko IKEDA[1], Kana KAMITANI[1], Minoru OKAMOTO[1], Haruyuki HIRATA[1], Kazuyuki SUZUKI[1]*

[1]The School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]FJ Cattle Brights LLC, Hokkaido, Japan
[3]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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ABSTRACT. To diagnose milk fever (MF) in dairy cows under clinical practice, measurement of ionized calcium concentrations in blood using point-of-care (POC) devices is required. However, blood sampling poses a significant financial burden on farmers and increases the workload of veterinarians who must visit farms to perform the examinations. The present study aimed to evaluate whether the onset of MF could be predicted using colostrum samples, which can be collected by farmers themselves. Total calcium (tCa) concentrations in colostrum were measured using a commercially available water quality test device. The results showed that cows treated for MF had significantly higher tCa concentrations in colostrum (2,141.1 ± 623.7 mg/L) compared to cows without MF (1,546.3 ± 494.4 mg/L) (P=0.006). Receiver operating characteristic (ROC) curve analysis suggested that measuring tCa concentration in colostrum could be used to predict the onset of MF. When the cut-off value was set at >2,000 mg/L, the sensitivity and specificity were 66.7% and 88.9%, respectively. Results from these findings, measuring tCa concentration in colostrum using a water quality test device offers a simple, inexpensive, and rapid method for predicting the onset of MF in dairy cows.

KEYWORDS: calcium, colostrum, dairy cow, milk fever, water quality test device

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Validation of point-of-care devices measuring calcium (iCa), potassium (K$^+$), and sodium (Na$^+$) concentrations in whole blood of cattle and horses using general-purpose water quality testing equipment

Nao Kondo[1], Ayana Iwasa[1], Minoru Okamoto[1], Kenji Tsukano[2], Haruyuki Hirata[1],
Kazuyuki Suzuki[1]

[1]Rakuno Gakuen University, The Graduate School of Veterinary Medicine, Bunnkyoudai, Ebetsu, Hokkaido, Japan
[2]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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Abstract

To clarify whether commercially available handheld iCa, K$^+$, and Na$^+$ devices may be used clinically, precise and accurate evaluations were performed using the handheld analyser i-STAT 1 as the standard device. Point-paired whole blood samples were obtained from 45 cattle and 19 horses. Data obtained using LAQUAtwin Ca-11C, K-11, and Na-11 correlated with those using i-STAT 1. LAQUAtwin devices were 'compatible' with i-STAT 1 because the frequency of differences between measurements within ± 20% of the mean was 95.5% in cattle and 94.7% in horses for iCa, 92.7% in cattle and 92.9% in horses for K$^+$, and 100% in both for Na$^+$. No proportional bias was observed between i-STAT 1 and LAQUAtwin Ca-11C and K-11 because the 95% CI for the intercept and slope were 0 and 1, respectively. LAQUAtwin Na-11 showed a good correlation with i-STAT 1 measurements of Na$^+$ concentrations in the whole blood of cattle and horses; however, measured values were approximately 4 mM lower with the former. Therefore, the LAQUAtwin series may be applied as a simplified system for measuring iCa, K$^+$, and Na$^+$ concentrations in the whole blood of cattle and horses under field conditions; however, caution is required because Na$^+$ values were lower than those with i-STAT 1.

Bovine, electrolyte, equine

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1828
AKADÉMIAI KIADÓ

Seasonal dynamics of *Ostertagia ostertagi* infection in lactating Holstein-Friesian cows on a dairy farm in Hokkaido, Japan

KEIKO IKEDA[1], YASUHITO ASAKURA[2], SHINICHIRO FUKUMOTO[1], KAZUYUKI SUZUKI[1] and HARUYUKI HIRATA[1]*

[1] The Graduate School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[2] Rumoi Hokubu Veterinary Clinic, Hokkaido Chuo Agricultural Mutual Relief Association, Teshio, Hokkaido, Japan

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RESEARCH ARTICLE

ABSTRACT

Gastrointestinal nematodes (GINs), such as *Ostertagia ostertagi* and *Cooperia* spp., are common in cattle worldwide. Although infected cattle are often asymptomatic, production is negatively affected. The present study investigated whether eggs per gram (EPG) of GINs in 32 lactating cows decreased during winter and increased as it became warmer. Larvae recovered by coprocultures mostly consisted of *O. ostertagi* in autumn, while three GINs, *Ostertagia*, *Trichostrongylus* and *Meistocirrus*, were collected in spring. Daily milk and bulk tank milk samples from cows previously infected with *O. ostertagi* contained *O. ostertagi* antibodies based on ELISA developed using SVANOVIER® *O. ostertagi*-Ab in all survey periods. Even if the faecal diagnosis was negative for GINs, infection was still possible. Therefore, the administration of anthelmintic drugs is recommended.

KEYWORDS

Gastrointestinal nematodes, *Ostertagia ostertagi*, ELISA

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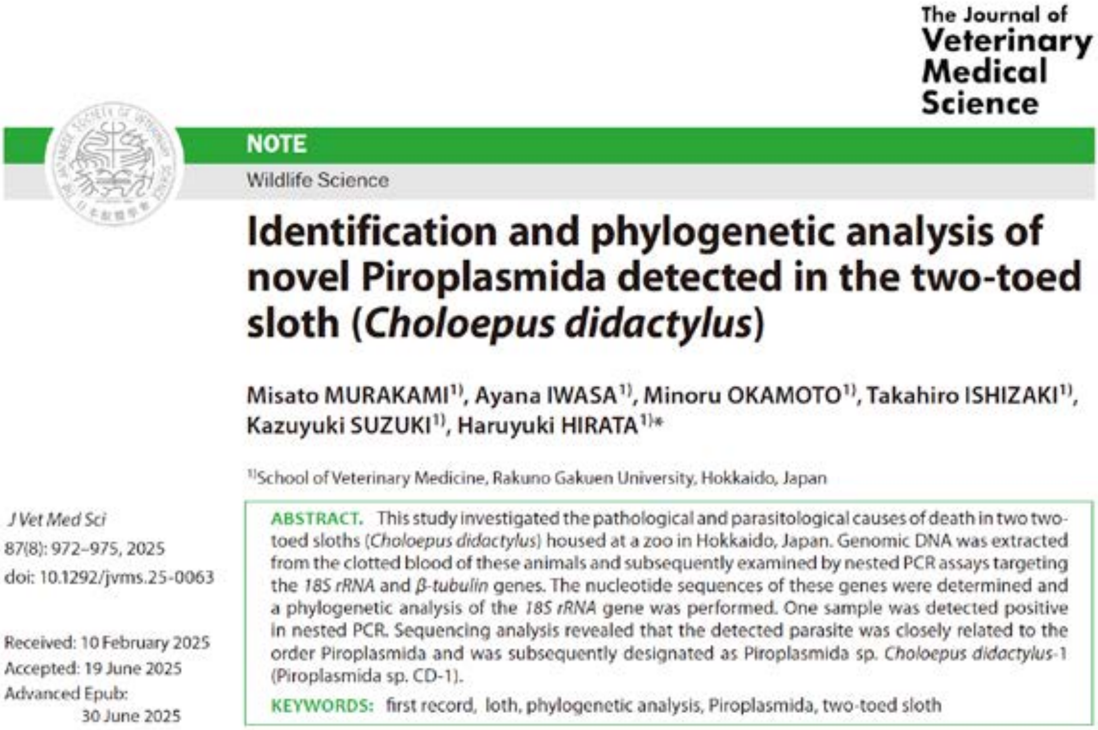

The Journal of
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NOTE
Wildlife Science

Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (*Choloepus didactylus*)

Misato MURAKAMI[1)], Ayana IWASA[1)], Minoru OKAMOTO[1)], Takahiro ISHIZAKI[1)], Kazuyuki SUZUKI[1)], Haruyuki HIRATA[1)]*

[1)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. This study investigated the pathological and parasitological causes of death in two two-toed sloths (*Choloepus didactylus*) housed at a zoo in Hokkaido, Japan. Genomic DNA was extracted from the clotted blood of these animals and subsequently examined by nested PCR assays targeting the *18S rRNA* and *β-tubulin* genes. The nucleotide sequences of these genes were determined and a phylogenetic analysis of the *18S rRNA* gene was performed. One sample was detected positive in nested PCR. Sequencing analysis revealed that the detected parasite was closely related to the order Piroplasmida and was subsequently designated as Piroplasmida sp. *Choloepus didactylus*-1 (Piroplasmida sp. CD-1).

KEYWORDS: first record, loth, phylogenetic analysis, Piroplasmida, two-toed sloth

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最終責任者 Haruyuki Hirata (Corresponding Author)

動物と人の関係学物（Animal Human Relations）

Yuko Takahashi

Professor

教授　髙橋　優子

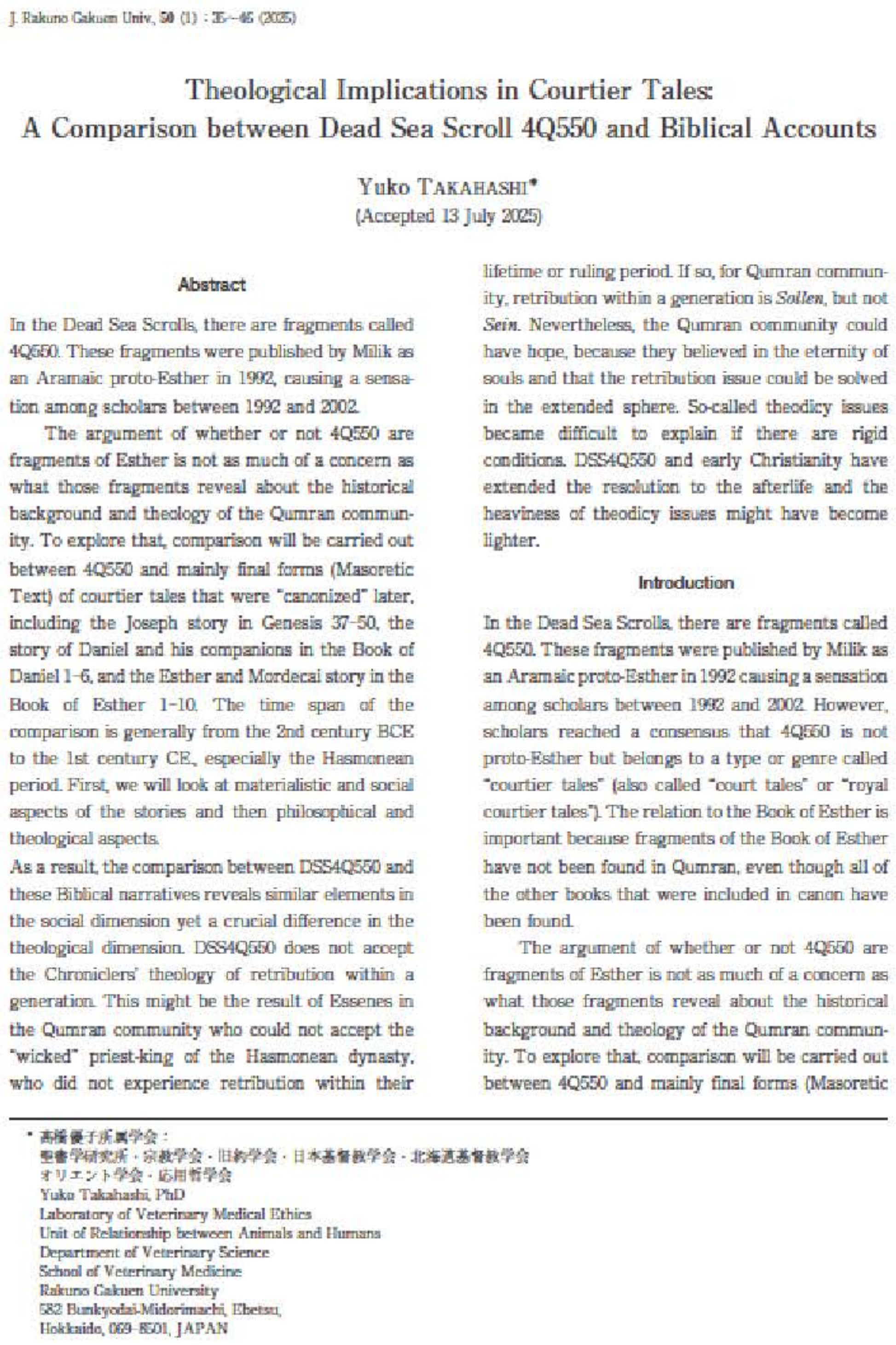
Ⅰ. 筆頭または責任著者　＜First or Corresponding Author＞

1) Theological Implications in Courtier Tales: A Comparison between Dead Sea Scroll 4Q550 and Biblical Accounts.
Yuko Takahashi.
Journal of Rakuno Gakuen University. 50: 35-46. 2025.
http://hdl.handle.net/10659/0002001067

2) Survey of Attitudes of Veterinary-related Students in Japan and the United States regarding the Donation of Deceased Pets for Anatomical and Pathological Education.
Takahashi Y, Takagi K, Kawamura, H., Suzuki, K.
J Vet Med Edun (accepted: Oct. 2025)

Ⅱ. その他　＜Others＞

該当なし



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Theological Implications in Courtier Tales: A Comparison between Dead Sea Scroll 4Q550 and Biblical Accounts

Yuko TAKAHASHI*

(Accepted 13 July 2025)

Abstract

In the Dead Sea Scrolls, there are fragments called 4Q550. These fragments were published by Milik as an Aramaic proto-Esther in 1992, causing a sensation among scholars between 1992 and 2002.

The argument of whether or not 4Q550 are fragments of Esther is not as much of a concern as what those fragments reveal about the historical background and theology of the Qumran community. To explore that, comparison will be carried out between 4Q550 and mainly final forms (Masoretic Text) of courtier tales that were "canonized" later, including the Joseph story in Genesis 37–50, the story of Daniel and his companions in the Book of Daniel 1–6, and the Esther and Mordecai story in the Book of Esther 1–10. The time span of the comparison is generally from the 2nd century BCE to the 1st century CE, especially the Hasmonean period. First, we will look at materialistic and social aspects of the stories and then philosophical and theological aspects.

As a result, the comparison between DSS4Q550 and these Biblical narratives reveals similar elements in the social dimension yet a crucial difference in the theological dimension. DSS4Q550 does not accept the Chroniclers' theology of retribution within a generation. This might be the result of Essenes in the Qumran community who could not accept the "wicked" priest-king of the Hasmonean dynasty, who did not experience retribution within their lifetime or ruling period. If so, for Qumran community, retribution within a generation is *Sollen*, but not *Sein*. Nevertheless, the Qumran community could have hope, because they believed in the eternity of souls and that the retribution issue could be solved in the extended sphere. So-called theodicy issues became difficult to explain if there are rigid conditions. DSS4Q550 and early Christianity have extended the resolution to the afterlife and the heaviness of theodicy issues might have become lighter.

Introduction

In the Dead Sea Scrolls, there are fragments called 4Q550. These fragments were published by Milik as an Aramaic proto-Esther in 1992 causing a sensation among scholars between 1992 and 2002. However, scholars reached a consensus that 4Q550 is not proto-Esther but belongs to a type or genre called "courtier tales" (also called "court tales" or "royal courtier tales"). The relation to the Book of Esther is important because fragments of the Book of Esther have not been found in Qumran, even though all of the other books that were included in canon have been found.

The argument of whether or not 4Q550 are fragments of Esther is not as much of a concern as what those fragments reveal about the historical background and theology of the Qumran community. To explore that, comparison will be carried out between 4Q550 and mainly final forms (Masoretic

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* 高橋優子所属学会：
聖書学研究所・宗教学会・旧約学会・日本基督教学会・北海道基督教学会
オリエント学会・応用哲学会
Yuko Takahashi, PhD
Laboratory of Veterinary Medical Ethics
Unit of Relationship between Animals and Humans
Department of Veterinary Science
School of Veterinary Medicine
Rakuno Gakuen University
582 Bunkyodai-Midorimachi, Ebetsu,
Hokkaido, 069-8501, JAPAN

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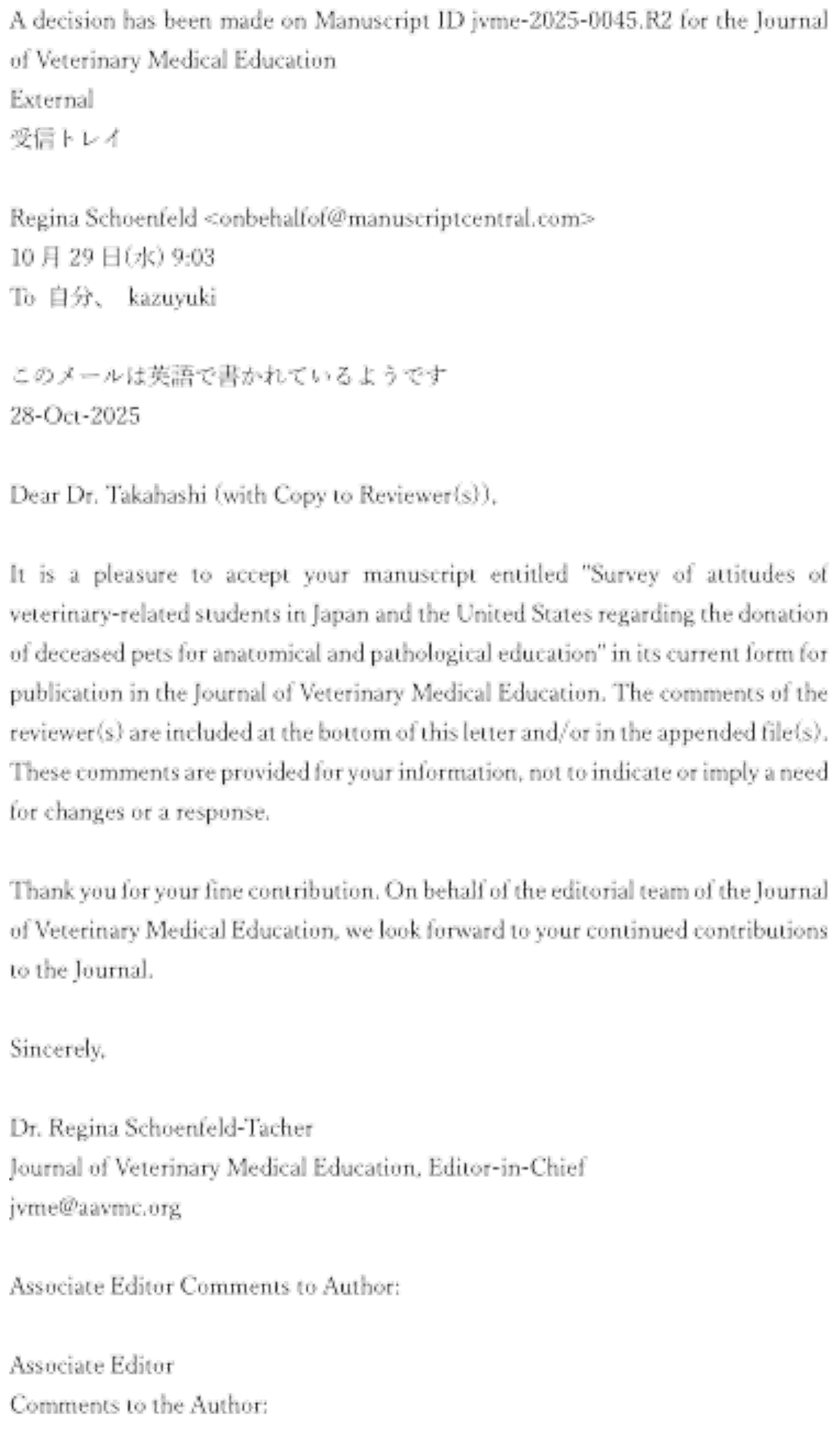

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Regina Schoenfeld <onbehalfof@manuscriptcentral.com>
10月29日(水) 9:03
To 自分、 kazuyuki

このメールは英語で書かれているようです
28-Oct-2025

Dear Dr. Takahashi (with Copy to Reviewer(s)),

It is a pleasure to accept your manuscript entitled "Survey of attitudes of veterinary-related students in Japan and the United States regarding the donation of deceased pets for anatomical and pathological education" in its current form for publication in the Journal of Veterinary Medical Education. The comments of the reviewer(s) are included at the bottom of this letter and/or in the appended file(s). These comments are provided for your information, not to indicate or imply a need for changes or a response.

Thank you for your fine contribution. On behalf of the editorial team of the Journal of Veterinary Medical Education, we look forward to your continued contributions to the Journal.

Sincerely,

Dr. Regina Schoenfeld-Tacher
Journal of Veterinary Medical Education, Editor-in-Chief
jvme@aavmc.org

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最終責任者　Yuko Takahashi (Corresponding Author)

獣医薬理学（Veterinary Pharmacology）

Hiroki Teraoka

Professor

教授　寺岡　宏樹

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1）Enhancement of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity by lipopolysaccharide in developing zebrafish lacking canonical pattern recognition pathways.
Hayato Kitamura, Katsuki Tanaka, Huan Wang, Tatsuro Nakamura, Makoto Kobayashi, **Hiroki Teraoka**.
Comp Biochem Physiol C Toxicol Pharmacol. 297:110261. 2025.
doi:10.1016/j.cbpc.

II. その他＜Others＞

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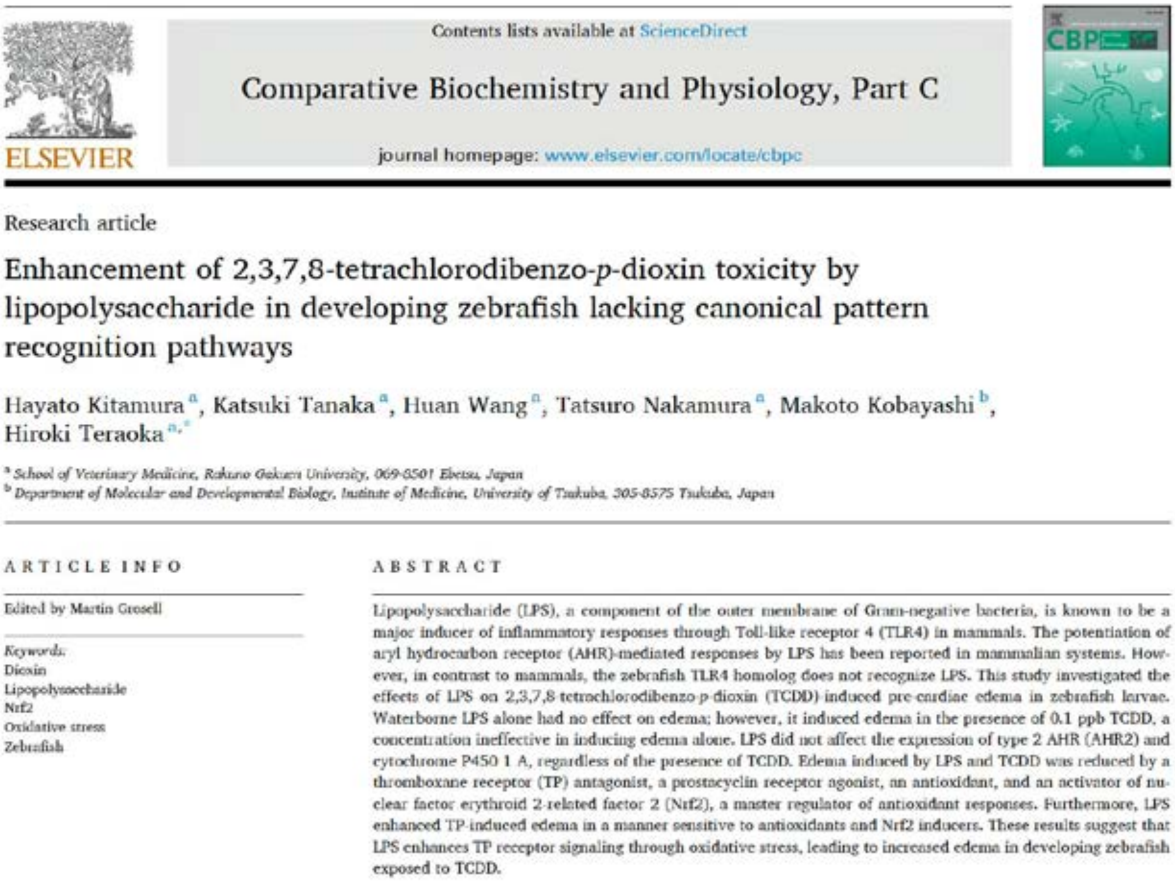

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Research article

Enhancement of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity by lipopolysaccharide in developing zebrafish lacking canonical pattern recognition pathways

Hayato Kitamura[a], Katsuki Tanaka[a], Huan Wang[a], Tatsuro Nakamura[a], Makoto Kobayashi[b], Hiroki Teraoka[a,*]

[a] *School of Veterinary Medicine, Rakuno Gakuen University, 069-8501 Ebetsu, Japan*
[b] *Department of Molecular and Developmental Biology, Institute of Medicine, University of Tsukuba, 305-8575 Tsukuba, Japan*

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ABSTRACT

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is known to be a major inducer of inflammatory responses through Toll-like receptor 4 (TLR4) in mammals. The potentiation of aryl hydrocarbon receptor (AHR)-mediated responses by LPS has been reported in mammalian systems. However, in contrast to mammals, the zebrafish TLR4 homolog does not recognize LPS. This study investigated the effects of LPS on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced pre-cardiac edema in zebrafish larvae. Waterborne LPS alone had no effect on edema; however, it induced edema in the presence of 0.1 ppb TCDD, a concentration ineffective in inducing edema alone. LPS did not affect the expression of type 2 AHR (AHR2) and cytochrome P450 1 A, regardless of the presence of TCDD. Edema induced by LPS and TCDD was reduced by a thromboxane receptor (TP) antagonist, a prostacyclin receptor agonist, an antioxidant, and an activator of nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of antioxidant responses. Furthermore, LPS enhanced TP-induced edema in a manner sensitive to antioxidants and Nrf2 inducers. These results suggest that LPS enhances TP receptor signaling through oxidative stress, leading to increased edema in developing zebrafish exposed to TCDD.

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伴侶動物外科学（Companion Animal Surgery）

Shidow Torisu

Professor

教授　鳥巣　至道

I. 筆頭または責任著者　＜First or Corresponding Author＞

1) Single Case of a Cat Suspected of Having Congenital Biliary Dilatation Associated with Pancreaticobiliary Maljunction.
Fujimoto S, **Torisu S**, Nakatani K, Amiya R, Hosaka Y, Kaneko Y, Nakamura K, Tanabe M.
J Am Anim Hosp Assoc. 61:50-55. 2025. doi: 10.5326/JAAHA-MS-7423.

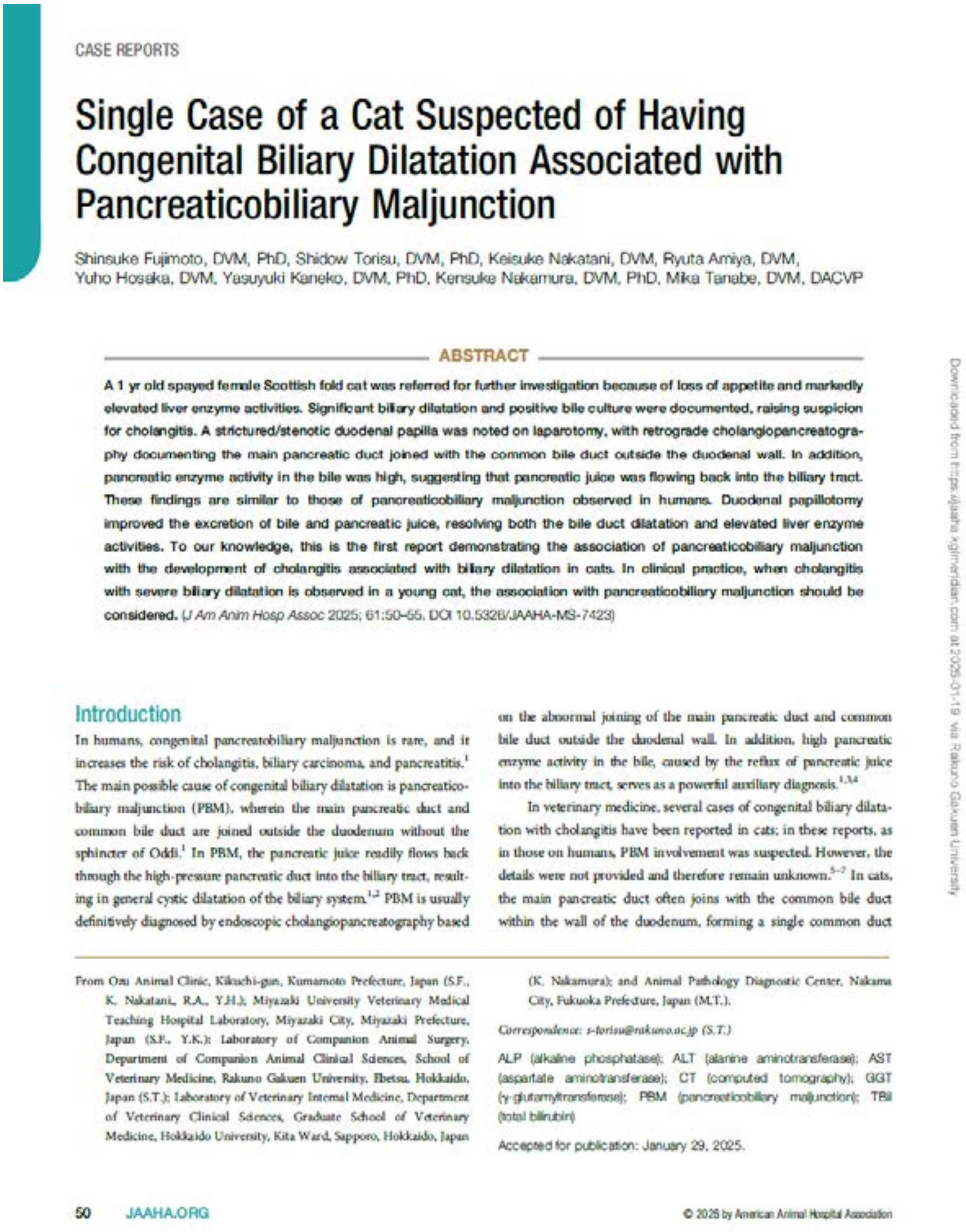
2) Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study.
Goda Y, Morimura M, Yamamoto S, Sakai T, Miyoshi K, Tamura M, Hanazono K, Deguchi T, Endo Y, Kadosawa T, Fujita M, Itami T, **Torisu S.**
Open Vet J. 15:5739-5747. 2025. doi: 10.5455/OVJ.2025.v15.i11.29

II. その他　＜Others＞

1) Dynamic Computed Tomography for detecting pulmonary cysts in canine Paragonimiasis: a case report.
Mizutani S, Mizutani Y, Goda Y, Satoh H, Asanuma T, Yoshida A, **Torisu S.**
J Vet Med Sci. 87:986-989. 2025. doi: 10.1292/jvms.25-0169.

2) Usefulness of transnasal volume-reduction treatment for nasal tumors by ultrasonic emulsification suction in 2 dogs.
Mizutani S, Mizutani Y, Goda Y, Asanuma T, **Torisu S**.
Can Vet J. 6:267-272. 2025.

3) Risk factors for complications associated with canine hepatic mass resection: A study of 96 cases.
Konno R, Kaneko Y, Osuga T, **Torisu S**, Yamamoto S, Okadera R, Naganobu K.
Vet Surg. 55:165-175. 2025. doi: 10.1111/vsu.70020.



CASE REPORTS

Single Case of a Cat Suspected of Having Congenital Biliary Dilatation Associated with Pancreaticobiliary Maljunction

Shinsuke Fujimoto, DVM, PhD, Shidow Torisu, DVM, PhD, Keisuke Nakatani, DVM, Ryuta Amiya, DVM, Yuho Hosaka, DVM, Yasuyuki Kaneko, DVM, PhD, Kensuke Nakamura, DVM, PhD, Mika Tanabe, DVM, DACVP

ABSTRACT

A 1 yr old spayed female Scottish fold cat was referred for further investigation because of loss of appetite and markedly elevated liver enzyme activities. Significant biliary dilatation and positive bile culture were documented, raising suspicion for cholangitis. A strictured/stenotic duodenal papilla was noted on laparotomy, with retrograde cholangiopancreatography documenting the main pancreatic duct joined with the common bile duct outside the duodenal wall. In addition, pancreatic enzyme activity in the bile was high, suggesting that pancreatic juice was flowing back into the biliary tract. These findings are similar to those of pancreaticobiliary maljunction observed in humans. Duodenal papillotomy improved the excretion of bile and pancreatic juice, resolving both the bile duct dilatation and elevated liver enzyme activities. To our knowledge, this is the first report demonstrating the association of pancreaticobiliary maljunction with the development of cholangitis associated with biliary dilatation in cats. In clinical practice, when cholangitis with severe biliary dilatation is observed in a young cat, the association with pancreaticobiliary maljunction should be considered. (*J Am Anim Hosp Assoc* 2025; 61:50–55. DOI 10.5326/JAAHA-MS-7423)

Introduction

In humans, congenital pancreatobiliary maljunction is rare, and it increases the risk of cholangitis, biliary carcinoma, and pancreatitis.[1] The main possible cause of congenital biliary dilatation is pancreaticobiliary maljunction (PBM), wherein the main pancreatic duct and common bile duct are joined outside the duodenum without the sphincter of Oddi.[1] In PBM, the pancreatic juice readily flows back through the high-pressure pancreatic duct into the biliary tract, resulting in general cystic dilatation of the biliary system.[1,2] PBM is usually definitively diagnosed by endoscopic cholangiopancreatography based on the abnormal joining of the main pancreatic duct and common bile duct outside the duodenal wall. In addition, high pancreatic enzyme activity in the bile, caused by the reflux of pancreatic juice into the biliary tract, serves as a powerful auxiliary diagnosis.[1,3,4]

In veterinary medicine, several cases of congenital biliary dilatation with cholangitis have been reported in cats; in these reports, as in those on humans, PBM involvement was suspected. However, the details were not provided and therefore remain unknown.[5–7] In cats, the main pancreatic duct often joins with the common bile duct within the wall of the duodenum, forming a single common duct

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From Ozu Animal Clinic, Kikuchi-gun, Kumamoto Prefecture, Japan (S.F., K. Nakatani, R.A., Y.H.); Miyazaki University Veterinary Medical Teaching Hospital Laboratory, Miyazaki City, Miyazaki Prefecture, Japan (S.F., Y.K.); Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan (S.T.); Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita Ward, Sapporo, Hokkaido, Japan (K. Nakamura); and Animal Pathology Diagnostic Center, Nakama City, Fukuoka Prefecture, Japan (M.T.).

Correspondence: s-torisu@rakuno.ac.jp (S.T.)

ALP (alkaline phosphatase); ALT (alanine aminotransferase); AST (aspartate aminotransferase); CT (computed tomography); GGT (γ-glutamyltransferase); PBM (pancreaticobiliary maljunction); TBil (total bilirubin)

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Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study

Yoshimichi Goda[1,#], Shushi Yamamoto[1,#], Kenjiro Miyoshi[1], Kiwamu Hanazono[1],
Mai Morimura[1], Toshikazu Sakai[1], Masahiro Tamura[1], Tatsuya Deguchi[1], Yoshifumi Endo[1,2],
Tsuyoshi Kadosawa[1,3], Mayu Fujita[1], Takaharu Itami[1] and Shidow Torisu[1]*

[1]*Department of Companion Animal Clinical Sciences, School of Veterinary Medicine,*
Rakuno Gakuen University, Ebetsu, Japan
[2]*Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, Japan*
[3]*Japan Small Animal Medical Center, Tokorozawa-Shi, Japan*
[#]*Both authors are equally contributed to this work*

ABSTRACT

Background: Recently, the usefulness of triple-phase contrast-enhanced computed tomography (CT) scans for liver tumors in canines has been reported. However, detailed information on the CT findings of cholangiocarcinoma (CCA) and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) remains limited.

Aim: This study aimed to retrospectively evaluate triple-phase contrast-enhanced CT findings in canine CCA and cHCC-CCA using objective parameters, such as CT values and morphological characteristics.

Methods: This study included eight dogs that underwent triple-phase contrast-enhanced CT scans and surgical removal and were pathologically diagnosed. Three CCA cases and five cHCC-CCA cases were analyzed. Parameters included CT values and mass sizes, CT values and lymph node sizes, and rim enhancement. "Rim enhancement" was defined as the contrast enhancement effect at the liver parenchyma–mass boundary.

Results: CT values for CCA were as follows: pre-contrast: 43.9 ± 3.6 Hounsfield units (HUs); arterial phase: 76.9 ± 22.5 HU; portal phase: 98.8 ± 37.7 HU; equilibrium phase: 90.9 ± 27.1 HU. For cHCC-CCA, the mean CT values were as follows: pre-contrast: 50.7 ± 6.1 HU; arterial phase: 80.2 ± 19.2 HU; portal phase: 95.0 ± 21.3 HU; equilibrium phase: 86.1 ± 13.4 HU. The peak contrast enhancement for both CCA and cHCC-CCA was in the portal phase. Rim enhancement appeared in the arterial phase in all CCA cases, whereas it appeared in the portal phase in 80% of cHCC-CCA cases, indicating a significant difference.

Conclusion: Rim enhancement may help distinguish between CCA and cHCC-CCA. However, this study has a limited number of cases, and future large-scale, multicenter studies are warranted.

Keywords: Canine, Cholangiocarcinoma, Combined hepatocellular-cholangiocarcinoma, Computed tomography, Dog.

Introduction

Primary hepatic tumors in canines are rare, accounting for 0.6%–1.3% of all canine tumors (Patnaik *et al.*, 1981). The most common malignant liver tumor is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma (CCA) (Liptak *et al.*, 2019). Unlike in humans, the cause of HCC in dogs remains unknown, and the risk of occurrence based on breed or sex is unclear. However, there are reports indicating a higher incidence in male dogs. Infection with trematodes has been reported as a risk factor for the occurrence of CCA, but cases have also been reported outside the infection area, and the relationship remains unclear (Liptak *et al.*, 2019). Combined hepatocellular-cholangiocarcinoma (cHCC-CCA), which shows the histological features of both HCC and CCA, has also been reported (Patnaik *et al.*, 1980; Trigo *et al.*, 1982; Terai *et al.*, 2022). It has been reported that among malignant canine hepatic tumors, the incidence of HCC is 77%, CCA is 9%–41%, and HCC-cCCA is 4.6%, respectively (Liptak *et al.*, 2019; Terai *et al.*, 2022). The diagnosis of HCC and CCA involves blood tests, X-ray, and ultrasonography. As advanced examinations, triple-phase contrast-enhanced computed tomography (CT) and magnetic

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***Corresponding Author**: Shidow Torisu. Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan. Email: *s-torisu@rakuno.ac.jp*

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NOTE

Parasitology

Dynamic Computed Tomography for detecting pulmonary cysts in canine Paragonimiasis: a case report

Shinya MIZUTANI[1)]*, Yuko MIZUTANI[2)], Yoshimichi GODA[3)], Hiroyuki SATOH[4)], Taketoshi ASANUMA[1)], Ayako YOSHIDA[5)], Shidow TORISU[6)]

[1)]Department of Small Animal Surgery, Field of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Ehime, Japan
[2)]University of Miyazaki Veterinary Teaching Hospital, Miyazaki, Japan
[3)]Laboratory of Veterinary Diagnostic Imaging, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[4)]Laboratory of Veterinary Clinical Radiology, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
[5)]Centre for Animal Disease Control (CADIC), University of Miyazaki, Miyazaki, Japan; Laboratory of Veterinary Parasitic Diseases, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
[6)]Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. An 11-year-old male French bulldog presented with incidental cystic lesions by the pulmonary CT. CT revealed two cysts in the right caudal lobe of the lung that were suspicious for emphysematous lesions. These cysts were divided into an air layer and a soft tissue-like layer. Dynamic computed tomography (DCT) of cysts revealed undulating motion in the soft tissue-like layer. The dog's rearing environment included brackish water, and it was evident that the dog had been eating brackish water crabs from the river. The dog was diagnosed with paragonimiasis. This DCT imaging revealed movement of live worms inside the cysts. This study is the first report that DCT may be useful for differentiating paragonimiasis from other pulmonary lesions.

KEYWORDS: dog, dynamic computed tomography, endemic disease, paragonimiasis, parasitic disease

Paragonimiasis is an important zoonosis caused by *Paragonimus* spp. in humans, dogs, and cats and is endemic, particularly in Asian countries [2, 18]. Paragonimiasis in dogs is primarily caused by the ingestion of a second intermediate host. However, cases involving the consumption of raw meat from wild boars and deer, which serve as paratenic hosts, have also been reported [2, 4, 6, 19]. *Paragonimus* spp. parasitize the lungs of definitive hosts (e.g., humans, dogs and cats), forming worm cysts. Symptoms of paragonimiasis include chronic respiratory symptoms such as coughing, respiratory urgency, pneumothorax, and bloody sputum [19].

Dynamic computed tomography (DCT) is an imaging technique that allows dynamic evaluation through a series of images of a single tomogram [8, 10–13]. DCT has been used to dynamically evaluate hemodynamics in tumors, information on blood flow in organs and the presence of inflammation, following contrast agent administration in humans and dogs [8, 10–13]. It has also been used to evaluate dynamic stenosis in the human lung and nasopharyngeal collapse in dogs [12, 13]. In this study, we report the use of DCT for the dynamic evaluation of *Paragonimus* spp. within the worm cysts of a dog with paragonimiasis due to natural infection.

An 11-year-old male French bulldog weighing 11.1 kg presented to our veterinary teaching hospital with epistaxis from the left nostril. At the time of presentation, the dog was in good condition, had a good appetite and had no obvious respiratory symptoms other than epistaxis from the left nostril. The dog's house was near brackish water area, and he often ate brackish water crabs from the river. Blood tests revealed no major abnormalities, and eosinophil counts were normal in our veterinary teaching hospital examination. Chest X-ray (right lateral, left lateral, ventral-dorsal) revealed no significant abnormalities, and abdominal X-ray revealed a high-density body, similar to a metallic body in the stomach. These X-ray evaluations were performed by a veterinarian trained in diagnostic imaging in our veterinary teaching hospital.

CT was then performed to examine the epistaxis in this dog. CT was performed under general anesthesia in the prone position under

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*Correspondence to: Mizutani S: s-mizutani@ous.ac.jp, Field of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan

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CASE REPORT
RAPPORT DE CAS

Shinya Mizutani, Yuko Mizutani,
Yoshimichi Goda, Taketoshi Asanuma,
Shidow Torisu

Usefulness of transnasal volume-reduction treatment for nasal tumors by ultrasonic emulsification suction in 2 dogs

ABSTRACT

Intranasal tumors in dogs are rare neoplastic diseases with obvious clinical signs, such as epistaxis, nasal congestion, and facial deformity. Radiation therapy is the treatment of choice but is not always accessible due to geographical location, logistics, or financial constraints. Other treatments may not be used because of various restrictions. We performed transnasal intranasal tumor volume reduction in 2 dogs with intranasal tumors; 1 dog had an adenocarcinoma and the other had a fibrosarcoma. In both cases, improvement in the quality of life (QOL), including reduced clinical signs and increased activity, was observed ~1 wk after treatment. No obvious complications were associated with this treatment. Although tumor recurrence was noted in both cases, this treatment could be applied multiple times. In conclusion, transnasal nasal tumor volume reduction is a palliative method that contributes to improvement of clinical signs and QOL.

Key clinical message:
We report palliative treatment of intranasal tumors in dogs. The treatment described in this report is available to most veterinarians and helps improve the QOL of dogs with nasal tumors, including improving respiratory status.

RÉSUMÉ

Utilité du traitement de réduction transnasale du volume pour les tumeurs nasales par émulsification et succion chez 2 chiens

Les tumeurs intranasales chez le chien sont des maladies néoplasiques rares qui présentent des symptômes cliniques évidents, tels que l'épistaxis, la congestion nasale et la déformation de la face. La radiothérapie est le traitement de choix, mais elle n'est pas toujours accessible en raison de la situation géographique ou de contraintes logistiques et financières. Cependant, ces traitements peuvent ne pas être réalisés en raison de diverses restrictions. Nous avons procédé à une réduction du volume de la tumeur intranasale par voie transnasale chez deux chiens atteints de tumeurs intranasales. Les tumeurs intranasales de la cavité nasale étaient des adénocarcinomes et des fibrosarcomes. Dans les deux cas, une amélioration de la qualité de vie, telle que l'amélioration des symptômes cliniques et de l'activité, a été observée environ une semaine après le traitement. Aucune complication évidente n'a été associée à ce traitement. Une récidive tumorale a été constatée dans les deux cas. Ce traitement a pu être réalisé plusieurs fois. Cette réduction transnasale du volume de la tumeur nasale est une méthode palliative. Elle est considérée comme un traitement qui contribue à l'amélioration des symptômes cliniques et de la qualité de vie.

Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan (Mizutani S, Asanuma); University of Miyazaki Veterinary Teaching Hospital, 1-1 Gakuen Kibana-dai Nishi, Miyazaki, Miyazaki 889-2192, Japan (Mizutani Y); Okayama University of Science Veterinary Medical Teaching Hospital, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan (Goda); Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan (Torisu).

Address all correspondence to Shinya Mizutani; email: s-mizutani@ous.ac.jp.

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CLINICAL RESEARCH

WILEY

Risk factors for complications associated with canine hepatic mass resection: A study of 96 cases

Ryusei Konno DVM[1] | **Yasuyuki Kaneko DVM, PhD**[1] | **Tatsuyuki Osuga DVM, PhD**[2] | **Shidow Torisu DVM, PhD**[3] | **Shushi Yamamoto DVM, PhD**[3] | **Ryota Okadera DVM**[1] | **Kiyokazu Naganobu DVM, PhD**[1]

[1]Faculty of Agriculture, Veterinary Medical Teaching Hospital, University of Miyazaki, Miyazaki, Japan
[2]Faculty of Agriculture, Veterinary Internal Medicine, University of Miyazaki, Miyazaki, Japan
[3]Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Correspondence
Yasuyuki Kaneko, Veterinary Teaching Hospital, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan.
Email: yasuyuki-kaneko@cc.miyazaki-u.ac.jp

Abstract

Objective: To identify pre- and intraoperative risk factors for complications occurring within 2 weeks following hepatic mass resection in dogs.

Study design: Retrospective case series.

Animals: A total of 96 client-owned dogs that underwent hepatic mass resection.

Methods: The evaluated preoperative variables were the signalment, clinical signs, presence of underlying diseases, blood test results (e.g., hematocrit), and computed tomography (CT) findings (mass location, maximum diameter, mass volume, and distance between the mass and the caudal vena cava [CVC]). The evaluated intraoperative variables were the surgical time, procedure details (e.g., surgical techniques), presence of intraoperative hypotension and hypoxemia, and blood transfusion. Comparisons were made between dogs with severe postoperative complications (including mortality) and those with mild or moderate complications. Univariable logistic regression was performed, and significant variables were used to construct multivariable models by combining them.

Results: Severe postoperative complications were observed in 17 dogs (17.7%), including six deaths (6.3%). Multivariable logistic regression analyses identified the presence of underlying diseases (OR: 2.703; $p = .007$), corrected distance from the mass to the CVC (OR: 0.666 per 0.1 cm/kg increase; $p = .017$), and intraoperative hypotension (OR: 3.589; $p = .019$) as risk factors for severe postoperative complications.

Conclusion: Among preoperative variables, both the presence of underlying diseases and the corrected distance from the mass to the CVC were associated with severe postoperative complications.

Clinical significance: Preoperative CT evaluation of the distance between the hepatic mass and the CVC, along with screening for underlying diseases, may contribute to improve the prediction of surgical risk.

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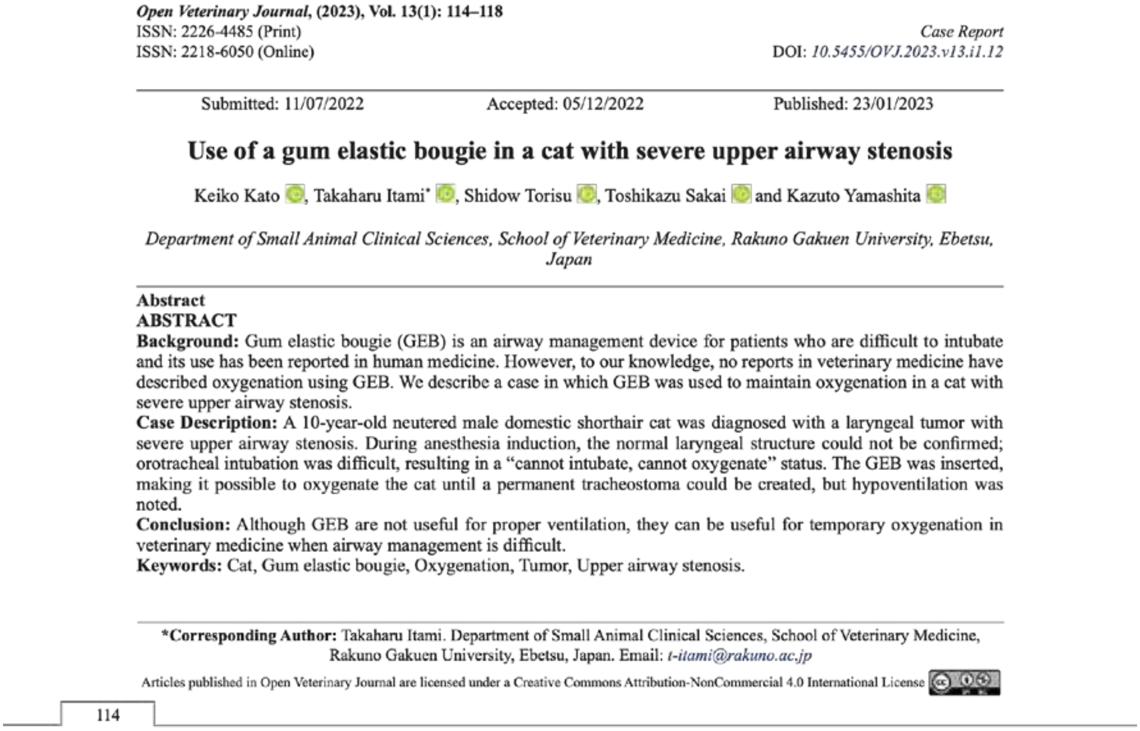

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Use of a gum elastic bougie in a cat with severe upper airway stenosis

Keiko Kato, Takaharu Itami*, Shidow Torisu, Toshikazu Sakai and Kazuto Yamashita

Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Abstract

ABSTRACT

Background: Gum elastic bougie (GEB) is an airway management device for patients who are difficult to intubate and its use has been reported in human medicine. However, to our knowledge, no reports in veterinary medicine have described oxygenation using GEB. We describe a case in which GEB was used to maintain oxygenation in a cat with severe upper airway stenosis.

Case Description: A 10-year-old neutered male domestic shorthair cat was diagnosed with a laryngeal tumor with severe upper airway stenosis. During anesthesia induction, the normal laryngeal structure could not be confirmed; orotracheal intubation was difficult, resulting in a "cannot intubate, cannot oxygenate" status. The GEB was inserted, making it possible to oxygenate the cat until a permanent tracheostoma could be created, but hypoventilation was noted.

Conclusion: Although GEB are not useful for proper ventilation, they can be useful for temporary oxygenation in veterinary medicine when airway management is difficult.

Keywords: Cat, Gum elastic bougie, Oxygenation, Tumor, Upper airway stenosis.

***Corresponding Author:** Takaharu Itami. Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan. Email: *t-itami@rakuno.ac.jp*

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最終責任者　Yasuyuki Kaneko (Corresponding Author)

食品衛生学 (Food Microbiology and Food Safety)

Masaru Usui

Professor

教授　臼井　優

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VETERINARY MICROBIOLOGY

Spreading Ability of *Tet(X)*-Harboring Plasmid and Effect of Tetracyclines as a Selective Pressure

Akira Fukuda,[1] Yuta Kozaki,[1] Cemil Kürekci,[2] Yasuhiko Suzuki,[3–5] Chie Nakajima,[3,4,6] and Masaru Usui[1]

Tigecycline is a last-resort antimicrobial in humans. Tetracyclines are the most widely used antimicrobials in livestock. Mobile tigecycline resistance genes [*tet(X)*] are disseminated worldwide, and tetracycline use may have promoted the selection of *tet(X)* genes. Thus, the selective pressure on *tet(X)* genes and their plasmids in livestock must be elucidated. We performed a retrospective study to clarify the prevalence of tigecycline-resistant *Escherichia coli* from pigs in Thailand. Screening for tigecycline resistance was performed on 107 *E. coli* strains from 25 samples, and *tet(X)*-carrying plasmids were characterized. *tet(X)* genes were cloned and expressed in *E. coli*. Bacterial growth rate in the presence of tetracycline as a result of the presence of *tet(X)* genes was also evaluated. Thirty-two *tet(X4)*-harboring tigecycline-resistant *E. coli* strains were detected in 10/25 samples (40%). The *tet(X4)* genes were carried on various Inc-type plasmids and flanked by IS*CR2*. The *tet(X)*-carrying plasmids were transferred to *E. coli* and *Klebsiella pneumoniae*. Acquisition of *tet(X)* genes and their plasmids improved bacterial growth in the presence of tetracycline. In summary, tetracycline use exerts selective pressure on *tet(X)* genes and their various backbone plasmids; therefore, a reduced amount of tetracycline use is important to limit the spreading of *tet(X)* genes.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, pig, plasmid, *tet(X)*, tigecycline

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Antimicrobial susceptibility and genetic diversity of staphylococcus pseudintermedius isolated from companion animals and human clinical patients in Japan: Potential zoonotic implications[☆]

Masaru Usui[a,*], Rana Fahmi Sabala[a,b], Sawa Morita[a], Akira Fukuda[a], Yuzo Tsuyuki[c], Kae Torii[c], Yuka Nakamura[d], Koichi Okamura[e], Tadato Komatsu[e], Junpei Sasaki[f], Chie Nakajima[g,h], Yasuhiko Suzuki[g,i]

[a] *School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*
[b] *Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*
[c] *Division of Clinical Laboratory, Sanritsu Zelkova Veterinary Laboratory, Tokyo, Japan*
[d] *Division of Clinical Laboratory, Sanritsu Laboratory, Chiba, Japan*
[e] *Bussan Animal Health Co., Ltd., Osaka, Japan*
[f] *Department of Clinical Laboratory, Teikyo University Hospital, Tokyo, Japan*
[g] *Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Hokkaido, Japan*
[h] *Division of Vaccinology for Clinical Development, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[i] *Division of Research Support, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*

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ABSTRACT

Objectives: *Staphylococcus pseudintermedius* is the primary pathogen that causes pyoderma in companion animals. The increasing number of multidrug-resistant strains, including methicillin-resistant *S. pseudintermedius* (MRSP), has become a major concern, highlighting the need for comprehensive data on antimicrobial susceptibility. Furthermore, with advancements in the accurate identification of *S. pseudintermedius* in human clinical patients, it is imperative to elucidate its definitive zoonotic potential.
Methods: We analyzed 111 strains of *S. pseudintermedius* derived from companion animals and 21 strains of *S. pseudintermedius* from human clinical patients to clarify antimicrobial susceptibility and correlation between strains derived from companion animals and humans.
Results: Approximately half of the animal-derived *S. pseudintermedius* isolates were MRSP. The isolates, particularly MRSP, exhibited high resistance to multiple antimicrobials used to treat pyoderma. Although florfenicol and fusidic acid are not approved for the treatment of pyoderma in companion animals in Japan, their efficacy has been demonstrated. Genetic analysis revealed that ST121, ST45, and ST71 were the most common ST types in animals. Additionally, ten novel STs were identified. ST45 and ST71 have frequently been identified in companion animals abroad, suggesting potential international transmission. However, ST121 has rarely been reported outside Japan, indicating its unique evolutionary trajectory within the country. Furthermore, these sequence types were identified in strains isolated from humans. Core genome analysis revealed nearly identical genotypes, suggesting transmission from companion animals to humans.
Conclusion: A limited number of approved antimicrobials are effective against *S. pseudintermedius* (particularly MRSP), which is being transmitted as a zoonotic infection from companion animals to humans.

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MICROBIOLOGY SOCIETY
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Selection and maintenance of mobile linezolid-resistance genes and plasmids carrying them in the presence of florfenicol, an animal-specific antimicrobial

Akira Fukuda and Masaru Usui*

Abstract

Mobile linezolid-resistance genes (*optrA*, *poxtA* and *cfr*) that confer resistance to linezolid and florfenicol have been detected globally in various sources. Linezolid is a last-resort antimicrobial used in human clinical settings, and florfenicol is commonly used in veterinary clinical settings. The present study sought to evaluate the potential of florfenicol in veterinary use to select for linezolid-resistant bacteria. The growth and fitness of linezolid-resistant bacteria harbouring mobile linezolid-resistance genes were assessed in the presence and absence of florfenicol using *Enterococcus faecalis* and *Enterococcus faecium*, respectively. The bacterial strains harboured wild and cloning plasmids carrying mobile linezolid-resistance genes, which reduced their susceptibility to linezolid and florfenicol. The acquisition of plasmids carrying mobile linezolid-resistance genes improved bacterial growth in the presence of florfenicol and conferred fitness costs in its absence. Florfenicol imposes a selection pressure on bacteria harbouring plasmids carrying mobile linezolid-resistance genes. Hence, the appropriate use of florfenicol in veterinary clinical settings is important to control the dissemination of mobile linezolid-resistance genes and to ensure the sustained effectiveness of linezolid against multidrug-resistant bacteria, including vancomycin-resistant enterococci in human clinical settings.

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| Bacteriology | Announcement

Complete genome sequence of *Treponema medium* isolated from foot of bovine digital dermatitis in Japan

Akira Fukuda,[1] Takashi Murakami,[2] Noritsugu Abe,[3] Yasuhiko Suzuki,[4,5,6] Chie Nakajima,[4,5,7] Masaru Usui[1]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Treponema* species are the primary causative bacteria of bovine digital dermatitis. This report describes the isolation and complete genome sequencing of *Treponema medium* strain T1 isolated from a hoof sole swab of a bovine in Japan. Genome sequences were obtained using a combination of long- and short-read sequencing technologies.

KEYWORDS *Treponema*, bovine, digital dermatitis, complete genome

Bovine digital dermatitis (BDD) is an infectious skin disease of the foot that leads to reduced dairy farm productivity. Molecular studies have identified *Treponema* species, particularly *T. medium*, *T. phagedenis*, *T. pedis*, and *T. denticola*, in BDD lesions (1, 2). Culturing and isolating *Treponema* under laboratory conditions is challenging because of their poor growth and the presence of other fast-growing bacteria in BDD lesions (3, 4). Studies on the isolation and complete genome analysis of bovine *Treponema* are very limited.

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Bacterial contamination level and characterization of antimicrobial-resistant bacteria in commercial pet foods in Japan

Akira Fukuda[a], Kazu Yamaura[a], Kazuyoshi Tokumoto[b], Yasuhiko Suzuki[c,d,e], Chie Nakajima[c,d,f], Shoichiro Yukawa[g], Masaru Usui[a,*]

[a] *Food Microbiology and Food Safety Unit, Division of Preventive Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*
[b] *Harmony Corporation, Tokyo, Japan*
[c] *Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Hokkaido, Japan*
[d] *International Collaboration Unit, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan*
[e] *Division of Research Support, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[f] *Vaccinology for Clinical Development, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[g] *Department of Comparative Animal Science, College of Life Science, Kurashiki University of Science and The Arts, Okayama, Japan*

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Pet food

ABSTRACT

With the growing pet market, various types of pet food become available. Pet foods, especially raw meat-based diets (RMBDs), are contaminated with pathogens and antimicrobial-resistant bacteria, leading to health concerns for pets and humans. The bacterial contamination levels in pet food, including the presence of antimicrobial-resistant bacteria, have not been extensively studied in Japan. To address this issue in commercial pet foods in Japan, we evaluated the standard plate counts (SPCs) of various pet food samples, and isolated and characterized *Escherichia coli* and *Enterococcus* spp. A total of 129 pet foods (48 RMBDs, 21 heat-treated foods, and 60 treats) were purchased, and SPC quantification and isolation of *E. coli* and *Enterococcus* spp. were performed. SPCs in RMBD were significantly higher than in heat-treated foods and treats. Specifically, 50.0 % of the RMBD samples had SPCs exceeding 10^6 CFU/g. *E. coli* was isolated only from 62.5 % of RMBDs. Four cephalosporin-resistant *E. coli* strains harboring *bla* genes were detected, among which, one carried *mcr* conferring resistance to colistin. *Enterococcus* spp. were isolated from 89.6 % of RMBDs and 23.3 % of treats. Linezolid-non-susceptible *E. faecalis* harboring *optrA* or *poxtA* genes were detected in four RMBDs. Pet food may be contaminated with bacteria; some RMBDs are contaminated at concentrations $>10^6$ CFU/g, and with veterinary and human clinically important antimicrobial-resistant bacteria. To prevent health risks in both humans and pets associated with pet food, ensuring the hygienic management of pet food, especially RMBDs, and promoting accurate knowledge among pet owners are important.

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Research Article

Surveillance of *Escherichia coli* From Frozen Chicken Meat in Fiji: Resistance Characteristics and Public Health Concerns

Deepika Darshani Lata,[1] Rana Fahmi Sabala,[1,2] Akira Fukuda,[1] Chie Nakajima,[3,4] Yasuhiko Suzuki,[3,5] and Masaru Usui[1]

[1]*School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*
[2]*Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*
[3]*Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Hokkaido, Japan*
[4]*Division of Vaccinology for Clinical Development, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[5]*Division of Research Support, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*

Correspondence should be addressed to Masaru Usui; usuima@rakuno.ac.jp

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Antimicrobial resistance (AMR) is a growing concern in human and veterinary medicine. Misuse and overuse of antimicrobials in human medicine, veterinary medicine, agriculture, and aquaculture are major drivers of AMR development, with resistant bacteria also being selected in livestock and transmitted through meat. Research on AMR in livestock and animal-derived foods is lacking in Fiji; thus, the associated risks remain unclear. Chicken is widely consumed in Fiji and is predominantly served frozen. This study is aimed at determining the prevalence and resistance profiles of *Escherichia coli* in frozen chicken meat from Fijian supermarkets. A total of 100 frozen chicken meat samples were purchased from supermarkets and retail outlets in Fiji for this study. *E. coli* was isolated from 72% of the samples. The *E. coli* isolates showed relatively high levels of resistance to ampicillin (36%), tetracycline (24%), and streptomycin (17%). Only one cefotaxime-resistant isolate was obtained, which was identified as an extended-spectrum β-lactamase (ESBL)–producing bacterium. This isolate harbored the ESBL-producing gene $bla_{\text{CTX-M-1}}$ and was classified as ST2522. One colistin-resistant isolate was obtained, and its resistance was attributed to a chromosomal mutation in the *pmrB* gene. The high level of intestinal bacterial contamination in frozen chicken meat suggests that improved hygiene management is necessary throughout the production and distribution chains. Furthermore, because resistance to antimicrobials is important in both human and veterinary medicine (cefotaxime- and colistin-resistant *E. coli*), careful monitoring of AMR trends in Fiji is essential. These results suggest that AMR surveillance in meat and livestock is necessary to prevent its spread in Fiji.

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FULL PAPER

Public Health

Prevalence and genetic characteristics of antimicrobial-resistant *Escherichia coli* in wild birds in Japan

Tetsuo ASAI[1)]*, Michiyo SUGIYAMA[1)], Tomoya MORIMOTO[1)], Akiko SUDO[2)], Junji MORIBE[3)], Masaru USUI[4)]

[1)]The United Graduate School of Veterinary Medicine, Gifu University, Gifu, Japan
[2)]Eaglet Office Inc., Shiga, Japan
[3)]School of Social System Management, Gifu University, Gifu, Japan
[4)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. Antimicrobial-resistant bacteria in wild animals are a multisectoral concern worldwide. In this study, we examined fecal samples collected from wild birds in two prefectures of Japan between 2021 and 2024 using both deoxycholate-hydrogen sulfide-lactose (DHL) agar media and antimicrobial-containing DHL agar media. Of the 252 fecal samples from 11 bird species, 159 *E. coli* were isolated from 70 samples (27.8%) of six species, and resistance was found in 4.4% of isolates from DHL media. Using nalidixic acid (NAL)- and cefotaxime (CTX)-containing media, NAL-resistant isolates were isolated from great cormorants and spot-billed ducks, and an extended-spectrum β-lactamase (ESBL)/AmpC β-lactamase producer was isolated from great cormorants. Next-generation sequencing analysis of 19 *E. coli* isolates exhibiting resistance to NAL and/or CTX indicated diverse genotypes. Genetic analysis indicated that amino acid substitution of quinolone-resistance-determining-regions and *qnrS* were responsible for NAL resistance, and CTX-Ms ($bla_{\text{CTX-M-14}}$ in three isolates, and $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ in each) and AmpC β-lactamase ($bla_{\text{MOX-4}}$ and $bla_{\text{CMY-2}}$ in each) were responsible for CTX resistance. Despite the presence of certain resistant strains, the overall prevalence of antimicrobial-resistant *E. coli* remains low in wild birds, suggesting limited environmental exposure to antimicrobials and antimicrobial resistant bacteria.

KEYWORDS: antimicrobial resistance, *Escherichia coli*, extended-spectrum β-lactamase, quinolone, wild bird

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最終責任者　Tetsuo ASAI (Corresponding Author)

獣医解剖学（Veterinary Anatomy）

Takafumi Watanabe

Professor

教授　渡邉　敬文

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Segmented ring-mesh model of glycosaminoglycan chains based on the 3D analysis of normal individual and musculocontractural Ehlers–Danlos syndrome skin using scanning transmission electron microscopy.
Takahashi N, Hirose T, Kametani K, Iwasaki T, Imamura Y, Kosho T, **Watanabe T**.
Microscopy. 74:358-366. 2025. doi:10.1093/jmicro/dfaf012.

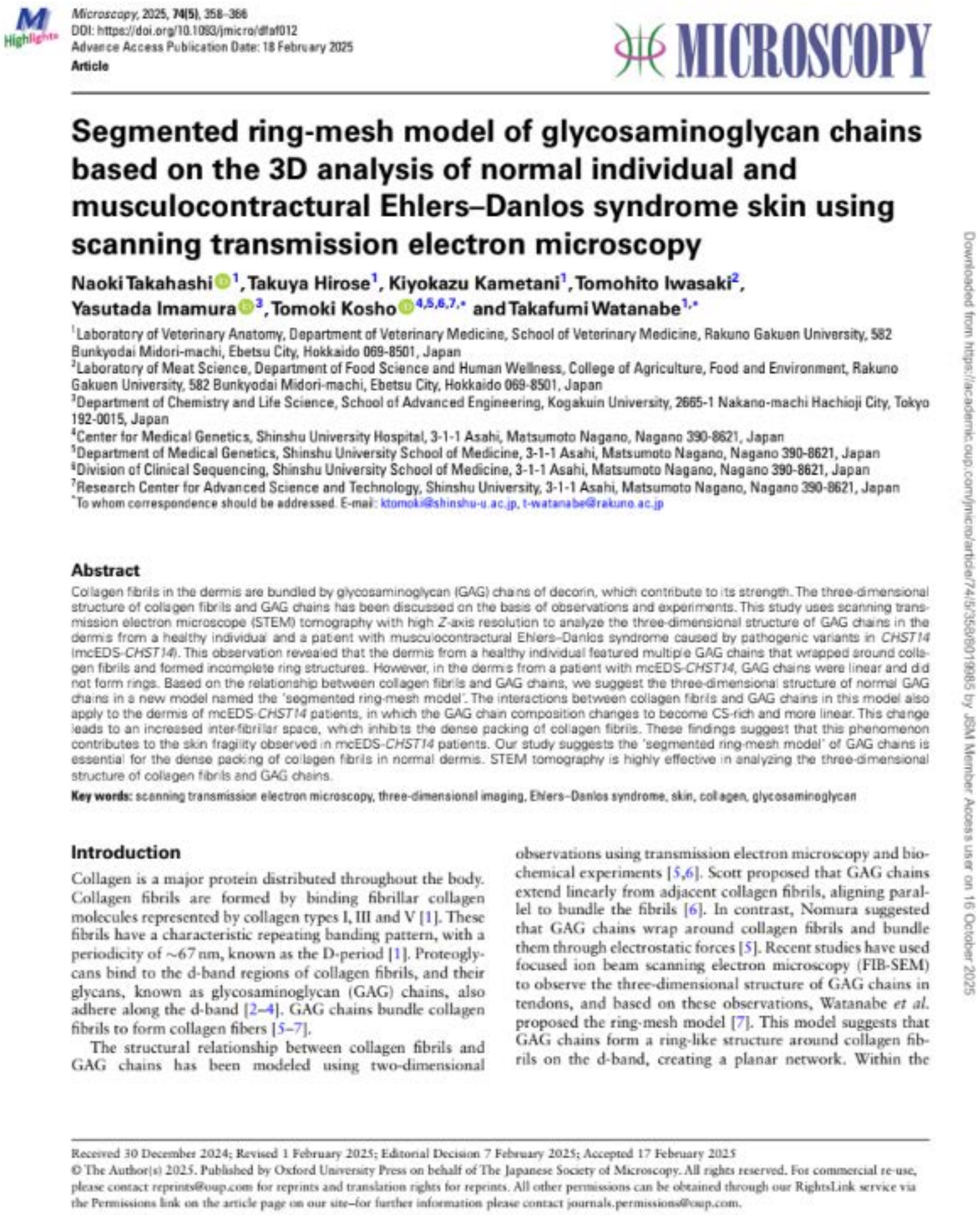
II. その他　＜Others＞

1）Assembly of collagen fibers into contiguous dense and loose regions of subcutaneous fascia.
Maeda N, **Watanabe T**, Suzuki D, Iwasaki T, Shin Y, Imamura Y.
Connect Tissue Res. 66:26-36. 2025.
doi:10.1080/03008207.2025.2455730.

2）CANT1 Is Involved in Collagen Fibrogenesis in Tendons by Regulating the Synthesis of Dermatan/Chondroitin Sulfate Attached to the Decorin Core Protein.
Yamashita R, Tsutsui S, Mizumoto S, **Watanabe T**, Yamamoto N, Nakano K, Yamada S, Okamura T, Furuichi T.
Int J Mol Sci. 26:2463. 2025. doi:10.3390/ijms26062463.

3）Injury and Fibrosis at the Myoaponeurotic Junction of Pectoralis Major and Supracoracoideus Muscles in Broiler Chickens.
Kawasaki T, Iwasaki T, **Watanabe T**, Yamada Y, Maeda N, Hasegawa Y, Takahashi N, Kobayashi R.
J Poult Sci. 62:2025014. 2025. doi:10.2141/jpsa.2025014.

4） Breast muscle myopathies: twists and turns in modern broilers.
Greene S E, Che S, Soglia F, Iwasaki T, **Watanabe T**, Kawasaki T, Susta L, Petracci M, Scanes C, Dridi S.
Avian Pathol. 54:669-692. 2025. doi:10.1080/03079457.2025.2533454.



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Article

Segmented ring-mesh model of glycosaminoglycan chains based on the 3D analysis of normal individual and musculocontractural Ehlers–Danlos syndrome skin using scanning transmission electron microscopy

Naoki Takahashi [1], Takuya Hirose [1], Kiyokazu Kametani [1], Tomohito Iwasaki [2], Yasutada Imamura [3], Tomoki Kosho [4,5,6,7,*] and Takafumi Watanabe [1,*]

[1] Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midori-machi, Ebetsu City, Hokkaido 069-8501, Japan
[2] Laboratory of Meat Science, Department of Food Science and Human Wellness, College of Agriculture, Food and Environment, Rakuno Gakuen University, 582 Bunkyodai Midori-machi, Ebetsu City, Hokkaido 069-8501, Japan
[3] Department of Chemistry and Life Science, School of Advanced Engineering, Kogakuin University, 2665-1 Nakano-machi Hachioji City, Tokyo 192-0015, Japan
[4] Center for Medical Genetics, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[5] Department of Medical Genetics, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[6] Division of Clinical Sequencing, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[7] Research Center for Advanced Science and Technology, Shinshu University, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[*] To whom correspondence should be addressed. E-mail: ktomoki@shinshu-u.ac.jp, t-watanabe@rakuno.ac.jp

Abstract

Collagen fibrils in the dermis are bundled by glycosaminoglycan (GAG) chains of decorin, which contribute to its strength. The three-dimensional structure of collagen fibrils and GAG chains has been discussed on the basis of observations and experiments. This study uses scanning transmission electron microscope (STEM) tomography with high *Z*-axis resolution to analyze the three-dimensional structure of GAG chains in the dermis from a healthy individual and a patient with musculocontractural Ehlers–Danlos syndrome caused by pathogenic variants in *CHST14* (mcEDS-*CHST14*). This observation revealed that the dermis from a healthy individual featured multiple GAG chains that wrapped around collagen fibrils and formed incomplete ring structures. However, in the dermis from a patient with mcEDS-*CHST14*, GAG chains were linear and did not form rings. Based on the relationship between collagen fibrils and GAG chains, we suggest the three-dimensional structure of normal GAG chains in a new model named the 'segmented ring-mesh model'. The interactions between collagen fibrils and GAG chains in this model also apply to the dermis of mcEDS-*CHST14* patients, in which the GAG chain composition changes to become CS-rich and more linear. This change leads to an increased inter-fibrillar space, which inhibits the dense packing of collagen fibrils. These findings suggest that this phenomenon contributes to the skin fragility observed in mcEDS-*CHST14* patients. Our study suggests the 'segmented ring-mesh model' of GAG chains is essential for the dense packing of collagen fibrils in normal dermis. STEM tomography is highly effective in analyzing the three-dimensional structure of collagen fibrils and GAG chains.

Key words: scanning transmission electron microscopy, three-dimensional imaging, Ehlers–Danlos syndrome, skin, collagen, glycosaminoglycan

Introduction

Collagen is a major protein distributed throughout the body. Collagen fibrils are formed by binding fibrillar collagen molecules represented by collagen types I, III and V [1]. These fibrils have a characteristic repeating banding pattern, with a periodicity of ~67 nm, known as the D-period [1]. Proteoglycans bind to the d-band regions of collagen fibrils, and their glycans, known as glycosaminoglycan (GAG) chains, also adhere along the d-band [2–4]. GAG chains bundle collagen fibrils to form collagen fibers [5–7].

The structural relationship between collagen fibrils and GAG chains has been modeled using two-dimensional observations using transmission electron microscopy and biochemical experiments [5,6]. Scott proposed that GAG chains extend linearly from adjacent collagen fibrils, aligning parallel to bundle the fibrils [6]. In contrast, Nomura suggested that GAG chains wrap around collagen fibrils and bundle them through electrostatic forces [5]. Recent studies have used focused ion beam scanning electron microscopy (FIB-SEM) to observe the three-dimensional structure of GAG chains in tendons, and based on these observations, Watanabe *et al.* proposed the ring-mesh model [7]. This model suggests that GAG chains form a ring-like structure around collagen fibrils on the d-band, creating a planar network. Within the

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Assembly of collagen fibers into contiguous dense and loose regions of subcutaneous fascia

Natsuki Maeda [1], Takafumi Watanabe [2], Daisuke Suzuki [3], Tomohito Iwasaki [4], Yongchol Shin [1] [5], Yasutada Imamura [1] [5]

Affiliations + expand

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Abstract

Objective: This study aimed to investigate the collagen fiber structure of the subcutaneous fascia, a connective tissue layer between the skin and epimysium.

Methods: Fascia samples with varying extensibility were examined using biochemical and microscopic methods.

Results: Loose fascia, the more extensible type, displayed sparsely distributed collagen fibers, while dense fascia showed tightly packed collagen fiber bundles. Elastase treatment, after urea pretreatment, caused the loosening of collagen fiber bundles and increased collagen fiber generation as the treatment time increased. This suggests that elastic fibers contribute to collagen fiber bundle formation. Additionally, elastase treatment stretched the fascia, indicating the presence of twodimensional tensile stress generated by elastic fibers. Either enzymes capable of cleaving elastic fibers may be activated or the stretching of elastic fibers accompanying tissue deformation may increase the enzyme sensitivity to elastic fibers, leading to the formation of localized collagen fibers in vivo. Tissue staining confirmed that loose and dense fascia corresponded to areas with sparse and dense collagen fibers, respectively. Some dense collagen fibers appeared to migrate and disperse into loose areas.

Conclusion: These findings provide insights into the structural organization and functional significance of collagen fibers within the subcutaneous fascia. They particularly highlight the role of elastic fibers in maintaining tissue integrity and facilitating dynamic remodeling.

Keywords: Fascia; collagen fiber; elastic fiber; elastin; subcutaneous connective tissue.

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Article

CANT1 Is Involved in Collagen Fibrogenesis in Tendons by Regulating the Synthesis of Dermatan/Chondroitin Sulfate Attached to the Decorin Core Protein

Rina Yamashita [1,2], Saki Tsutsui [2], Shuji Mizumoto [3], Takafumi Watanabe [4], Noritaka Yamamoto [5], Kenta Nakano [6], Shuhei Yamada [3], Tadashi Okamura [6] and Tatsuya Furuichi [1,2,*]

[1] Laboratory of Laboratory Animal Science and Medicine, Graduate School of Veterinary Sciences, Iwate University, Morioka 020-8550, Japan; a3124005@iwate-u.ac.jp
[2] Laboratory of Laboratory Animal Science and Medicine, Co-Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka 020-8550, Japan; shitanaga19@gmail.com
[3] Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University, Nagoya 468-8503, Japan; mizumoto@meijo-u.ac.jp (S.M.)
[4] Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; t-watanabe@rakuno.ac.jp
[5] Department of Mechanical Engineering, Ritsumeikan University, Kusatsu 525-8577, Japan; noritaka@se.ritsumei.ac.jp
[6] Department of Laboratory Animal Medicine, Research Institute, National Center for Global Health and Medicine (NCGM), Shinjuku-ku 162-8655, Japan; okamurat@ri.ncgm.go.jp (T.O.)
* Correspondence: furuichi@iwate-u.ac.jp; Tel.: +81-19-621-6222

Abstract: Tendons are connective tissues that join muscles and bones and are rich in glycosaminoglycans (GAGs). Decorin is a proteoglycan with one dermatan sulfate (DS) or chondroitin sulfate (CS) chain (a type of GAG) attached to its core protein and is involved in regulating the assembly of collagen fibrils in the tendon extracellular matrix (ECM). Calcium-activated nucleotidase 1 (CANT1), a nucleotidase that hydrolyzes uridine diphosphate into uridine monophosphate and phosphate, plays an important role in GAG synthesis in cartilage. In the present study, we performed detailed histological and biochemical analyses of the tendons from *Cant1* knockout ($Cant1^{-/-}$) mice. No abnormalities were observed in the tendons on postnatal day 1 (P1); however, remarkable hypoplasia was observed on P30 and P180. The collagen fibrils were more angular and larger in the $Cant1^{-/-}$ tendons than in the control (Ctrl) tendons. In the $Cant1^{-/-}$ tendons, the DS/CS content was significantly reduced, and the DC/CS chains attached to the decorin core protein became shorter than those in the Ctrl tendons. No abnormalities were observed in the proliferation and differentiation of tendon fibroblasts (tenocytes) in the $Cant1^{-/-}$ mice. These results strongly suggest that CANT1 dysfunction causes defective DS/CS synthesis, followed by impairment of decorin function, which regulates collagen fibrogenesis in the tendon ECM. Multiple joint dislocations are a clinical feature of Desbuquois dysplasia type 1 caused by human *CANT1* mutations. The multiple joint dislocations associated with this genetic disorder may be attributed to tendon fragility resulting from CANT1 dysfunction.

Keywords: CANT1; tendon; decorin; proteoglycan; glycosaminoglycan; dermatan sulfate; chondroitin sulfate; collagen fibril; Desbuquois dysplasia

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1. Introduction

Tendons connect muscles to bones and transmit the mechanical force of muscle contraction to the skeleton, thereby allowing mobility and joint stability. Tendons have a unique

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最終責任者　Tatsuya Furuichi (Corresponding Author)

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Injury and Fibrosis at the Myoaponeurotic Junction of Pectoralis Major and Supracoracoideus Muscles in Broiler Chickens

Takeshi Kawasaki[1], Tomohito Iwasaki[2], Takafumi Watanabe[3], Michi Yamada[4], Naoyuki Maeda[2], Yasuhiro Hasegawa[2], Naoki Takahashi[3], and Ryosuke Kobayashi[3]

[1] Research Office Concerning the Health of Humans and Birds, Abashiri, 099-3119, Japan
[2] Department of Food Science and Human Wellness, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[3] Department of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[4] Department of Sustainable Agriculture, Rakuno Gakuen University, Ebetsu, 069-8501, Japan

In this study, we aimed to identify the mechanism responsible for tissue degeneration and fibrosis in pectoral and supracoracoideus muscles. Ten chickens fed ad libitum broiler feed (Bro) were compared to 10 chickens fed breeding feed (Adj), which has lower metabolizable energy. The median body weight of Bro and Adj birds at 48 days of age was 4.9 and 0.9 kg, respectively. In Bro birds, hind legs were farther apart and tended to abduct, whereas their standing posture was often tilted forward, making them unstable. The two Bro males were heavier than the average, markedly less stable when standing or walking, and often flapped their wings vigorously to maintain balance. Myofiber damage and fibrosis were observed at the myoaponeurotic junction of the pectoralis major and supracoracoideus muscles in Bro birds. Myofiber damage and fibrosis were detected also in areas distal to the myoaponeurotic junction in the two heavier males but were otherwise less evident. By contrast, in Adj birds, almost no degeneration or fibrosis of muscle tissue was observed at the myoaponeurotic junction. In addition, the supracoracoideus muscle of one of the Bro birds showed coagulative necrosis of muscle tissue, surrounded by prominent fibrous tissue. Numerous incompletely formed blood vessels with irregular shapes and prominent branching proliferated in the fibrous tissue. These findings suggest that injury at the myoaponeurotic junction and abnormal capillary proliferation may be closely related to the formation of lesions, along with prominent fibrosis in the pectoralis major and supracoracoideus muscles.

Key words: broilers, fibrosis, myoaponeurotic junction, pectoralis major muscles, supracoracoideus muscles, wooden breast

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Introduction

The primary function of the pectoralis major and supracoracoideus muscles in flying birds is to generate and control flight forces by the wings. The pectoralis major muscles present an intramuscular aponeurosis that originates from the deltopectoral crest of the humerus and is composed of a sternobrachial (extending from the aponeurosis to the keel) and thoracobrachial (extending to the dorsal area of the ribs) portions[1,2]. The supracoracoideus muscle originates from the sternum, coracoid bone, and sternocoracoclavicular membrane to connect the dorsal tubercle of the humerus via the triosseal canal[2]. The wing downstroke is caused mainly by contraction of the pectoralis major muscle, in which the intramuscular aponeurosis acts as an axis. By contrast, wing elevation is initiated by supination of the humerus via contraction of the supracoracoideus muscle at the end of the wing downstroke[3]. Lifting the wing does not require a large force; therefore, supracoracoideus muscles are smaller than the pectoralis major muscles[2]. Indeed, the pectoralis major muscles account for 17% of body weight, whereas the supracoracoideus for 2%–4% across various species[4]. Chickens can only fly short distances at low altitudes; hence, their pectoralis major muscles account for a lower proportion of body weight (<10%), although it has risen to >20% in newer breeds of broiler chickens[5,6]. Pectoralis major muscles develop more significantly in broilers than in flying birds; however, this is not due to functional necessity but rather due to breeding selection for birds with hyperplasia

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Correspondence: Takeshi Kawasaki, DVM, Ph.D., Research Office Concerning the Health of Humans and Birds, 2-7-1, Masu-ura, Abashiri, 099-3119, Japan. (E-mail: takeshi@kawavet.com)

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Breast muscle myopathies: twists and turns in modern broilers

Elizabeth S Greene [1], Sunoh Che [2], Francesca Soglia [3], Tomohito Iwasaki [4], Takafumi Watanabe [5], Takeshi Kawasaki [6], Leonardo Susta [7], Massimiliano Petracci [3], Colin Scanes [8], Sami Dridi [1]

Affiliations — collapse

Affiliations

1 Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR, USA.
2 Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA.
3 Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy.
4 Department of Food Science and Human Wellness, Rakuno Gakuen University, Ebetsu, Japan.
5 School of Veterinary Science, Rakuno Gakuen University, Ebetsu, Japan.
6 Research Office Concerning the Health of Humans and Birds, Abashiri, Japan.
7 Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Canada.
8 Department of Biological Science, University of Wisconsin Milwaukee, Milwaukee, WI, USA.

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Abstract

Although poultry meat production supports the livelihood and provides food security for billions of people worldwide, it is facing substantial challenges. The emergence of broiler breast myopathies (white striping, woody breast, spaghetti meat) at large scale is one of the most significant economic and welfare challenges that menace poultry production sustainability and for which there is currently no effective prevention, due to its unknown aetiologies. Here, by inviting and gathering several experts with diverse, but complementary disciplines, the objective of the present review is to highlight the current progress and knowledge on these myopathies. Five sections are presented, describing in detail the history and geographic occurrence of these breast myopathies, their macroscopic morphologies and microscopic characteristics, their putative aetiologies and causes as well as their underlying molecular mechanisms, and potential strategies and solutions. The is review summarizes both descriptive and functional mechanistic studies, highlights the complexity of these myopathies and the kinship between broiler genome, nutrition, and management, and outlines some of the promising molecular signatures. It aims to offer new fundamental frameworks for future investigations.

Keywords: Breast myopathies; aetiology; characteristics; history; molecular mechanisms; strategies.

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最終責任者　Sami Dridi (Corresponding Author)

獣医麻酔学（Veterinary Anesthesiology）

Kazuto Yamashita

Professor

教授　山下　和人

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他　＜Others＞

1） Evaluation of drug distribution, sensory and motor blockade regions in canine coccygeal epidural anesthesia.
Aso R, Itami T, Hashimoto M, Hori A, Wei Y, Chen IY, **Yamashita K**.
J Vet Med Sci. 2025 Dec 8. doi: 10.1292/jvms.25-0239. Online ahead of print.

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FULL TEXT LINKS

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Evaluation of drug distribution, sensory and motor blockade regions in canine coccygeal epidural anesthesia

Ryota Aso [1], Takaharu Itami [1], Masayuki Hashimoto [1], Ai Hori [1], Yixian Wei [1], I-Ying Chen [1], Kazuto Yamashita [1]

Affiliations

Affiliation

[1] Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University.

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Free article

Abstract

The distribution of anesthetic solutions, sensory blockade (SB), and motor blockade (MB) following coccygeal epidural anesthesia in dogs were evaluated. Six dogs received three different doses (0.1, 0.2, and 0.3 mL/kg) of a mixture containing equal volumes of ropivacaine and iohexol, administered via the first and second coccygeal intervertebral spaces. Drug spread was assessed using computed tomography, whereas SB and MB were evaluated using standardized scoring methods. The cranial extent of contrast medium spread reached L5, the L4-5 interspace, and L4 in the 0.1, 0.2, and 0.3 mL/kg groups, respectively, with no significant differences among groups. SB extended from the tail to the cranial femoral region in the 0.1 mL/kg group, to the caudal femoral-pubic region in the 0.2 mL/kg group, and to the cranial femoral-pubic region in the 0.3 mL/kg group. The 0.2 and 0.3 mL/kg groups showed significantly higher SB than the 0.1 mL/kg group did (P=0.039 and P=0.019, respectively). MB extended to the femoral nerve territory in dogs receiving 0.2 or 0.3 mL/kg, while dogs in the 0.1 mL/kg group maintained motor function. The 0.3 mL/kg group showed significantly higher MB than the 0.1 mL/kg group did (P=0.019). Despite the increased volume at 0.3 mL/kg, greater leakage through the neural foramina resulted in a net epidural dosage comparable to 0.2 mL/kg. These findings indicate that coccygeal anesthesia at 0.1 mL/kg provides SB from the tail to the perineal region while maintaining hindlimb motor function in dogs.

Keywords: canine; coccygeal epidural anesthesia; contrast medium; drug distribution; neural blockade.

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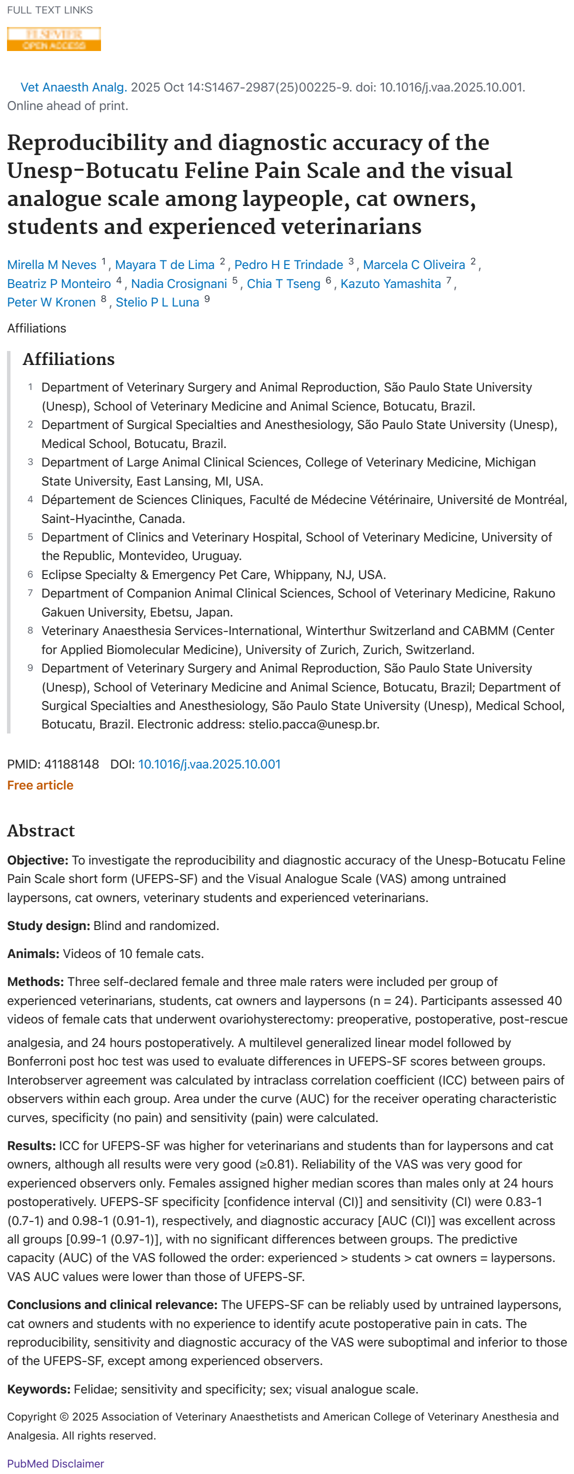

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Reproducibility and diagnostic accuracy of the Unesp-Botucatu Feline Pain Scale and the visual analogue scale among laypeople, cat owners, students and experienced veterinarians

Mirella M Neves [1], Mayara T de Lima [2], Pedro H E Trindade [3], Marcela C Oliveira [2], Beatriz P Monteiro [4], Nadia Crosignani [5], Chia T Tseng [6], Kazuto Yamashita [7], Peter W Kronen [8], Stelio P L Luna [9]

Affiliations

Affiliations

1 Department of Veterinary Surgery and Animal Reproduction, São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Brazil.
2 Department of Surgical Specialties and Anesthesiology, São Paulo State University (Unesp), Medical School, Botucatu, Brazil.
3 Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.
4 Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Canada.
5 Department of Clinics and Veterinary Hospital, School of Veterinary Medicine, University of the Republic, Montevideo, Uruguay.
6 Eclipse Specialty & Emergency Pet Care, Whippany, NJ, USA.
7 Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan.
8 Veterinary Anaesthesia Services-International, Winterthur Switzerland and CABMM (Center for Applied Biomolecular Medicine), University of Zurich, Zurich, Switzerland.
9 Department of Veterinary Surgery and Animal Reproduction, São Paulo State University (Unesp), School of Veterinary Medicine and Animal Science, Botucatu, Brazil; Department of Surgical Specialties and Anesthesiology, São Paulo State University (Unesp), Medical School, Botucatu, Brazil. Electronic address: stelio.pacca@unesp.br.

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Free article

Abstract

Objective: To investigate the reproducibility and diagnostic accuracy of the Unesp-Botucatu Feline Pain Scale short form (UFEPS-SF) and the Visual Analogue Scale (VAS) among untrained laypersons, cat owners, veterinary students and experienced veterinarians.

Study design: Blind and randomized.

Animals: Videos of 10 female cats.

Methods: Three self-declared female and three male raters were included per group of experienced veterinarians, students, cat owners and laypersons (n = 24). Participants assessed 40 videos of female cats that underwent ovariohysterectomy: preoperative, postoperative, post-rescue analgesia, and 24 hours postoperatively. A multilevel generalized linear model followed by Bonferroni post hoc test was used to evaluate differences in UFEPS-SF scores between groups. Interobserver agreement was calculated by intraclass correlation coefficient (ICC) between pairs of observers within each group. Area under the curve (AUC) for the receiver operating characteristic curves, specificity (no pain) and sensitivity (pain) were calculated.

Results: ICC for UFEPS-SF was higher for veterinarians and students than for laypersons and cat owners, although all results were very good (≥0.81). Reliability of the VAS was very good for experienced observers only. Females assigned higher median scores than males only at 24 hours postoperatively. UFEPS-SF specificity [confidence interval (CI)] and sensitivity (CI) were 0.83-1 (0.7-1) and 0.98-1 (0.91-1), respectively, and diagnostic accuracy [AUC (CI)] was excellent across all groups [0.99-1 (0.97-1)], with no significant differences between groups. The predictive capacity (AUC) of the VAS followed the order: experienced > students > cat owners = laypersons. VAS AUC values were lower than those of UFEPS-SF.

Conclusions and clinical relevance: The UFEPS-SF can be reliably used by untrained laypersons, cat owners and students with no experience to identify acute postoperative pain in cats. The reproducibility, sensitivity and diagnostic accuracy of the VAS were suboptimal and inferior to those of the UFEPS-SF, except among experienced observers.

Keywords: Felidae; sensitivity and specificity; sex; visual analogue scale.

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FULL PAPER

Surgery

Evaluation of oxygen reserve index as an early warning indicator of hypoxemia in anesthetized dogs

Taku HIROKAWA[1,2], Takaharu ITAMI[1]*, Keiko KATO[1], Yujiro MINAMOTO[1], I-Ying CHEN[1], Chihiro SUGITA[1], Yusuke ENDO[1,3], Taku MIYASHO[1], Kazuto YAMASHITA[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]Nirenoki Animal Hospital, Hokkaido, Japan
[3]Feinstein Institutes for Medical Research, Manhasset, NY, USA

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ABSTRACT. The oxygen reserve index (ORi) is a non-invasive parameter recently introduced in human medicine that detects impending hypoxemia earlier than peripheral oxygen saturation (SpO_2). This study evaluated the utility of ORi as an early warning indicator of hypoxemia in anesthetized dogs. Six beagle dogs were subjected to planned apnea under two oxygen conditions (FiO_2 1.0 and 0.4). Changes in ORi, SpO_2, and arterial oxygen partial pressure (PaO_2) were monitored. Warning times (WT) were calculated as the time difference between each timepoint and SpO_2 reaching 90%. We defined Added warning times (AWT) as the time between an ORi warning point and the moment SpO_2 fell to 96%. When ORi decreased to half of its baseline value or reached 0.4, it provided early warnings of 43.2 ± 24.6 sec and 44.7 ± 37.0 sec, under FiO_2 1.0, and 29.5 ± 21.9 sec and 37.0 ± 22.5 sec under FiO_2 0.4, before SpO_2 began to decrease. A significant positive correlation was observed between ORi and PaO_2 when SpO_2 was ≥97% (r=0.74, P<0.0001). Receiver operating characteristic (ROC) analysis showed high diagnostic accuracy of ORi for predicting PaO_2 ≥150 mmHg (area under the curve [AUC]=0.92), with an optimal cutoff value of 0.48 (sensitivity 92%, specificity 78.8%). Unlike in humans, ORi continued to change at PaO_2 levels above 200 mmHg in dogs, suggesting species-specific differences. In conclusion, ORi can detect deterioration in oxygenation earlier than SpO_2 in anesthetized dogs and provides valuable non-invasive real-time monitoring of oxygen reserves during controlled apnea under anesthesia.

KEYWORDS: anesthesia, apnea, hypoxemia, oxygen reserve index, pulse oximetry

*Correspondence to: Itami T: t-itami@rakuno.ac.jp, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

(Supplementary material: refer to J-STAGE: https://www.jstage.jst.go.jp/browse/jvms/)

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The Journal of
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NOTE

Internal Medicine

Inter-device variability in lactate measurement in dogs: comparison of blood gas analyzer and portable lactate meter

Mayu FUJITA[1], Takaharu ITAMI[1]*, Kodai KAWASE[2], Tomoya MORITA[2], Kazuto YAMASHITA[1]

[1]Department of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]Sapporo Veterinary Emergency Hospital, Hokkaido, Japan

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ABSTRACT. Lactate levels are critical indicators of severe, life-threatening conditions and are widely used as prognostic markers in veterinary emergency care. Blood gas analyzers and portable lactate meters are common tools, but discrepancies between devices, especially at high lactate levels, have been reported. This study aimed to evaluate inter-device variability in canine lactate measurement. Lactate levels in 118 dogs were measured using a blood gas analyzer and a portable lactate meter. A strong correlation (R=0.906) was observed; however, the portable meter exhibited an 18% positive bias primarily characterized by additive error. These findings emphasize the need for careful device selection and the standardization of lactate measurement methods.

KEYWORDS: blood gas analyzer, canine, inter-device variability, lactate, portable lactate meter

*Correspondence to: Itami T: t-itami@rakuno.ac.jp, Department of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

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最終責任者　Takaharu Itami (Corresponding Author)

獣医ウイルス学ユニット (Veterinary Virology)

Tomo Daidoji

Associate Professor

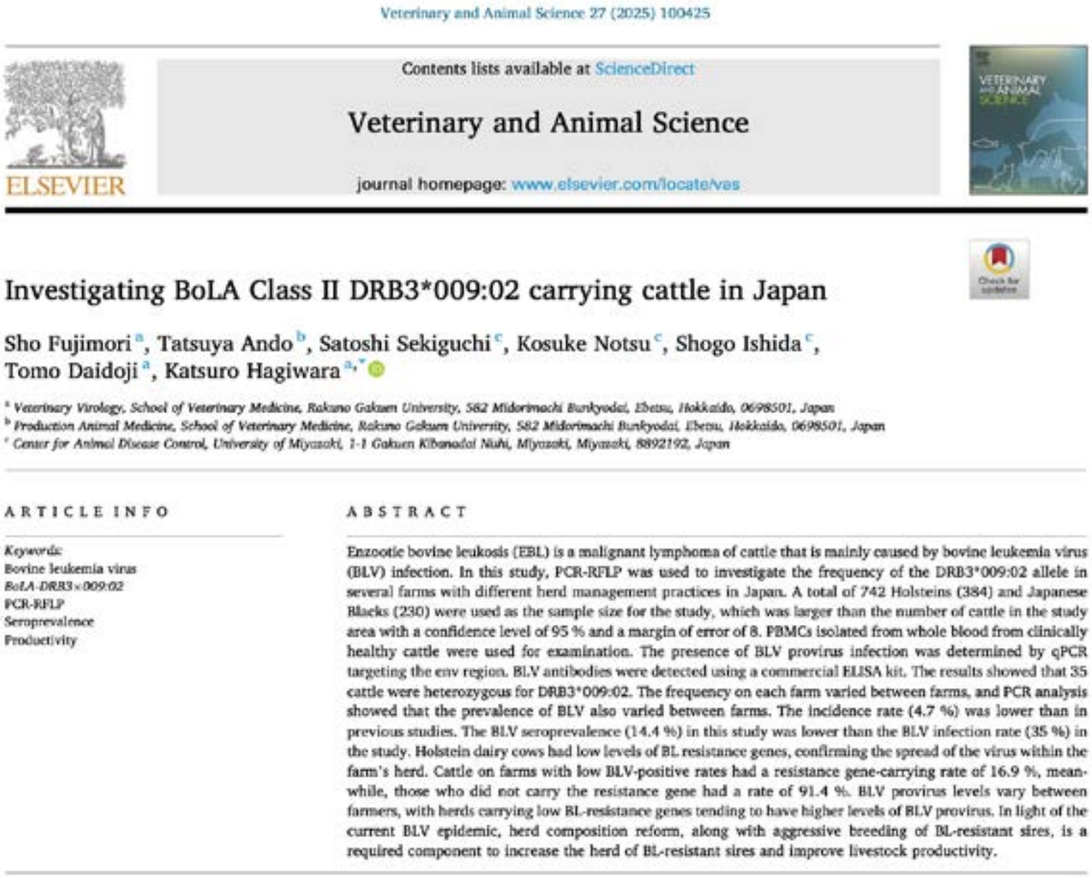
准教授　大道寺　智

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他＜Others＞

1）Investigating BoLA Class II DRB3* 009: 02 carrying cattle in Japan.
Fujimori S, Ando T, Sekiguchi S, Notsu K, Ishida S, **Daidoji T**, Hagiwara K.
Vet Anim Sci. 27:100425. 2025



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Investigating BoLA Class II DRB3*009:02 carrying cattle in Japan

Sho Fujimori[a], Tatsuya Ando[b], Satoshi Sekiguchi[c], Kosuke Notsu[c], Shogo Ishida[c],
Tomo Daidoji[a], Katsuro Hagiwara[a,*]

[a] *Veterinary Virology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi Bunkyodai, Ebetsu, Hokkaido, 0698501, Japan*
[b] *Production Animal Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi Bunkyodai, Ebetsu, Hokkaido, 0698501, Japan*
[c] *Center for Animal Disease Control, University of Miyazaki, 1-1 Gakuen Kibanadai Nishi, Miyazaki, Miyazaki, 8892192, Japan*

ARTICLE INFO

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Bovine leukemia virus
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ABSTRACT

Enzootic bovine leukosis (EBL) is a malignant lymphoma of cattle that is mainly caused by bovine leukemia virus (BLV) infection. In this study, PCR-RFLP was used to investigate the frequency of the DRB3*009:02 allele in several farms with different herd management practices in Japan. A total of 742 Holsteins (384) and Japanese Blacks (230) were used as the sample size for the study, which was larger than the number of cattle in the study area with a confidence level of 95 % and a margin of error of 8. PBMCs isolated from whole blood from clinically healthy cattle were used for examination. The presence of BLV provirus infection was determined by qPCR targeting the env region. BLV antibodies were detected using a commercial ELISA kit. The results showed that 35 cattle were heterozygous for DRB3*009:02. The frequency on each farm varied between farms, and PCR analysis showed that the prevalence of BLV also varied between farms. The incidence rate (4.7 %) was lower than in previous studies. The BLV seroprevalence (14.4 %) in this study was lower than the BLV infection rate (35 %) in the study. Holstein dairy cows had low levels of BL resistance genes, confirming the spread of the virus within the farm's herd. Cattle on farms with low BLV-positive rates had a resistance gene-carrying rate of 16.9 %, meanwhile, those who did not carry the resistance gene had a rate of 91.4 %. BLV provirus levels vary between farmers, with herds carrying low BL-resistance genes tending to have higher levels of BLV provirus. In light of the current BLV epidemic, herd composition reform, along with aggressive breeding of BL-resistant sires, is a required component to increase the herd of BL-resistant sires and improve livestock productivity.

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最終責任者　Katsuhiro Hagiwara (Corresponding Auth

ハードヘルス学（Veterinary Herd Health）

Rika Fukumori

Associate professor

准教授　福森　理加

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Effects of amount of lactose in milk replacer on gastrointestinal function of dairy calves.
Fukumori R, Nakayama T, Hirose M, Norimura I, Izumi K, Shimada K, Mineo H, Steele MA, Gondaira S, Higuchi H, Watanabe T, Ueda H, Sano T, Chisato K, Oikawa S.
Animal Feed Science and Technology 319.116157. 2025.
doi.org/110.1016/j.anifeedsci.2024.1

2）Lipoprotein composition of calves before and after weaning and comparison with adult cows.
Satoh H, Kumano R, **Fukumori R**, Oikawa S.
J Vet Med Sci. 87 167-170. 2025.
doi.org/110.1292/jvms.24-0172

3）Effects of ruminal administration of propylene glycol or sucrose on ruminal, blood, and hepatic parameters in nonlactating cows with high plasma nonesterified fatty acid concentrations.
Chisato K, **Fukumori R**, Imaishi R, Gondaira S, Higuchi H, Izumi K, Oikawa S.
Anim Sci J. 96 e70100. 2025.

4）Effects of bypass sugar supplementation from the close-up period through 5 days after calving on milk production, blood profiles, and health conditions in dairy cows.
Hitomi Satoh, **Fukumori R**, Oikawa S.
Anim Biosci (in press) 2025. doi.org/110.5713/ab.250489

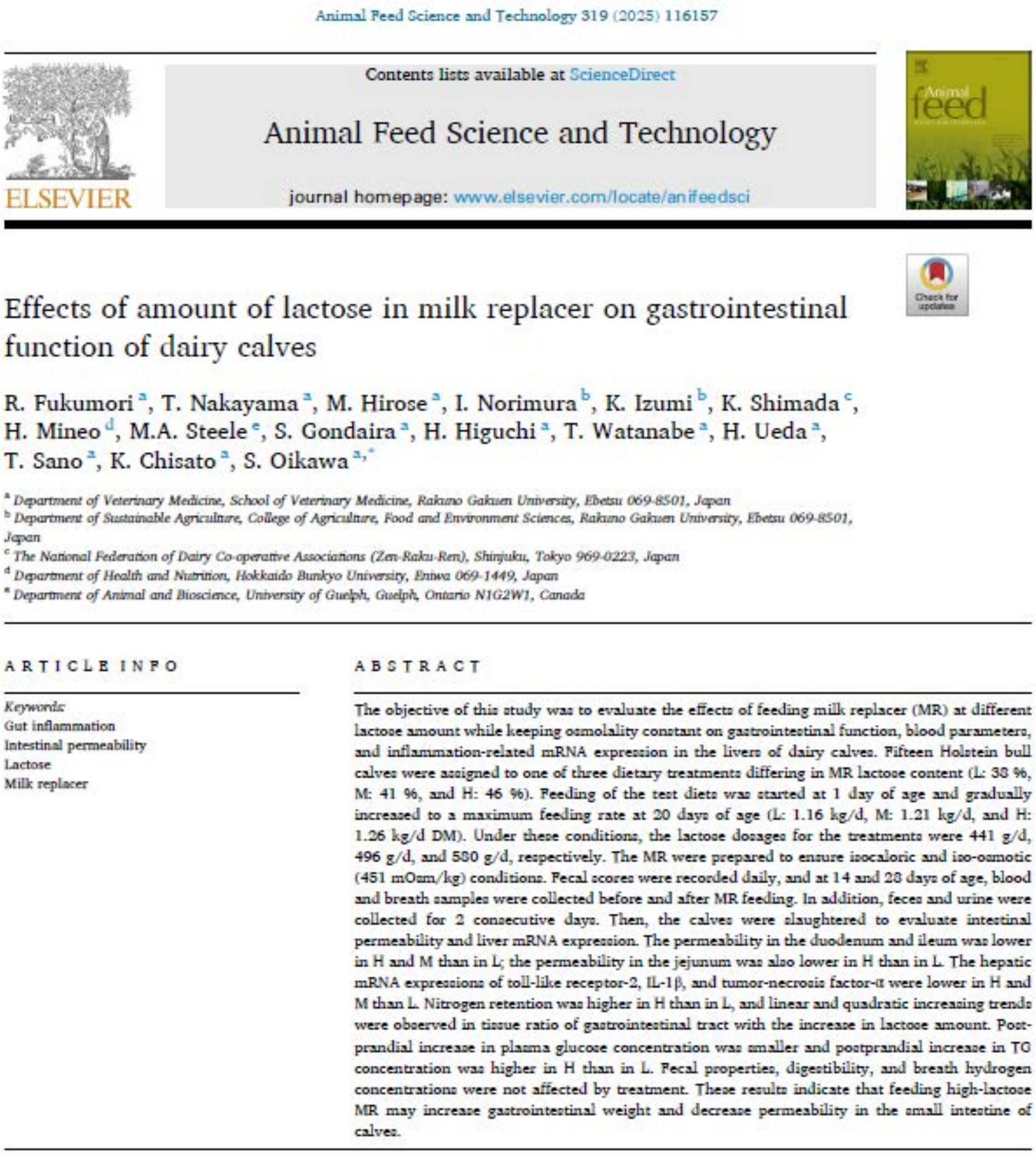
II. その他＜Others＞

1） Evaluation of Colostrum Components and Milking Status Affecting Colostrum IgG Concentration. Kayasaki S, Satoh H, Oguchi K, Chisato K, **Fukumori R**, Oikawa S. *Animals* 15 1-10. 2025.
doi.org/110.3390/ani15050718

2） Significance of serum pepsinogen value in close-up dairy cattle as a displaced abomasum predictive marker: a case-control study.
Nishinakagawa J, Oikawa S, Chisato K, **Fukumori R**, Tharwat M.
*Res Vet Sci.*105925-105925 2025.
doi.org/110.1016/j.rvsc.2025.105925

3） Effects of Preventive Administration of Propylene Glycol or Sucrose in Dairy Cows with Elevated Blood Non-Esterified Fatty Acids During the Close-Up Period.
Chisato K, Ishizaka M, Honjo T, Watanabe Y, **Fukumori R**, Oikawa S.
Animals 15 3211-3211 2025. doi.org/110.3390/ani15213211

4） Mitochondrial function and nutrient partitioning in high and low feed efficient multiparous Holstein dairy cows.
Kendall SJ, Martinez-Boggio G, Arshad U, Kennedy KM, **Fukumori R**, Rodrigues Wenzel M, France TL, Rostoll-Cangiano L, Weigel KA, Zhou Z, VandeHaar MJ, Peñagaricano F, White HM.
J Dairy Sci (in press) 2025. doi.org/110.3168/jds.2025-27001



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Effects of amount of lactose in milk replacer on gastrointestinal function of dairy calves

R. Fukumori[a], T. Nakayama[a], M. Hirose[a], I. Norimura[b], K. Izumi[b], K. Shimada[c], H. Mineo[d], M.A. Steele[e], S. Gondaira[a], H. Higuchi[a], T. Watanabe[a], H. Ueda[a], T. Sano[a], K. Chisato[a], S. Oikawa[a,*]

[a] *Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[b] *Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[c] *The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Shinjuku, Tokyo 969-0223, Japan*
[d] *Department of Health and Nutrition, Hokkaido Bunkyo University, Eniwa 069-1449, Japan*
[e] *Department of Animal and Bioscience, University of Guelph, Guelph, Ontario N1G2W1, Canada*

ARTICLE INFO

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Gut inflammation
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ABSTRACT

The objective of this study was to evaluate the effects of feeding milk replacer (MR) at different lactose amount while keeping osmolality constant on gastrointestinal function, blood parameters, and inflammation-related mRNA expression in the livers of dairy calves. Fifteen Holstein bull calves were assigned to one of three dietary treatments differing in MR lactose content (L: 38 %, M: 41 %, and H: 46 %). Feeding of the test diets was started at 1 day of age and gradually increased to a maximum feeding rate at 20 days of age (L: 1.16 kg/d, M: 1.21 kg/d, and H: 1.26 kg/d DM). Under these conditions, the lactose dosages for the treatments were 441 g/d, 496 g/d, and 580 g/d, respectively. The MR were prepared to ensure isocaloric and iso-osmotic (451 mOsm/kg) conditions. Fecal scores were recorded daily, and at 14 and 28 days of age, blood and breath samples were collected before and after MR feeding. In addition, feces and urine were collected for 2 consecutive days. Then, the calves were slaughtered to evaluate intestinal permeability and liver mRNA expression. The permeability in the duodenum and ileum was lower in H and M than in L; the permeability in the jejunum was also lower in H than in L. The hepatic mRNA expressions of toll-like receptor-2, IL-1β, and tumor-necrosis factor-α were lower in H and M than L. Nitrogen retention was higher in H than in L, and linear and quadratic increasing trends were observed in tissue ratio of gastrointestinal tract with the increase in lactose amount. Post-prandial increase in plasma glucose concentration was smaller and postprandial increase in TG concentration was higher in H than in L. Fecal properties, digestibility, and breath hydrogen concentrations were not affected by treatment. These results indicate that feeding high-lactose MR may increase gastrointestinal weight and decrease permeability in the small intestine of calves.

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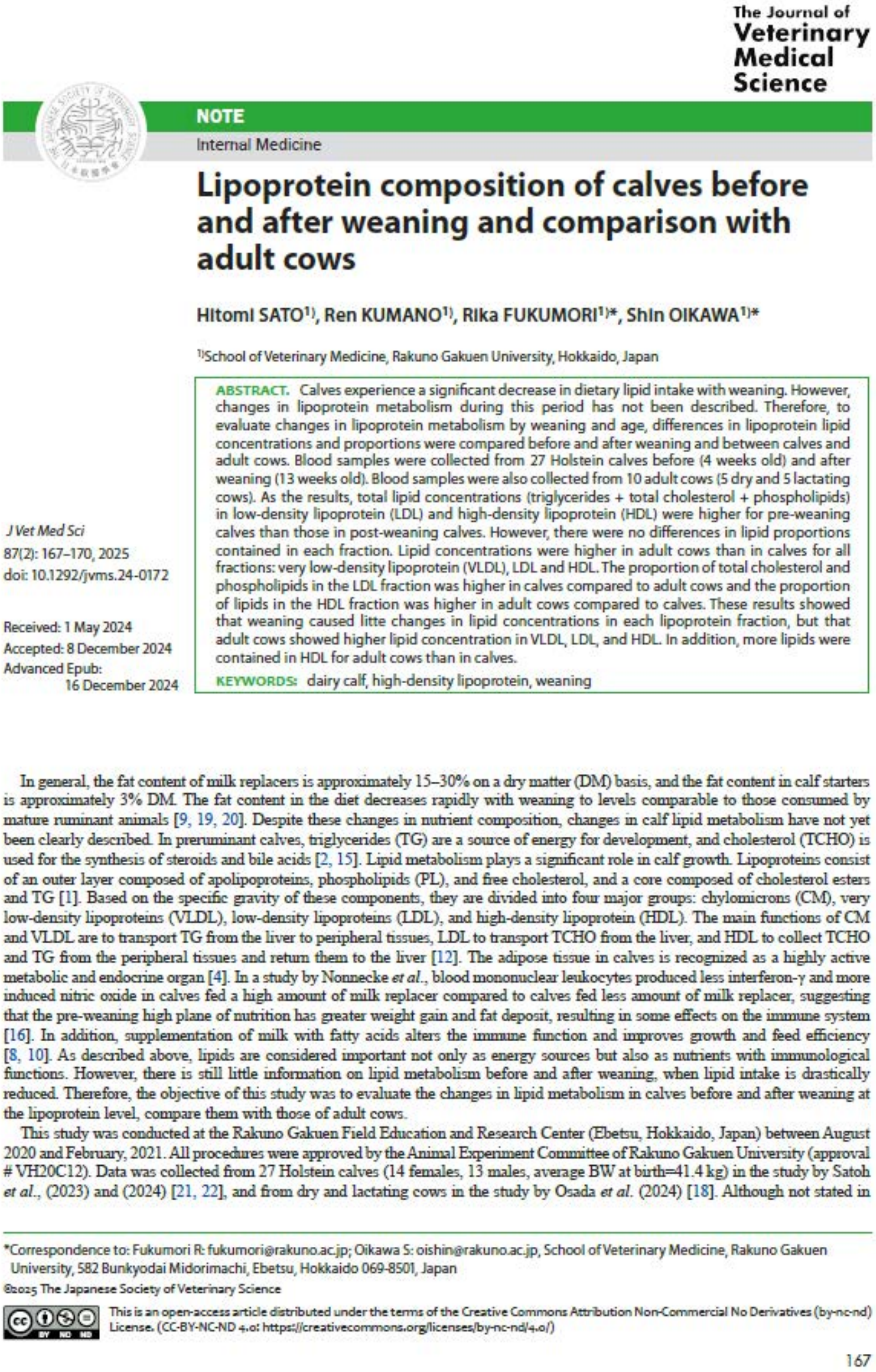
筆頭著者　Rika Fukumori
最終責任者　Shin Oikawa (Corresponding Author)



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NOTE

Internal Medicine

Lipoprotein composition of calves before and after weaning and comparison with adult cows

Hitomi SATO[1)], Ren KUMANO[1)], Rika FUKUMORI[1)]*, Shin OIKAWA[1)]*

[1)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. Calves experience a significant decrease in dietary lipid intake with weaning. However, changes in lipoprotein metabolism during this period has not been described. Therefore, to evaluate changes in lipoprotein metabolism by weaning and age, differences in lipoprotein lipid concentrations and proportions were compared before and after weaning and between calves and adult cows. Blood samples were collected from 27 Holstein calves before (4 weeks old) and after weaning (13 weeks old). Blood samples were also collected from 10 adult cows (5 dry and 5 lactating cows). As the results, total lipid concentrations (triglycerides + total cholesterol + phospholipids) in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were higher for pre-weaning calves than those in post-weaning calves. However, there were no differences in lipid proportions contained in each fraction. Lipid concentrations were higher in adult cows than in calves for all fractions: very low-density lipoprotein (VLDL), LDL and HDL. The proportion of total cholesterol and phospholipids in the LDL fraction was higher in calves compared to adult cows and the proportion of lipids in the HDL fraction was higher in adult cows compared to calves. These results showed that weaning caused litte changes in lipid concentrations in each lipoprotein fraction, but that adult cows showed higher lipid concentration in VLDL, LDL, and HDL. In addition, more lipids were contained in HDL for adult cows than in calves.

KEYWORDS: dairy calf, high-density lipoprotein, weaning

In general, the fat content of milk replacers is approximately 15–30% on a dry matter (DM) basis, and the fat content in calf starters is approximately 3% DM. The fat content in the diet decreases rapidly with weaning to levels comparable to those consumed by mature ruminant animals [9, 19, 20]. Despite these changes in nutrient composition, changes in calf lipid metabolism have not yet been clearly described. In preruminant calves, triglycerides (TG) are a source of energy for development, and cholesterol (TCHO) is used for the synthesis of steroids and bile acids [2, 15]. Lipid metabolism plays a significant role in calf growth. Lipoproteins consist of an outer layer composed of apolipoproteins, phospholipids (PL), and free cholesterol, and a core composed of cholesterol esters and TG [1]. Based on the specific gravity of these components, they are divided into four major groups: chylomicrons (CM), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoprotein (HDL). The main functions of CM and VLDL are to transport TG from the liver to peripheral tissues, LDL to transport TCHO from the liver, and HDL to collect TCHO and TG from the peripheral tissues and return them to the liver [12]. The adipose tissue in calves is recognized as a highly active metabolic and endocrine organ [4]. In a study by Nonnecke *et al.*, blood mononuclear leukocytes produced less interferon-γ and more induced nitric oxide in calves fed a high amount of milk replacer compared to calves fed less amount of milk replacer, suggesting that the pre-weaning high plane of nutrition has greater weight gain and fat deposit, resulting in some effects on the immune system [16]. In addition, supplementation of milk with fatty acids alters the immune function and improves growth and feed efficiency [8, 10]. As described above, lipids are considered important not only as energy sources but also as nutrients with immunological functions. However, there is still little information on lipid metabolism before and after weaning, when lipid intake is drastically reduced. Therefore, the objective of this study was to evaluate the changes in lipid metabolism in calves before and after weaning at the lipoprotein level, compare them with those of adult cows.

This study was conducted at the Rakuno Gakuen Field Education and Research Center (Ebetsu, Hokkaido, Japan) between August 2020 and February, 2021. All procedures were approved by the Animal Experiment Committee of Rakuno Gakuen University (approval # VH20C12). Data was collected from 27 Holstein calves (14 females, 13 males, average BW at birth=41.4 kg) in the study by Satoh *et al.*, (2023) and (2024) [21, 22], and from dry and lactating cows in the study by Osada *et al.* (2024) [18]. Although not stated in

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*Correspondence to: Fukumori R: fukumori@rakuno.ac.jp; Oikawa S: oishin@rakuno.ac.jp, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

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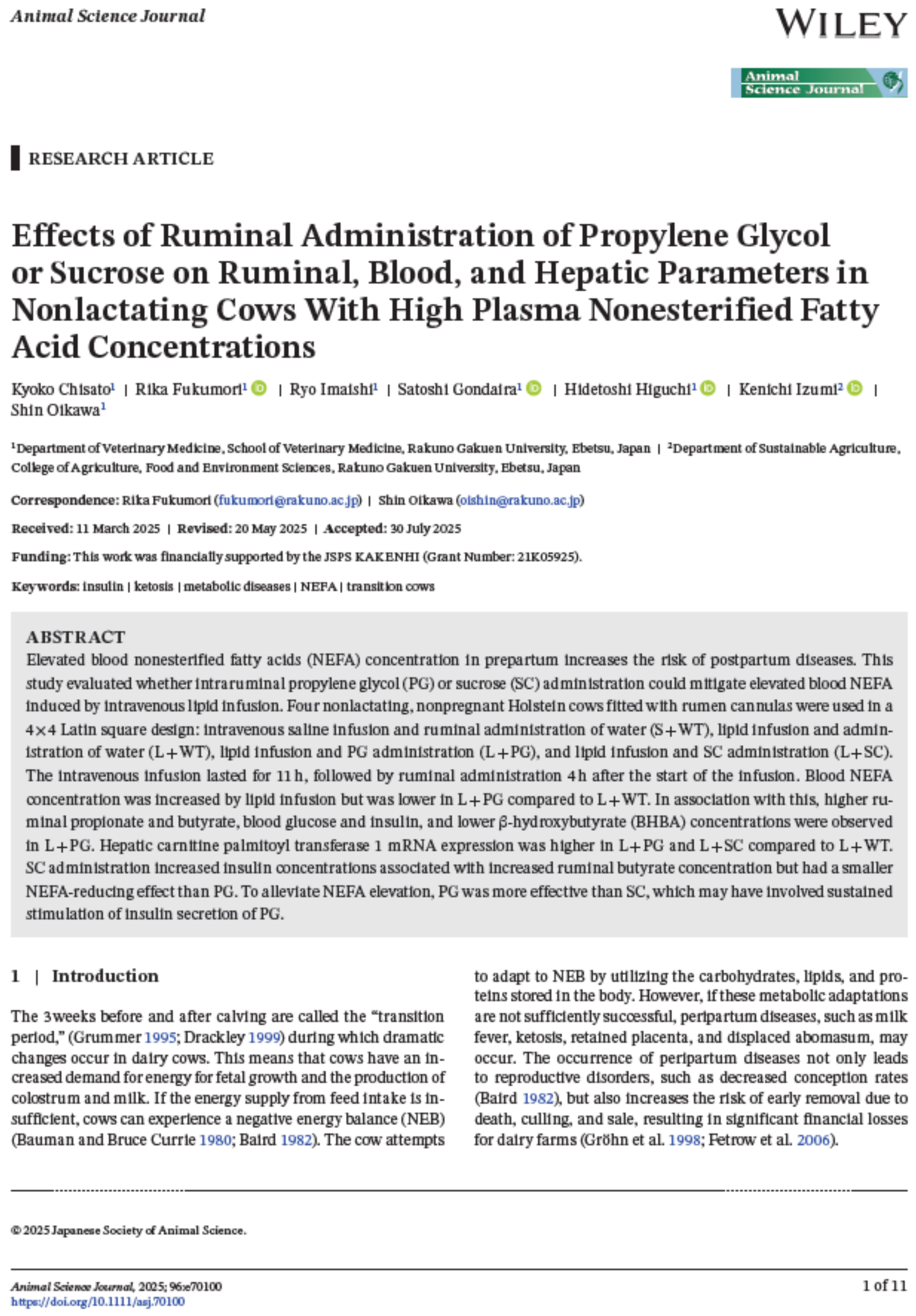

Animal Science Journal

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Animal Science Journal

RESEARCH ARTICLE

Effects of Ruminal Administration of Propylene Glycol or Sucrose on Ruminal, Blood, and Hepatic Parameters in Nonlactating Cows With High Plasma Nonesterified Fatty Acid Concentrations

Kyoko Chisato[1] | Rika Fukumori[1] | Ryo Imaishi[1] | Satoshi Gondaira[1] | Hidetoshi Higuchi[1] | Kenichi Izumi[2] | Shin Oikawa[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu, Japan

Correspondence: Rika Fukumori (fukumori@rakuno.ac.jp) | Shin Oikawa (oishin@rakuno.ac.jp)

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Keywords: insulin | ketosis | metabolic diseases | NEFA | transition cows

ABSTRACT

Elevated blood nonesterified fatty acids (NEFA) concentration in prepartum increases the risk of postpartum diseases. This study evaluated whether intraruminal propylene glycol (PG) or sucrose (SC) administration could mitigate elevated blood NEFA induced by intravenous lipid infusion. Four nonlactating, nonpregnant Holstein cows fitted with rumen cannulas were used in a 4×4 Latin square design: intravenous saline infusion and ruminal administration of water (S+WT), lipid infusion and administration of water (L+WT), lipid infusion and PG administration (L+PG), and lipid infusion and SC administration (L+SC). The intravenous infusion lasted for 11 h, followed by ruminal administration 4 h after the start of the infusion. Blood NEFA concentration was increased by lipid infusion but was lower in L+PG compared to L+WT. In association with this, higher ruminal propionate and butyrate, blood glucose and insulin, and lower β-hydroxybutyrate (BHBA) concentrations were observed in L+PG. Hepatic carnitine palmitoyl transferase 1 mRNA expression was higher in L+PG and L+SC compared to L+WT. SC administration increased insulin concentrations associated with increased ruminal butyrate concentration but had a smaller NEFA-reducing effect than PG. To alleviate NEFA elevation, PG was more effective than SC, which may have involved sustained stimulation of insulin secretion of PG.

1 | Introduction

The 3 weeks before and after calving are called the “transition period,” (Grummer 1995; Drackley 1999) during which dramatic changes occur in dairy cows. This means that cows have an increased demand for energy for fetal growth and the production of colostrum and milk. If the energy supply from feed intake is insufficient, cows can experience a negative energy balance (NEB) (Bauman and Bruce Currie 1980; Baird 1982). The cow attempts to adapt to NEB by utilizing the carbohydrates, lipids, and proteins stored in the body. However, if these metabolic adaptations are not sufficiently successful, peripartum diseases, such as milk fever, ketosis, retained placenta, and displaced abomasum, may occur. The occurrence of peripartum diseases not only leads to reproductive disorders, such as decreased conception rates (Baird 1982), but also increases the risk of early removal due to death, culling, and sale, resulting in significant financial losses for dairy farms (Gröhn et al. 1998; Fetrow et al. 2006).

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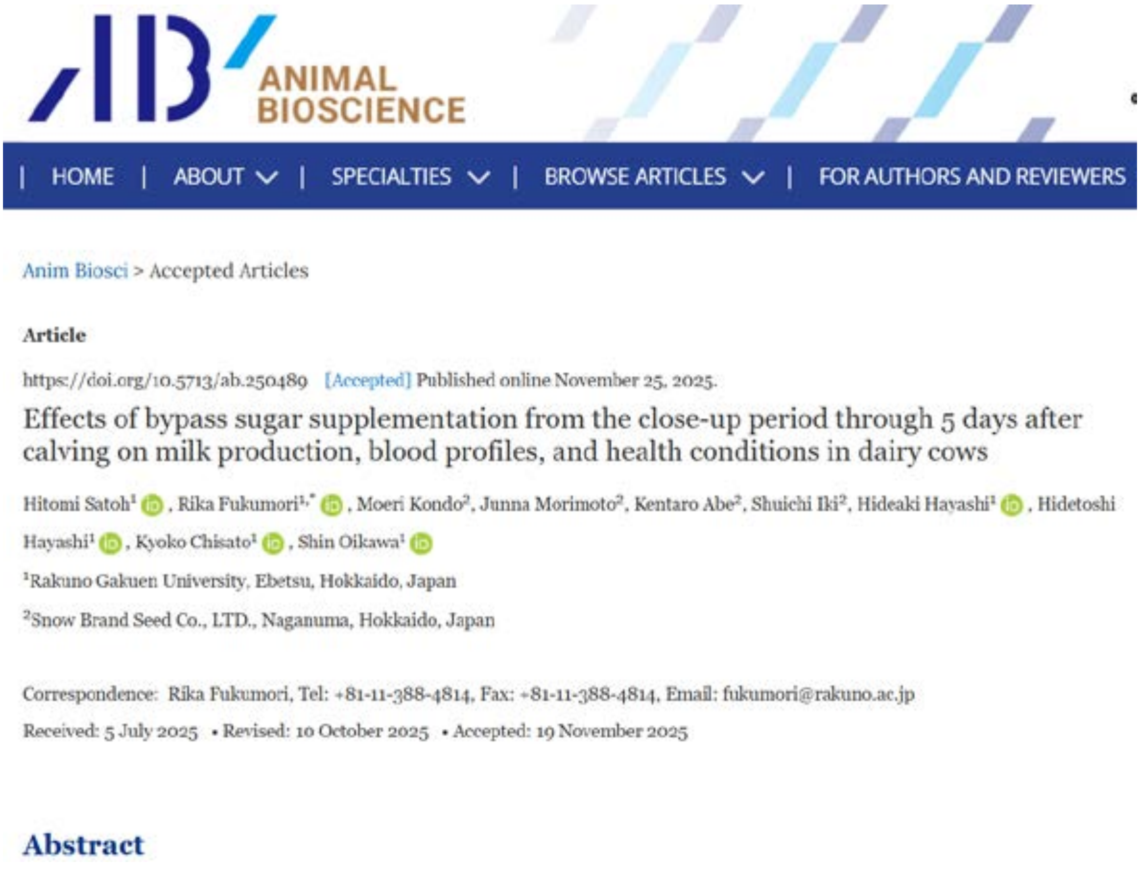

Article

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Effects of bypass sugar supplementation from the close-up period through 5 days after calving on milk production, blood profiles, and health conditions in dairy cows

Hitomi Satoh[1], Rika Fukumori[1,*], Moeri Kondo[2], Junna Morimoto[2], Kentaro Abe[2], Shuichi Iki[2], Hideaki Hayashi[1], Hidetoshi Hayashi[1], Kyoko Chisato[1], Shin Oikawa[1]

[1]Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[2]Snow Brand Seed Co., LTD., Naganuma, Hokkaido, Japan

Correspondence: Rika Fukumori, Tel: +81-11-388-4814, Fax: +81-11-388-4814, Email: fukumori@rakuno.ac.jp

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Abstract

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animals

MDPI

Article

Evaluation of Colostrum Components and Milking Status Affecting Colostrum IgG Concentration

Shuji Kayasaki [1,2], Hitomi Satoh [2], Keitaro Oguchi [2], Kyoko Chisato [2], Rika Fukumori [2] and Shin Oikawa [2,*]

[1] Nijibetsu Livestock Clinic, Kushiro Central Branch, East Regional Center, Hokkaido Agricultural Mutual Aid Association, 103-1, Line 67, Nijibetsu Genya, Shibecha-cho 088-2461, Hokkaido, Japan; s22041006@g.rakuno.ac.jp

[2] Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu-shi 069-8501, Hokkaido, Japan; s22241004@stu.rakuno.ac.jp (H.S.); s21761048@g.rakuno.ac.jp (K.O.); k-chisato@rakuno.ac.jp (K.C.); fukumori@rakuno.ac.jp (R.F.)

* Correspondence: oishin@rakuno.ac.jp; Tel.: +81-11-388-4825

Simple Summary: The objective of this study was to examine the factors that contribute to high-quality colostrum in dairy farms in eastern Hokkaido, Japan. The percentage of samples with a high colostrum IgG concentration, an indicator of passive transfer (≥50 g/L), was low, at 48.9%, but the percentage of those with a low total plate count, an indicator of bacterial contamination (<100,000 CFU/mL), was high, at 86.5%. Measurement of colostrum Brix value, a measure of solid component concentration, provided a practical estimate of colostrum IgG concentration. The criteria for obtaining colostrum with high IgG concentration were high parity, low milking volume, and a short time from calving to milking. The estimation of colostrum IgG concentration by Brix meter and the approaching of ideal milking status were thought to lead to the obtaining of high-IgG colostrum.

Abstract: This study investigated IgG concentrations, bacterial contamination, and nutrient components of colostrum on dairy farms in eastern Hokkaido, Japan, to examine the factors making it possible to obtain high-quality colostrum. Colostrum samples were collected from 266 Holstein cows. The general criteria for high-quality colostrum are an IgG concentration of 50 g/L or higher and a total plate count of less than 100,000 CFU/mL. The percentage of samples with the criteria for IgG concentration was low, at 48.9%, but the percentage with the criteria for total plate count was high, at 86.5%. The colostrum Brix value was significantly correlated with the colostrum IgG concentration ($r^2 = 0.233$, $p < 0.001$) and provided a rough estimate of that concentration. There was no association between the colostrum IgG concentration and milk components other than protein. For colostrum milking conditions, the time from calving to milking tended to be shorter for high-IgG colostrum than for low-IgG colostrum ($p < 0.01$). The likelihood of high-IgG colostrum increased 1.28-fold with first parity higher and decreased 0.92-fold with a 1 L higher milking volume ($p < 0.01$). The results suggest that, in addition to estimating the colostrum IgG concentration via the Brix value, the likelihood of obtaining high-IgG colostrum is increased by taking into account parity, milking volume, and time from calving to milking.

Keywords: cow; colostrum; immunoglobulin G (IgG); bacteria; Brix; milking status

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1. Introduction

In calf management, the health implications of colostrum feeding are highlighted. Due to their anatomical structure, newborn calves have no transfer of antibodies via

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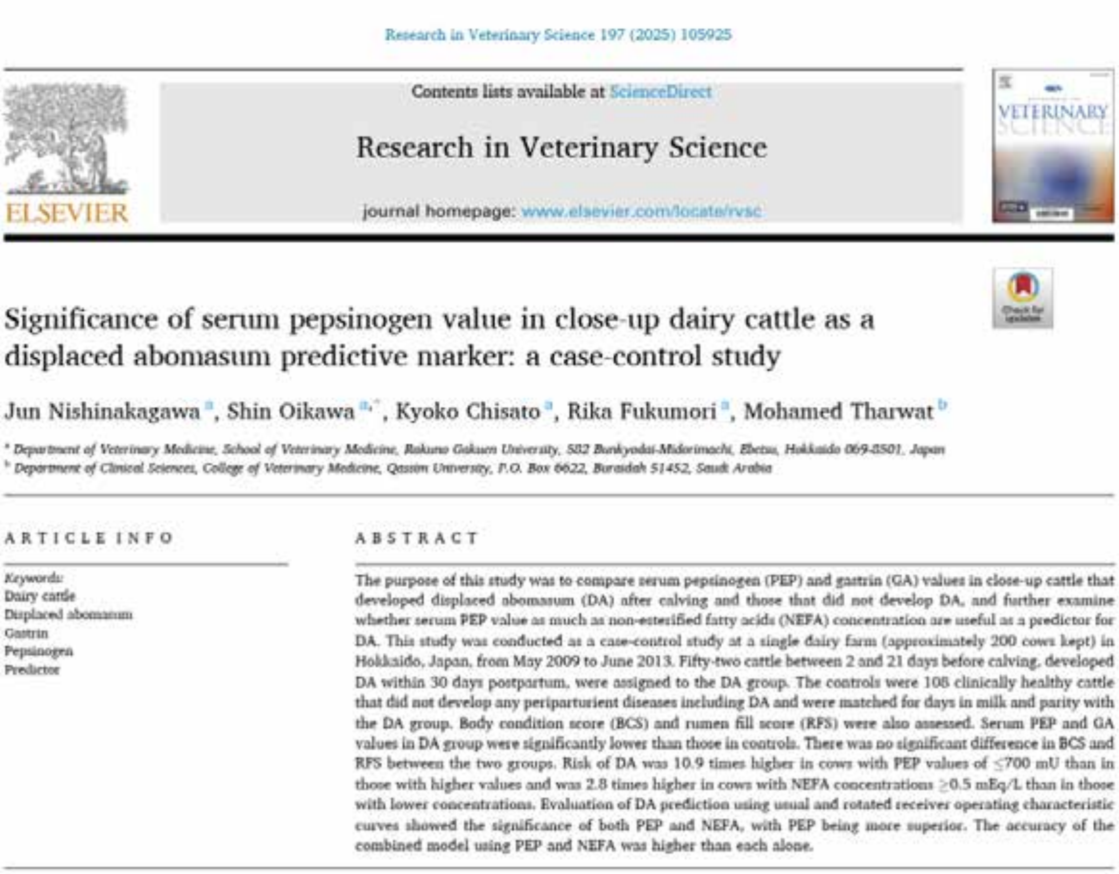

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Significance of serum pepsinogen value in close-up dairy cattle as a displaced abomasum predictive marker: a case-control study

Jun Nishinakagawa[a], Shin Oikawa[a,*], Kyoko Chisato[a], Rika Fukumori[a], Mohamed Tharwat[b]

[a] Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[b] Department of Clinical Sciences, College of Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah 51452, Saudi Arabia

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Keywords:
Dairy cattle
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ABSTRACT

The purpose of this study was to compare serum pepsinogen (PEP) and gastrin (GA) values in close-up cattle that developed displaced abomasum (DA) after calving and those that did not develop DA, and further examine whether serum PEP value as much as non-esterified fatty acids (NEFA) concentration are useful as a predictor for DA. This study was conducted as a case-control study at a single dairy farm (approximately 200 cows kept) in Hokkaido, Japan, from May 2009 to June 2013. Fifty-two cattle between 2 and 21 days before calving, developed DA within 30 days postpartum, were assigned to the DA group. The controls were 108 clinically healthy cattle that did not develop any periparturient diseases including DA and were matched for days in milk and parity with the DA group. Body condition score (BCS) and rumen fill score (RFS) were also assessed. Serum PEP and GA values in DA group were significantly lower than those in controls. There was no significant difference in BCS and RFS between the two groups. Risk of DA was 10.9 times higher in cows with PEP values of ≤700 mU than in those with higher values and was 2.8 times higher in cows with NEFA concentrations ≥0.5 mEq/L than in those with lower concentrations. Evaluation of DA prediction using usual and rotated receiver operating characteristic curves showed the significance of both PEP and NEFA, with PEP being more superior. The accuracy of the combined model using PEP and NEFA was higher than each alone.

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animals

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Article

Effects of Preventive Administration of Propylene Glycol or Sucrose in Dairy Cows with Elevated Blood Non-Esterified Fatty Acids During the Close-Up Period

Kyoko Chisato, Miki Ishizaka, Takumi Honjo, Yuta Watanabe, Rika Fukumori and Shin Oikawa *

Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan; k-chisato@rakuno.ac.jp (K.C.); fukumori@rakuno.ac.jp (R.F.)
* Correspondence: oishin@rakuno.ac.jp; Tel.: +81-11-388-4825

Simple Summary

The objective of this study was to evaluate the effects of administration of propylene glycol (PG) or sucrose (SC) on health and production outcomes in dairy cows with elevated non-esterified fatty acids (NEFA) levels of 0.3 mEq/L or higher during the close-up period. Thirty-five cows from two farms in Hokkaido were assigned to PG, SC, or untreated control groups, with treatments administered for 5 days starting from the blood testing. In PG and SC cows, blood profiles related to energy metabolism, including NEFA and β-hydroxybutyrate concentrations, improved after calving compared with controls, and liver function was maintained as well. Cows in both treatment groups exhibited significant decreases in postpartum culling rates. These findings suggest that prophylactic administration of PG or SC may contribute to postpartum productivity.

Abstract

The purpose of this study was to evaluate the preventive effects of propylene glycol (PG) or sucrose (SC) in dairy cows with high levels of non-esterified fatty acids (NEFAs) during the close-up period. From July 2021 to August 2022, blood samples were collected from 193 cows between 14 and 7 days prior to the expected calving date in two farms, and 35 multiparous cows with serum NEFA $\geq$ 0.3 mEq/L were randomly assigned to PG (500 mL/day, $n = 11$), SC (1000 mL/day of 50% solution, $n = 11$), and untreated control (HC; $n = 13$) groups. Treatments were administered orally for 5 consecutive days. Compared with HC cows, the serum NEFA concentration tended to be lower in SC cows at 3 days in milk (DIM) and was significantly lower in PG cows at 14 DIM. Serum β-hydroxybutyrate concentrations tended to be lower in SC cows at 21 DIM. Blood glucose concentrations were higher in both treatment groups at 3 DIM, and the serum total bilirubin concentration remained lower until 14 DIM in PG cows and until 7 DIM in SC cows. At 7 DIM, PG cows showed significantly higher total very low-density lipoprotein levels and PG and SC cows had significantly or tendentially higher low-density lipoprotein triglyceride concentrations. Cows in both treatment groups had significantly reduced culling after calving. These results suggest that prophylactic administration of PG or SC improves energy metabolism by supporting liver function, thereby reducing postpartum culling, with the PG group showing a more consistent effect.

Keywords: dairy cow; close-up period; propylene glycol; sucrose; prophylactic administration

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Mitochondrial function and nutrient partitioning in high and low feed efficient multiparous Holstein dairy cows

Sophia J. Kendall,[1] Guillermo Martínez-Boggio,[1] Usman Arshad,[1,2] Katherine M. Kennedy,[1,3] Rika Fukumori,[1,4] Mariana Rodrigues Wenzel,[1] Tanya L. France,[1] Lautaro Rostoll-Cangiano,[1] Kent A. Weigel,[1] Zheng Zhou,[5] Michael J. VandeHaar,[5] Francisco Peñagaricano,[1] and Heather M. White[1*]
[1]University of Wisconsin–Madison, Madison, WI
[2]ETH Zürich, Zürich, Switzerland
[3]Utah State University, Logan, UT
[4]Rakuno Gakuen University, Ebetsu, Japan
[5]Michigan State University, East Lansing, MI

ABSTRACT

Residual feed intake (RFI) is a metric to identify high feed efficient (negative RFI; −RFI) and low feed efficient (positive RFI; +RFI) animals within and across cohorts. The objective of this study was to quantify and determine potential differences between the most RFI divergent cows in (1) metabolites, hormones, and fatty acids in tail vessel and mammary vein blood; (2) hepatic gene expression; (3) CH_4, CO_2, O_2, and related metrics; and (4) hepatic mitochondrial function. Mid-lactation Holstein dairy cows (n = 64/trial) were enrolled in two 8-wk trials to quantify intake, BW, and milk production. The top and bottom 25% (n = 64; analyzed for all variables) and the most divergent 15% (n = 19; additional analysis of gene expression, mitochondrial function, and gas emissions and consumption) RFI subgroups were analyzed across the trials. Milk yield and milk energy output were similar, but milk lactose percentage was greater and MUN and SCS was lower in −RFI cows than +RFI cows. Tail vessel concentrations of C16:0, C16:1 *cis*, C17:0, C18:0, and C22:0 were (or tended to be) greater, and mammary vein C16:1 *cis* and C18:1 *cis* were greater in −RFI cows than +RFI cows. Mammary vein BUN was lower and tail vessel insulin concentration tended to be greater in −RFI cows than +RFI cows. Expression of uncoupling, mitochondrial, or tricarboxylic acid cycle genes were similar across RFI status. Mitochondrial complex IV, when quantified with complex I substrate, had lower oxygen consumption rates in −RFI cows than +RFI cows. Emissions of CH_4 and overall CO_2 were lower in −RFI cows than +RFI cows. Overall, mitochondrial function and nutrient partitioning differed in dairy cows of divergent RFI status, although differences did not appear to be due to changes in hepatic gene expression.

Key words: residual feed intake, methane, energetics, metabolites, Seahorse

INTRODUCTION

Selective breeding for improved feed efficiency through genetic selection could improve economic and environmental sustainability by reducing feed cost and by potentially reducing gas emissions. Regardless of species, feed costs represent 50% or greater of all farm expenditures (Kolath et al., 2006; Ojano-Dirain et al., 2007; Herd and Arthur, 2009), presenting opportunities for tangible improvements through selection for highly efficient, high-producing dairy cows. Inefficiencies in feed utilization can be manifested in the rumen (e.g., methane production), digestive tract (e.g., conversion of gross energy to digestible energy), or body tissues (e.g., futile cycling, metabolic efficiency). Improvements in efficiency within any of these points could improve the relative proportion of feed nutrients lost or used for productive purposes, yet differences in these fractions between high feed efficient (**HE**) and low feed efficient (**LE**) cows are not well understood. Although broad classifications exist of what contributes to feed efficiency status (Herd and Arthur, 2009), the molecular etiologies behind these classifications are poorly characterized. Understanding nutrient utilization and fundamental metabolic differences, such as hepatic mitochondrial function in dairy cattle, may allow for advances in feed efficiency to feed the world's growing population more sustainably and with lower methane and carbon dioxide emissions.

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*Corresponding author: heather.white@wisc.edu

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獣医衛生学（Animal Health）

Satoshi Gondaira

Associate Professor

准教授　権平　智

I. 筆頭または責任著者　＜First or Corresponding Author＞

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II. その他　＜Others＞

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Veterinary Research

RESEARCH ARTICLE **Open Access**

Biofilm characterisation of *Mycoplasma bovis* co-cultured with *Trueperella pyogenes*

Koji Nishi[1,2], Satoshi Gondaira[1*], Yuki Hirano[3], Masahide Ohashi[1], Ayano Sato[4], Kazuya Matsuda[5], Tomohito Iwasaki[6], Takuya Kanda[7], Ryoko Uemura[8] and Hidetoshi Higuchi[1*]

Abstract

Mycoplasma pneumonia, caused by *Mycoplasma bovis* (*Mycoplasmopsis bovis*; *M. bovis*), is linked with severe inflammatory reactions in the lungs and can be challenging to treat with antibiotics. Biofilms play a significant role in bacterial persistence and contribute to the development of chronic lesions. A recent study has shown that polymicrobial interactions between species are an important factor in biofilm formation, yet the precise mechanism of biofilm formation in *M. bovis* remains unknown. By assuming multiple pathogen infections in the bovine respiratory disease complex (BRDC), this study examined the characterisation of the polymicrobial relationship between *M. bovis* and *Trueperella pyogenes* (*T. pyogenes*) during biofilm formation. Autopsies were performed on four Holstein calves (two chronic *Mycoplasma* pneumonia calves and two control calves). Bacterium-like aggregation structures (> 10 µm), which were assumed to be biofilms of *M. bovis* in vivo, were observed adhering to the cilia in calves with *Mycoplasma* pneumonia. *M. bovis* released an extracellular matrix to connect with neighbouring bacteria and form a mature biofilm on the plate. Biofilm formation in the co-culture of *M. bovis* and *T. pyogenes* (strain T1: 1×10^5 and 1×10^6 CFU/well) significantly increased ($p < 0.05$ and $p < 0.01$; 64.1% and 64.8% increase) compared to that in a single culture of these bacteria. Furthermore, some large aggregates (> 40 µm), composed of *M. bovis* and *T. pyogenes*, were observed. The morphological characteristics of this biofilm were similar to those observed in vivo compared to a single culture. In conclusion, the polymicrobial interaction between *M. bovis* and *T. pyogenes* induces biofilm formation, which is associated with increased resistance to antimicrobial agents, and this exacerbates the progression of chronic *Mycoplasma* pneumonia.

Keywords Antibiotics, antimicrobial agents, bovine respiratory disease, extracellular matrix, trachea

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*Correspondence:
Satoshi Gondaira
s-gondaira@rakuno.ac.jp
Hidetoshi Higuchi
higuchi@rakuno.ac.jp
[1] Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[2] Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu, Hokkaido, Japan
[3] Animal Research Center, Agricultural Research Department, Hokkaido Research Organization, Shintoku, Hokkaido, Japan
[4] Large Animal Clinical Science, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[5] Department of Veterinary Pathology, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[6] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[7] Food Safety Laboratory, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan
[8] Animal Health, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

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Journal of Veterinary Science

Research Report
Microbiology

RNA sequencing of *Mycoplasma bovis* infecting bovine mammary epithelial cells and bovine mononuclear cells

Noriko Imaizumi [1], **Satoshi Gondaira** [1,*], **Tomochika Sugiura** [2], **Ayako Eguchi** [1], **Koji Nishi** [1,3], **Jumpei Fujiki** [4], **Hidetomo Iwano** [4], **Hidetoshi Higuchi** [1,*]

[1]Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[2]Theriogenology Unit, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[3]Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu 099-5171, Japan
[4]Veterinary Biochemistry Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan

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***Corresponding authors:**

Satoshi Gondaira
Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Japan.
Email: s-gondaira@rakuno.ac.jp
https://orcid.org/0000-0001-9745-7792

Hidetoshi Higuchi
Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Japan.
Email: higuchi@rakuno.ac.jp
https://orcid.org/0000-0002-7476-8544

ABSTRACT

Importance: *Mycoplasma bovis* is the major pathogen of bovine mycoplasmosis, such as mastitis, pneumonia, otitis media, and arthritis. Understanding the pathophysiology of mycoplasmal infections from the pathogen-side is as important as elucidating the host-side effects; however, few studies have focused on the variations in the expression of *M. bovis* genes.
Objective: In this study, we performed RNA sequencing of *M. bovis* infecting bovine mammary epithelial cells (bMECs) and mononuclear cells (MNCs) to elucidate pathogen-side factors involved in the pathophysiology of *M. bovis* mastitis.
Methods: *M. bovis* was cocultured with bMEC and MNC using a transwell system, and total RNA was extracted for RNA sequencing analysis.
Results: We identified 162 differentially expressed genes (DEGs), 104 upregulated and 58 downregulated, in *M. bovis* infecting bMEC, whereas 220 DEGs, 154 upregulated and 66 downregulated, in *M. bovis* infecting MNC. Enrichment analysis of upregulated DEGs in *M. bovis* infecting bMEC revealed multiple pathways with overlapping genes involved in lipid metabolism. The expression of six virulence genes were significantly upregulated in *M. bovis* infecting bMEC and MNC, whereas the expression of three virulence genes were significantly downregulated in *M. bovis* infecting MNC.
Conclusions and Relevance: Our findings indicate that *M. bovis* exhibits infecting cell-specific gene expression, and its virulence varies with the infecting cell type.

Keywords: Cattle; *Mycoplasma bovis*; bovine mastitis; virulence; gene expression profiling

INTRODUCTION

Mycoplasma bovis (also known as *Mycoplasmopsis bovis*) causes bovine mycoplasmosis, a group of severe bovine diseases that include mastitis, pneumonia, otitis media, and arthritis [1]. Mycoplasmal mastitis can be differentiated from mastitis caused by other bacteria because it is highly contagious, affects multiple quarters, is often resistant to antibiotics, and affected cows apparently appear normal even in severe cases [2]. *M. bovis* mastitis is sometimes

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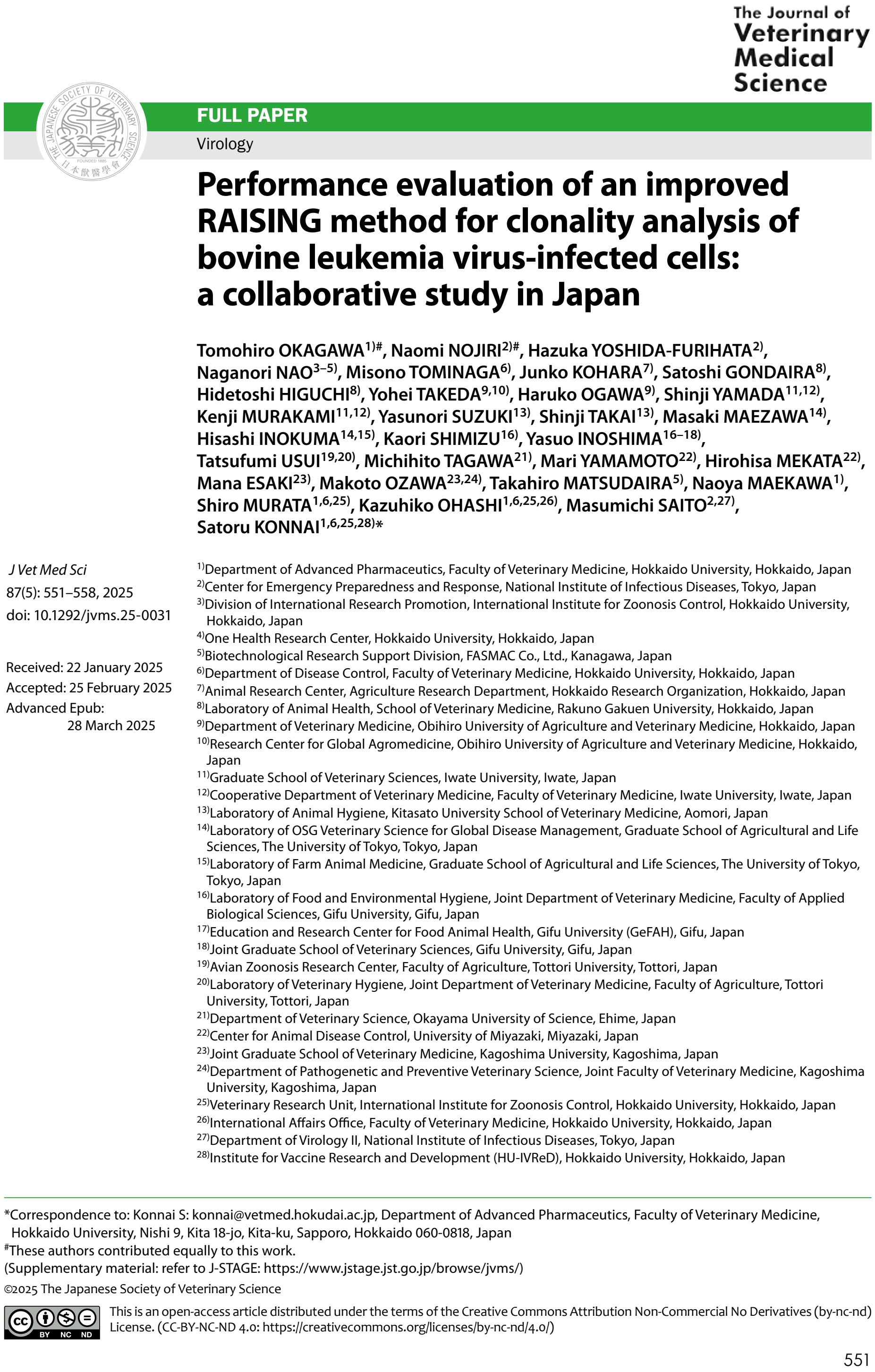

The Journal of
Veterinary
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FULL PAPER

Virology

Performance evaluation of an improved RAISING method for clonality analysis of bovine leukemia virus-infected cells: a collaborative study in Japan

Tomohiro OKAGAWA[1)#], Naomi NOJIRI[2)#], Hazuka YOSHIDA-FURIHATA[2)], Naganori NAO[3–5)], Misono TOMINAGA[6)], Junko KOHARA[7)], Satoshi GONDAIRA[8)], Hidetoshi HIGUCHI[8)], Yohei TAKEDA[9,10)], Haruko OGAWA[9)], Shinji YAMADA[11,12)], Kenji MURAKAMI[11,12)], Yasunori SUZUKI[13)], Shinji TAKAI[13)], Masaki MAEZAWA[14)], Hisashi INOKUMA[14,15)], Kaori SHIMIZU[16)], Yasuo INOSHIMA[16–18)], Tatsufumi USUI[19,20)], Michihito TAGAWA[21)], Mari YAMAMOTO[22)], Hirohisa MEKATA[22)], Mana ESAKI[23)], Makoto OZAWA[23,24)], Takahiro MATSUDAIRA[5)], Naoya MAEKAWA[1)], Shiro MURATA[1,6,25)], Kazuhiko OHASHI[1,6,25,26)], Masumichi SAITO[2,27)], Satoru KONNAI[1,6,25,28)]*

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[1)]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[2)]Center for Emergency Preparedness and Response, National Institute of Infectious Diseases, Tokyo, Japan
[3)]Division of International Research Promotion, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan
[4)]One Health Research Center, Hokkaido University, Hokkaido, Japan
[5)]Biotechnological Research Support Division, FASMAC Co., Ltd., Kanagawa, Japan
[6)]Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[7)]Animal Research Center, Agriculture Research Department, Hokkaido Research Organization, Hokkaido, Japan
[8)]Laboratory of Animal Health, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[9)]Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[10)]Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[11)]Graduate School of Veterinary Sciences, Iwate University, Iwate, Japan
[12)]Cooperative Department of Veterinary Medicine, Faculty of Veterinary Medicine, Iwate University, Iwate, Japan
[13)]Laboratory of Animal Hygiene, Kitasato University School of Veterinary Medicine, Aomori, Japan
[14)]Laboratory of OSG Veterinary Science for Global Disease Management, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[15)]Laboratory of Farm Animal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[16)]Laboratory of Food and Environmental Hygiene, Joint Department of Veterinary Medicine, Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan
[17)]Education and Research Center for Food Animal Health, Gifu University (GeFAH), Gifu, Japan
[18)]Joint Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan
[19)]Avian Zoonosis Research Center, Faculty of Agriculture, Tottori University, Tottori, Japan
[20)]Laboratory of Veterinary Hygiene, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori, Japan
[21)]Department of Veterinary Science, Okayama University of Science, Ehime, Japan
[22)]Center for Animal Disease Control, University of Miyazaki, Miyazaki, Japan
[23)]Joint Graduate School of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[24)]Department of Pathogenetic and Preventive Veterinary Science, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan
[25)]Veterinary Research Unit, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan
[26)]International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[27)]Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan
[28)]Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Hokkaido, Japan

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*Correspondence to: Konnai S: konnai@vetmed.hokudai.ac.jp, Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Nishi 9, Kita 18-jo, Kita-ku, Sapporo, Hokkaido 060-0818, Japan

[#]These authors contributed equally to this work.

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Original article

Anti-inflammatory effects of glycyrrhizin on lipoteichoic acid and lipopolysaccharide-induced bovine mastitis

T. Kurumisawa[1,2†], K. Kazama[1†], S. Gondaira[3], H. Higuchi[3], A. Eguchi[3], K. Onda[1], S-G. Roh[4], K. Kawai[1,2]

[1] School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe Chuo-ku, Sagamihara 252-5201, Japan
[2] Azabu University Mastitis Research Center, 1-17-71 Fuchinobe Chuo-ku, Sagamihara 252-5201, Japan
[3] Graduate School of Veterinary Medicine,
Rakuno Gakuen University, 852 Midorimachi Bunkyoudai, Ebetsu 069-8501, Japan
[4] Graduate School of Agricultural Science,
Tohoku University, 468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0842, Japan

Correspondence to: K. Kawai, e-mail: kawai@azabu-u.ac.jp
† These authors contributed equally to this work.

Abstract

Bovine mastitis is primarily treated with antimicrobial agents. Anti-inflammatory agents are also used to alleviate clinical symptoms or reduce antimicrobial use. Glycyrrhizin is an anti-inflammatory agent used in the treatment of bovine mastitis, but its effects are not fully understood. We therefore examined the anti-inflammatory effects of glycyrrhizin both in vivo and in vitro. We first tested whether glycyrrhizin exerts anti-inflammatory effects using MAC-T cells, an immortalized bovine mammary epithelial cell line. Glycyrrhizin decreased the expression of interleukin (IL)-1β mRNA in a concentration-dependent manner in MAC-T cells stimulated with lipoteichoic acid (LTA). We then investigated the effects of glycyrrhizin in bovine mammary epithelial cells (bMECs), which seem to retain more of the characteristics of actual mammary epithelial cells. Stimulation with LTA or lipopolysaccharide significantly increased cytokine mRNA expression in bMECs. Glycyrrhizin exhibited a slight inhibitory effect, but no significant difference was observed. The effect of glycyrrhizin on LTA-induced mastitis was examined in lactating cows. Quarters were divided into test and control areas (test quarter: n=8, control quarter: n=7). All quarters were stimulated with LTA at the start of the trial (0 h). In the test quarter group, glycyrrhizin was administered via intramammary infusion. The somatic cell count and relative gene expression of IL-1β and tumor necrosis factor–α were significantly lower in test quarters than control quarters. Both the in vitro and in vivo studies showed that glycyrrhizin reduces the expression of proinflammatory cytokine genes in response to LTA-induced inflammation and partially revealed the mechanism of the anti-inflammatory effect of glycyrrhizin on mastitis. Further investigations involving field cases of mastitis with bacterial infections are needed to demonstrate the anti-inflammatory effect of glycyrrhizin on bovine mastitis.

Keywords: anti-inflammatory effect, bovine mammary epithelial cells, bovine mastitis, glycyrrhizin, lipopolysaccharide, lipoteichoic acid

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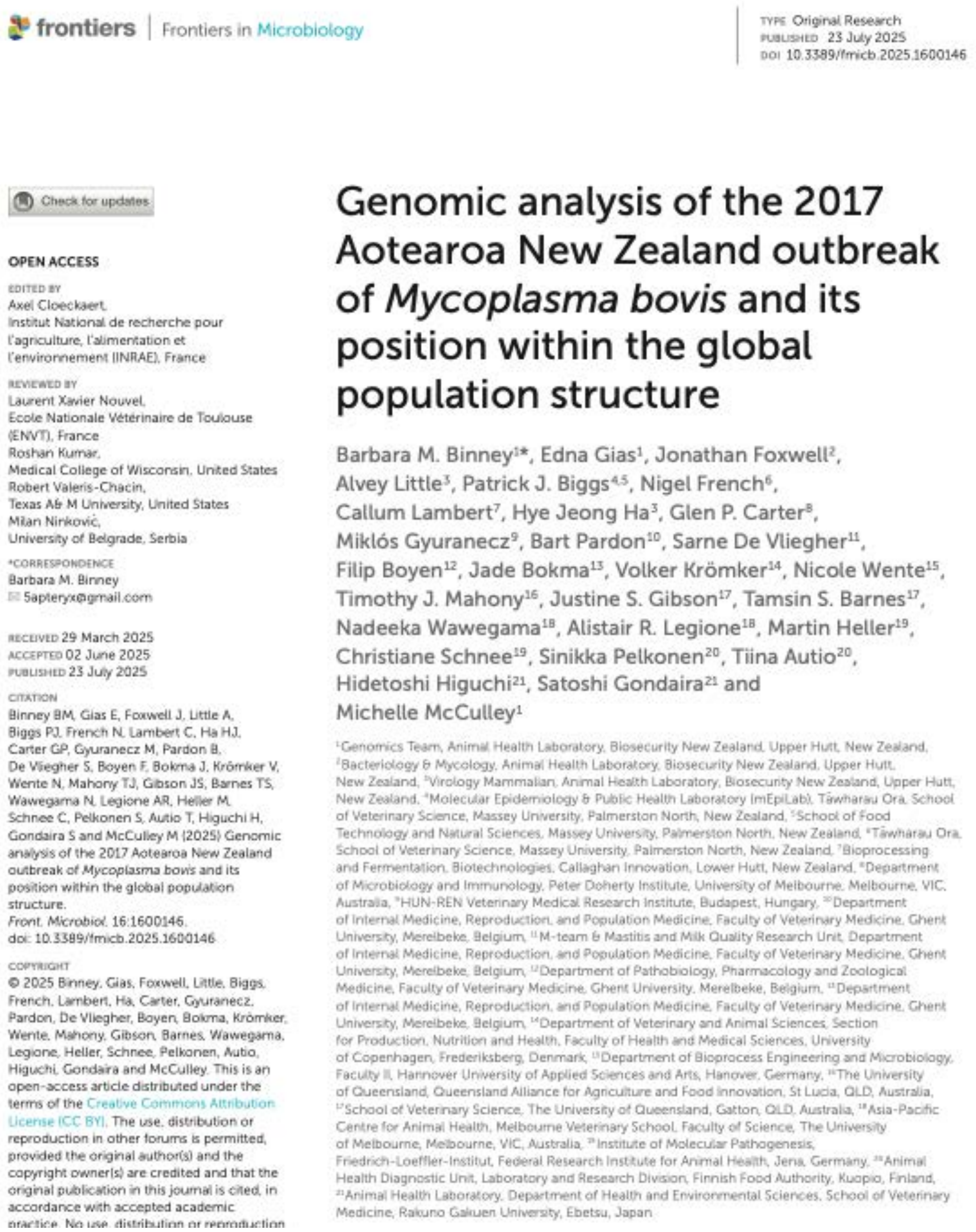

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Genomic analysis of the 2017 Aotearoa New Zealand outbreak of *Mycoplasma bovis* and its position within the global population structure

Barbara M. Binney[1]*, Edna Gias[1], Jonathan Foxwell[2], Alvey Little[3], Patrick J. Biggs[4,5], Nigel French[6], Callum Lambert[7], Hye Jeong Ha[3], Glen P. Carter[8], Miklós Gyuranecz[9], Bart Pardon[10], Sarne De Vliegher[11], Filip Boyen[12], Jade Bokma[13], Volker Krömker[14], Nicole Wente[15], Timothy J. Mahony[16], Justine S. Gibson[17], Tamsin S. Barnes[17], Nadeeka Wawegama[18], Alistair R. Legione[18], Martin Heller[19], Christiane Schnee[19], Sinikka Pelkonen[20], Tiina Autio[20], Hidetoshi Higuchi[21], Satoshi Gondaira[21] and Michelle McCulley[1]

[1]Genomics Team, Animal Health Laboratory, Biosecurity New Zealand, Upper Hutt, New Zealand, [2]Bacteriology & Mycology, Animal Health Laboratory, Biosecurity New Zealand, Upper Hutt, New Zealand, [3]Virology Mammalian, Animal Health Laboratory, Biosecurity New Zealand, Upper Hutt, New Zealand, [4]Molecular Epidemiology & Public Health Laboratory (mEpiLab), Tāwharau Ora, School of Veterinary Science, Massey University, Palmerston North, New Zealand, [5]School of Food Technology and Natural Sciences, Massey University, Palmerston North, New Zealand, [6]Tāwharau Ora, School of Veterinary Science, Massey University, Palmerston North, New Zealand, [7]Bioprocessing and Fermentation, Biotechnologies, Callaghan Innovation, Lower Hutt, New Zealand, [8]Department of Microbiology and Immunology, Peter Doherty Institute, University of Melbourne, Melbourne, VIC, Australia, [9]HUN-REN Veterinary Medical Research Institute, Budapest, Hungary, [10]Department of Internal Medicine, Reproduction, and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [11]M-team & Mastitis and Milk Quality Research Unit, Department of Internal Medicine, Reproduction, and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [12]Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [13]Department of Internal Medicine, Reproduction, and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, [14]Department of Veterinary and Animal Sciences, Section for Production, Nutrition and Health, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark, [15]Department of Bioprocess Engineering and Microbiology, Faculty II, Hannover University of Applied Sciences and Arts, Hanover, Germany, [16]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, St Lucia, QLD, Australia, [17]School of Veterinary Science, The University of Queensland, Gatton, QLD, Australia, [18]Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, Faculty of Science, The University of Melbourne, Melbourne, VIC, Australia, [19]Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Jena, Germany, [20]Animal Health Diagnostic Unit, Laboratory and Research Division, Finnish Food Authority, Kuopio, Finland, [21]Animal Health Laboratory, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

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EDITED BY
Axel Cloeckaert,
Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), France

REVIEWED BY
Laurent Xavier Nouvel,
Ecole Nationale Vétérinaire de Toulouse (ENVT), France
Roshan Kumar,
Medical College of Wisconsin, United States
Robert Valeris-Chacin,
Texas A& M University, United States
Milan Ninković,
University of Belgrade, Serbia

*CORRESPONDENCE
Barbara M. Binney
✉ 5apteryx@gmail.com

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Effects of amount of lactose in milk replacer on gastrointestinal function of dairy calves

R. Fukumori [a], T. Nakayama [a], M. Hirose [a], I. Norimura [b], K. Izumi [b], K. Shimada [c], H. Mineo [d], M.A. Steele [e], S. Gondaira [a], H. Higuchi [a], T. Watanabe [a], H. Ueda [a], T. Sano [a], K. Chisato [a], S. Oikawa [a],*

[a] *Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[b] *Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan*
[c] *The National Federation of Dairy Co-operative Associations (Zen-Raku-Ren), Shinjuku, Tokyo 969-0223, Japan*
[d] *Department of Health and Nutrition, Hokkaido Bunkyo University, Eniwa 069-1449, Japan*
[e] *Department of Animal and Bioscience, University of Guelph, Guelph, Ontario N1G2W1, Canada*

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ABSTRACT

The objective of this study was to evaluate the effects of feeding milk replacer (MR) at different lactose amount while keeping osmolality constant on gastrointestinal function, blood parameters, and inflammation-related mRNA expression in the livers of dairy calves. Fifteen Holstein bull calves were assigned to one of three dietary treatments differing in MR lactose content (L: 38 %, M: 41 %, and H: 46 %). Feeding of the test diets was started at 1 day of age and gradually increased to a maximum feeding rate at 20 days of age (L: 1.16 kg/d, M: 1.21 kg/d, and H: 1.26 kg/d DM). Under these conditions, the lactose dosages for the treatments were 441 g/d, 496 g/d, and 580 g/d, respectively. The MR were prepared to ensure isocaloric and iso-osmotic (451 mOsm/kg) conditions. Fecal scores were recorded daily, and at 14 and 28 days of age, blood and breath samples were collected before and after MR feeding. In addition, feces and urine were collected for 2 consecutive days. Then, the calves were slaughtered to evaluate intestinal permeability and liver mRNA expression. The permeability in the duodenum and ileum was lower in H and M than in L; the permeability in the jejunum was also lower in H than in L. The hepatic mRNA expressions of toll-like receptor-2, IL-1β, and tumor-necrosis factor-α were lower in H and M than L. Nitrogen retention was higher in H than in L, and linear and quadratic increasing trends were observed in tissue ratio of gastrointestinal tract with the increase in lactose amount. Postprandial increase in plasma glucose concentration was smaller and postprandial increase in TG concentration was higher in H than in L. Fecal properties, digestibility, and breath hydrogen concentrations were not affected by treatment. These results indicate that feeding high-lactose MR may increase gastrointestinal weight and decrease permeability in the small intestine of calves.

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* Corresponding author.
E-mail address: oishin@rakuno.ac.jp (S. Oikawa).

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RESEARCH ARTICLE

Effects of Ruminal Administration of Propylene Glycol or Sucrose on Ruminal, Blood, and Hepatic Parameters in Nonlactating Cows With High Plasma Nonesterified Fatty Acid Concentrations

Kyoko Chisato[1] | Rika Fukumori[1] | Ryo Imaishi[1] | Satoshi Gondaira[1] | Hidetoshi Higuchi[1] | Kenichi Izumi[2] | Shin Oikawa[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu, Japan

Correspondence: Rika Fukumori (fukumori@rakuno.ac.jp) | Shin Oikawa (oishin@rakuno.ac.jp)

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Keywords: insulin | ketosis | metabolic diseases | NEFA | transition cows

ABSTRACT

Elevated blood nonesterified fatty acids (NEFA) concentration in prepartum increases the risk of postpartum diseases. This study evaluated whether intraruminal propylene glycol (PG) or sucrose (SC) administration could mitigate elevated blood NEFA induced by intravenous lipid infusion. Four nonlactating, nonpregnant Holstein cows fitted with rumen cannulas were used in a 4×4 Latin square design: intravenous saline infusion and ruminal administration of water (S+WT), lipid infusion and administration of water (L+WT), lipid infusion and PG administration (L+PG), and lipid infusion and SC administration (L+SC). The intravenous infusion lasted for 11 h, followed by ruminal administration 4 h after the start of the infusion. Blood NEFA concentration was increased by lipid infusion but was lower in L+PG compared to L+WT. In association with this, higher ruminal propionate and butyrate, blood glucose and insulin, and lower β-hydroxybutyrate (BHBA) concentrations were observed in L+PG. Hepatic carnitine palmitoyl transferase 1 mRNA expression was higher in L+PG and L+SC compared to L+WT. SC administration increased insulin concentrations associated with increased ruminal butyrate concentration but had a smaller NEFA-reducing effect than PG. To alleviate NEFA elevation, PG was more effective than SC, which may have involved sustained stimulation of insulin secretion of PG.

1 | Introduction

The 3 weeks before and after calving are called the “transition period,” (Grummer 1995; Drackley 1999) during which dramatic changes occur in dairy cows. This means that cows have an increased demand for energy for fetal growth and the production of colostrum and milk. If the energy supply from feed intake is insufficient, cows can experience a negative energy balance (NEB) (Bauman and Bruce Currie 1980; Baird 1982). The cow attempts to adapt to NEB by utilizing the carbohydrates, lipids, and proteins stored in the body. However, if these metabolic adaptations are not sufficiently successful, peripartum diseases, such as milk fever, ketosis, retained placenta, and displaced abomasum, may occur. The occurrence of peripartum diseases not only leads to reproductive disorders, such as decreased conception rates (Baird 1982), but also increases the risk of early removal due to death, culling, and sale, resulting in significant financial losses for dairy farms (Gröhn et al. 1998; Fetrow et al. 2006).

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最終責任者　Shin Oikawa (Corresponding Author)

画像診断学（Veterinary Diagnostic Imaging）

Kiwamu Hanazono

Associate Professor

准教授　華園　究

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他＜Others＞

1) Prospective Estimation of the Prevalence of Thromboembolism in Dogs with Inflammatory Protein-Losing Enteropathy.
   Oishi N, Ohta H, Tamura M, **Hanazono K**, Miyoshi K, Yokoyama N, Shinbo G.
   J Vet Intern Med. 39: e70098. 2025. doi: 10.1111/jvim.70098.

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Prospective Estimation of the Prevalence of Thromboembolism in Dogs With Inflammatory Protein-Losing Enteropathy

Nene Oishi, Hiroshi Ohta, Masahiro Tamura, Kiwamu Hanazono, Kenjiro Miyoshi, Nozomu Yokoyama, Genya Shinbo

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Haruyuki Hirata

Associate Professor

准教授　平田　晴之

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1） Validation of point-of care devices measuring calcium (iCa), potassium (K^+), and sodium (Na^+) concentrations in whole blood of cattle and horses using general-purpose water quality testing equipmet.
Kondo N, Iwasa A, Okamoto M, Tsukano K, **Hirata H**, Suzuki K.
Acta Veterinaria Brno. 94:3-7. 2025. doi: 10.2754/avb202594010003

2） Seasonal dynamic of *Ostertagia ostertagi* infection in lactating Holstein-Friesian on a dairy farm in Hokkaido, Japan.
Ikeda K., Asakura Y., Fukumoto S., Suzuki K., **Hirata H.**.
Acta Vet Hung 73:130-133. 2025. doi: 10.1556/004.2025.01144

3） Identification and phylogenetic analysis of novel Piroplasma detected in the two-toed sloth (*Choloepus didactylus*).
Murakami M, Iwasa A, Okamoto M, Suzuki K, **Hirata H**.
J Vet Med Sci. 87:972-975. 2025. doi: 10.1292/jvms.25-0063

II. その他＜Others＞

1） A simple, inexpensive, and rapid method for measuring calcium concentrations in colostrum to predict postpartum milk fever in cows using a commercially available water quality test device.
Kondo N, Maeda M, Murotsuki R, Ryoha T, Otsuka M, Tsukano K, Kozutsumi H, Ikeda K, Takami N, Kamitani K, Okamoto M, **Hirata H.**, Suzuki K.
J Vet Med Sci. 87:1186-1193. 2025. doi: 10.1292/jvms.25-0298

2） Intestinal expression of diamine oxidase (DAO) and the relationship between blood activity and haptoglobin concentration in diarrheal calves.
Kozutsumi A, Okamoto M, Takeuchi H, Otsuka M, Tsukano K, Kondo N, Kamitani K, Ikeda K, Takami N, **Hirata H**, Suzuki K.
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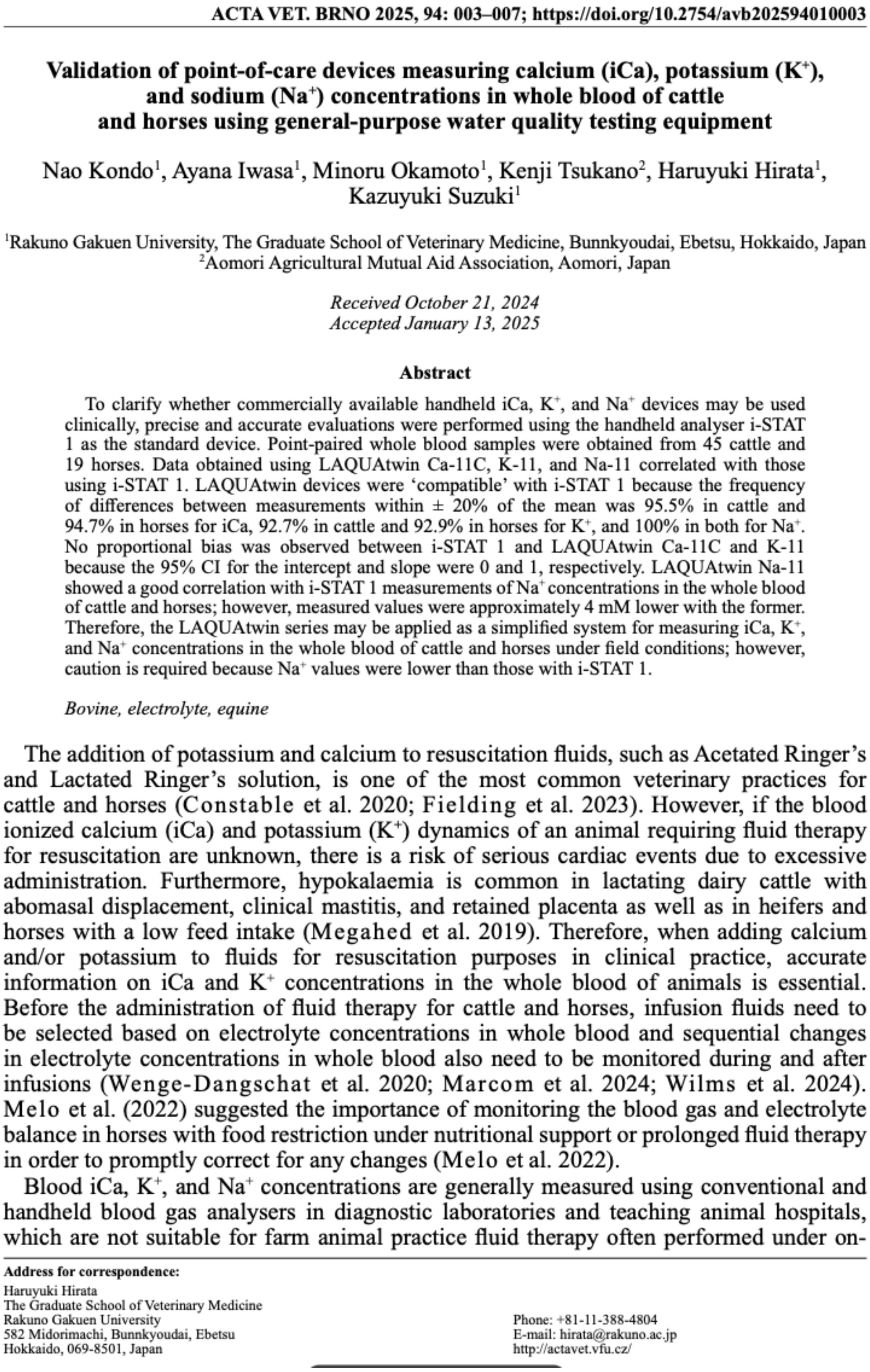

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Validation of point-of-care devices measuring calcium (iCa), potassium (K^+), and sodium (Na^+) concentrations in whole blood of cattle and horses using general-purpose water quality testing equipment

Nao Kondo[1], Ayana Iwasa[1], Minoru Okamoto[1], Kenji Tsukano[2], Haruyuki Hirata[1],
Kazuyuki Suzuki[1]

[1]Rakuno Gakuen University, The Graduate School of Veterinary Medicine, Bunnkyoudai, Ebetsu, Hokkaido, Japan
[2]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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Abstract

To clarify whether commercially available handheld iCa, K^+, and Na^+ devices may be used clinically, precise and accurate evaluations were performed using the handheld analyser i-STAT 1 as the standard device. Point-paired whole blood samples were obtained from 45 cattle and 19 horses. Data obtained using LAQUAtwin Ca-11C, K-11, and Na-11 correlated with those using i-STAT 1. LAQUAtwin devices were 'compatible' with i-STAT 1 because the frequency of differences between measurements within ± 20% of the mean was 95.5% in cattle and 94.7% in horses for iCa, 92.7% in cattle and 92.9% in horses for K^+, and 100% in both for Na^+. No proportional bias was observed between i-STAT 1 and LAQUAtwin Ca-11C and K-11 because the 95% CI for the intercept and slope were 0 and 1, respectively. LAQUAtwin Na-11 showed a good correlation with i-STAT 1 measurements of Na^+ concentrations in the whole blood of cattle and horses; however, measured values were approximately 4 mM lower with the former. Therefore, the LAQUAtwin series may be applied as a simplified system for measuring iCa, K^+, and Na^+ concentrations in the whole blood of cattle and horses under field conditions; however, caution is required because Na^+ values were lower than those with i-STAT 1.

Bovine, electrolyte, equine

The addition of potassium and calcium to resuscitation fluids, such as Acetated Ringer's and Lactated Ringer's solution, is one of the most common veterinary practices for cattle and horses (Constable et al. 2020; Fielding et al. 2023). However, if the blood ionized calcium (iCa) and potassium (K^+) dynamics of an animal requiring fluid therapy for resuscitation are unknown, there is a risk of serious cardiac events due to excessive administration. Furthermore, hypokalaemia is common in lactating dairy cattle with abomasal displacement, clinical mastitis, and retained placenta as well as in heifers and horses with a low feed intake (Megahed et al. 2019). Therefore, when adding calcium and/or potassium to fluids for resuscitation purposes in clinical practice, accurate information on iCa and K^+ concentrations in the whole blood of animals is essential. Before the administration of fluid therapy for cattle and horses, infusion fluids need to be selected based on electrolyte concentrations in whole blood and sequential changes in electrolyte concentrations in whole blood also need to be monitored during and after infusions (Wenge-Dangschat et al. 2020; Marcom et al. 2024; Wilms et al. 2024). Melo et al. (2022) suggested the importance of monitoring the blood gas and electrolyte balance in horses with food restriction under nutritional support or prolonged fluid therapy in order to promptly correct for any changes (Melo et al. 2022).

Blood iCa, K^+, and Na^+ concentrations are generally measured using conventional and handheld blood gas analysers in diagnostic laboratories and teaching animal hospitals, which are not suitable for farm animal practice fluid therapy often performed under on-

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Address for correspondence:
Haruyuki Hirata
The Graduate School of Veterinary Medicine
Rakuno Gakuen University
582 Midorimachi, Bunnkyoudai, Ebetsu
Hokkaido, 069-8501, Japan

Phone: +81-11-388-4804
E-mail: hirata@rakuno.ac.jp
http://actavet.vfu.cz/

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Seasonal dynamics of *Ostertagia ostertagi* infection in lactating Holstein-Friesian cows on a dairy farm in Hokkaido, Japan

KEIKO IKEDA[1], YASUHITO ASAKURA[2], SHINICHIRO FUKUMOTO[1], KAZUYUKI SUZUKI[1] and HARUYUKI HIRATA[1]*

[1] The Graduate School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[2] Rumoi Hokubu Veterinary Clinic, Hokkaido Chuo Agricultural Mutual Relief Association, Teshio, Hokkaido, Japan

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RESEARCH ARTICLE

ABSTRACT

Gastrointestinal nematodes (GINs), such as *Ostertagia ostertagi* and *Cooperia* spp., are common in cattle worldwide. Although infected cattle are often asymptomatic, production is negatively affected. The present study investigated whether eggs per gram (EPG) of GINs in 32 lactating cows decreased during winter and increased as it became warmer. Larvae recovered by coprocultures mostly consisted of *O. ostertagi* in autumn, while three GINs, *Ostertagia*, *Trichostrongylus* and *Meistocirrus*, were collected in spring. Daily milk and bulk tank milk samples from cows previously infected with *O. ostertagi* contained *O. ostertagi* antibodies based on ELISA developed using SVANOVIER® *O. ostertagi*-Ab in all survey periods. Even if the faecal diagnosis was negative for GINs, infection was still possible. Therefore, the administration of anthelmintic drugs is recommended.

KEYWORDS

Gastrointestinal nematodes, *Ostertagia ostertagi*, ELISA

Gastrointestinal nematodes (GINs), such as *Ostertagia ostertagi* and *Cooperia* spp., are common in cattle worldwide (Louw, 1999; Williams et al., 1993). Although infected cattle are often asymptomatic, cattle production is negatively affected (Younie et al., 2004). Many species of GINs cause economical losses, such as the retardation of calf growth and reductions in the pregnancy rate and milk production in dairy cows (Ballweber et al., 1997; Charlier et al., 2005; Stromberg et al., 1997).

A previous study has reported that low temperatures in autumn and early winter in the Northern hemisphere contributed to the arrested larval development of GINs (hypobiosis) (Smeal et al., 1980). Third-stage larvae (L3) of *O. ostertagi* exposed to a low temperature in autumn prior to infection has prevented the development of early fourth-stage larvae in the abomasum, decreasing the number of eggs of *O. ostertagi* in faeces in winter. This phenomenon is referred to as "spring rise". However, few studies have examined the 'hypobiosis' and 'spring rise' of GINs in dairy cows on farms in Japan (Fukumoto et al., 1990).

The aim of the present study was to confirm the seasonal dynamics of GIN infections in dairy cows on a farm in Japan. We also measured *O. ostertagi* antibody levels in the milk of lactating cows and bulk tank milk using solid-phase indirect ELISA.

MATERIALS AND METHODS

Sample collection

All lactating cows (of Holstein-Friesian breed) on a dairy farm located in Horonobe in Northern Hokkaido grazed on pastures between May and October 2018 and were housed

*Corresponding author. Department of Veterinary Clinicopathology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan.
Tel.: +81 11 388 4804.
E-mail: hirata@rakuno.ac.jp

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NOTE

Wildlife Science

Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (*Choloepus didactylus*)

Misato MURAKAMI[1], Ayana IWASA[1], Minoru OKAMOTO[1], Takahiro ISHIZAKI[1], Kazuyuki SUZUKI[1], Haruyuki HIRATA[1]*

[1]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. This study investigated the pathological and parasitological causes of death in two two-toed sloths (*Choloepus didactylus*) housed at a zoo in Hokkaido, Japan. Genomic DNA was extracted from the clotted blood of these animals and subsequently examined by nested PCR assays targeting the *18S rRNA* and *β-tubulin* genes. The nucleotide sequences of these genes were determined and a phylogenetic analysis of the *18S rRNA* gene was performed. One sample was detected positive in nested PCR. Sequencing analysis revealed that the detected parasite was closely related to the order Piroplasmida and was subsequently designated as Piroplasmida sp. *Choloepus didactylus*-1 (Piroplasmida sp. CD-1).

KEYWORDS: first record, loth, phylogenetic analysis, Piroplasmida, two-toed sloth

Sloths are well known for their deliberate, slow movements and their use of suspensorial modes of arboreal locomotion. Despite similarities in their behaviors, the evolutionary lineages of two-toed sloths (*Choloepodidae*) and three-toed sloths (*Bradypodidae*) are distantly related [2, 4]. Two-toed sloths are distrubuted throughout Central and South America [3] and occupy a larger northern range than three-toed sloths, whose distribution markedly overlaps [5]. Since two-toed sloths are kept in numerous zoos worldwide, relatively detailed information is available on various diseases that develop in these animals, including chronic renal disease [12]. Although several parasites have been reported to infect sloths [14], only Debet *et al.* described an infection with *Piroplasma* in 1988 [1].

The order Piroplasmida, including the genera *Babesia*, *Cytauxzoon*, and *Theileria*, is often referred to collectively. These organisms consist belonging to dixenous hemoprotozoans transmitted by ticks to mammalian or avian hosts [13]. To date, more than 600 species of Piroplasmida have been reported, with infections resulting in hemolytic anemia, jaundice, and hemoglobinuria in case of babesiosis, although such symptoms are not observed with theileriosis [7, 10]. Recent molecular techniques have facilitated the detection and identification of Piroplasmida in wild animals and those housed in aquariums and zoos, such as raccoons, elk, opossums, monkeys, and rhinoceroses [6, 8, 11, 18]. In the present study, autopsies were performed on two two-toed sloths to identify the pathological cause of death, and molecular as well as phylogenetic analyses were subsequently conducted on the intra-erythrocytic parasites detected in these animals.

In January and February 2024, a petting zoo at Hokkaido, Japan submitted two two-toed sloths (Patient nos. 24-0206-1: CR235, and 24-0403-1: CR261) to the Department of Veterinary Clinicopathology of Rakuno Gakuen University for a pathological diagnosis by autopsy. Both animals were imported females, kept housed indoors, and showed no clinical symptoms in a petting zoo. Furthermore, no ectoparasites, such as ticks or lice, were also detected on the skins. Pathological autopsies were performed to identify the cause of death. No abnormalities, including splenomegaly, were detected in either sloth. The spleen, liver, and kidney were collected from each animal and fixed in 20% neutral-buffered formalin. Paraffin-embedded sections were prepared, stained with Berlin blue to detect the hemosiderin deposition, and then counterstained with hematoxylin and eosin. One sloth had hemosiderin deposits in hepatic and splenic cells (Fig. 1A and 1B), and only sparse deposits in renal cells (Fig. 1C), indicating erythrocyte destruction. Even though the pathological findings suggest a hemoprotozoan infection, the Giemsa-stained blood smear prepared by stamping the spleen did not reveal any clear protozoa forms in microscopic examination (data not shown). This indicates that while an infection is suspected, the specific protozoa could not be morphologically identified.

Total genomic DNA (gDNA) was extracted from clotted blood using phenol-chloroform-isoamyl alcohol (25:24:1) (Merck KGaA, Darmstadt, Germany) following the manufacturer's instructions [9]. The extracted gDNA was stored at −20°C and subsequently used as the template for nested PCR assays targeting the *18S rRNA* and *β-tubulin* genes, specific to the genera *Babesia* and *Theileria*

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*Correspondence to: Hirata H: hirata@rakuno.ac.jp, Department of Veterinary Clinicopathology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midori-machi, Bunkyodai, Ebetsu, Hokkaido 069-0836, Japan

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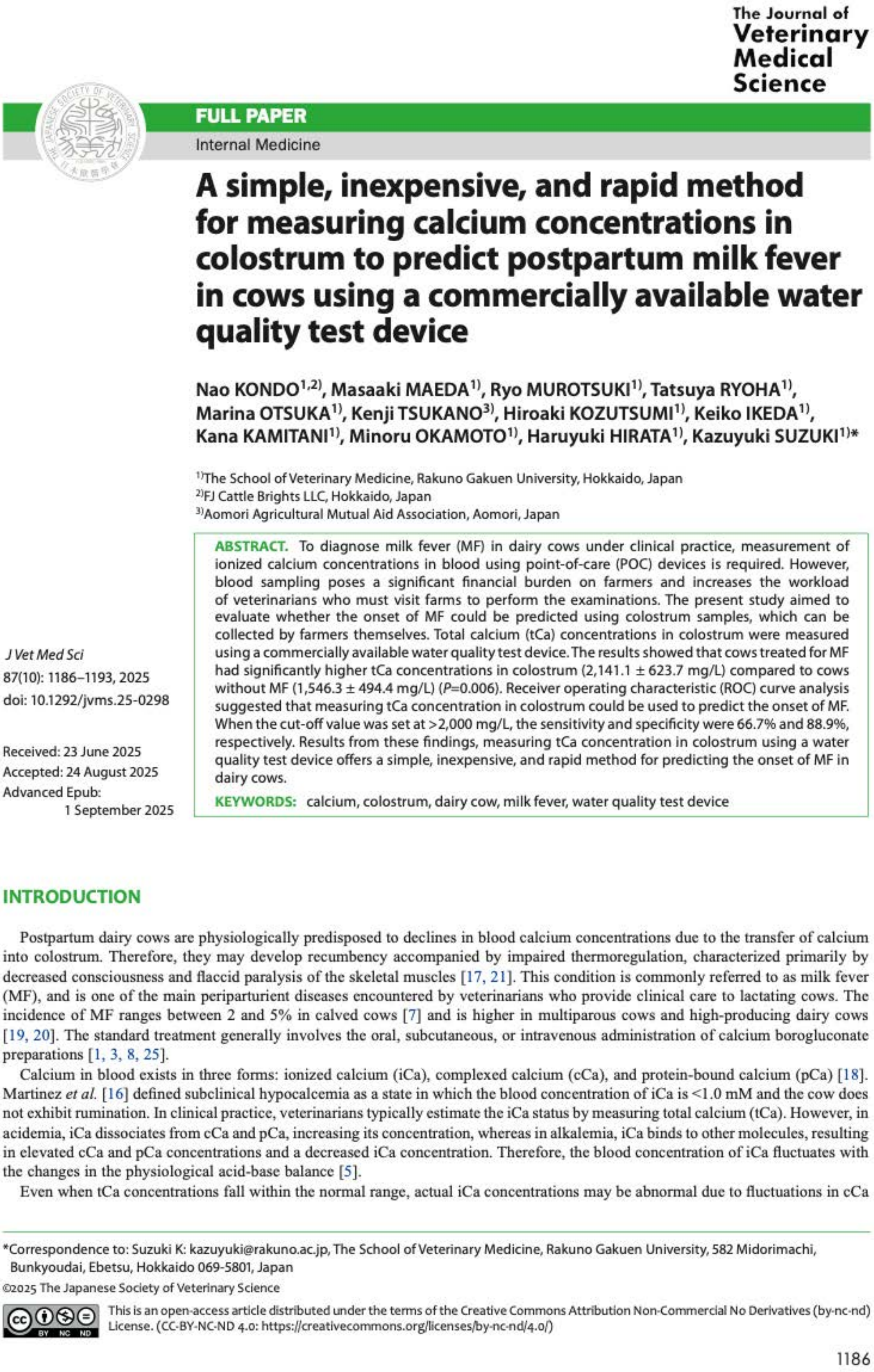

The Journal of
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FULL PAPER

Internal Medicine

A simple, inexpensive, and rapid method for measuring calcium concentrations in colostrum to predict postpartum milk fever in cows using a commercially available water quality test device

Nao KONDO[1,2], Masaaki MAEDA[1], Ryo MUROTSUKI[1], Tatsuya RYOHA[1], Marina OTSUKA[1], Kenji TSUKANO[3], Hiroaki KOZUTSUMI[1], Keiko IKEDA[1], Kana KAMITANI[1], Minoru OKAMOTO[1], Haruyuki HIRATA[1], Kazuyuki SUZUKI[1]*

[1]The School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]FJ Cattle Brights LLC, Hokkaido, Japan
[3]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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ABSTRACT. To diagnose milk fever (MF) in dairy cows under clinical practice, measurement of ionized calcium concentrations in blood using point-of-care (POC) devices is required. However, blood sampling poses a significant financial burden on farmers and increases the workload of veterinarians who must visit farms to perform the examinations. The present study aimed to evaluate whether the onset of MF could be predicted using colostrum samples, which can be collected by farmers themselves. Total calcium (tCa) concentrations in colostrum were measured using a commercially available water quality test device. The results showed that cows treated for MF had significantly higher tCa concentrations in colostrum (2,141.1 ± 623.7 mg/L) compared to cows without MF (1,546.3 ± 494.4 mg/L) (*P*=0.006). Receiver operating characteristic (ROC) curve analysis suggested that measuring tCa concentration in colostrum could be used to predict the onset of MF. When the cut-off value was set at >2,000 mg/L, the sensitivity and specificity were 66.7% and 88.9%, respectively. Results from these findings, measuring tCa concentration in colostrum using a water quality test device offers a simple, inexpensive, and rapid method for predicting the onset of MF in dairy cows.

KEYWORDS: calcium, colostrum, dairy cow, milk fever, water quality test device

INTRODUCTION

Postpartum dairy cows are physiologically predisposed to declines in blood calcium concentrations due to the transfer of calcium into colostrum. Therefore, they may develop recumbency accompanied by impaired thermoregulation, characterized primarily by decreased consciousness and flaccid paralysis of the skeletal muscles [17, 21]. This condition is commonly referred to as milk fever (MF), and is one of the main periparturient diseases encountered by veterinarians who provide clinical care to lactating cows. The incidence of MF ranges between 2 and 5% in calved cows [7] and is higher in multiparous cows and high-producing dairy cows [19, 20]. The standard treatment generally involves the oral, subcutaneous, or intravenous administration of calcium borogluconate preparations [1, 3, 8, 25].

Calcium in blood exists in three forms: ionized calcium (iCa), complexed calcium (cCa), and protein-bound calcium (pCa) [18]. Martinez *et al.* [16] defined subclinical hypocalcemia as a state in which the blood concentration of iCa is <1.0 mM and the cow does not exhibit rumination. In clinical practice, veterinarians typically estimate the iCa status by measuring total calcium (tCa). However, in acidemia, iCa dissociates from cCa and pCa, increasing its concentration, whereas in alkalemia, iCa binds to other molecules, resulting in elevated cCa and pCa concentrations and a decreased iCa concentration. Therefore, the blood concentration of iCa fluctuates with the changes in the physiological acid-base balance [5].

Even when tCa concentrations fall within the normal range, actual iCa concentrations may be abnormal due to fluctuations in cCa

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*Correspondence to: Suzuki K: kazuyuki@rakuno.ac.jp, The School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyoudai, Ebetsu, Hokkaido 069-5801, Japan

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FULL PAPER

Internal Medicine

Intestinal expression of diamine oxidase (DAO) and the relationship between blood DAO activity and haptoglobin concentration in diarrheal calves

Akihiro KOZUTSUMI1,2)#, Minoru OKAMOTO1)#, Hana TAKEUCHI1), Marina OTSUKA1), Kenji TSUKANO3), Nao KONDO1), Kana KAMITANI1), Keiko IKEDA1), Natsuki TAKAMI1), Haruyuki HIRATA1), Kazuyuki SUZUKI1)*

1)The School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
2)Miyagi Agricultural Mutual Aid Association, Miyagi, Japan
3)Aomori Agricultural Mutual Aid Association, Aomori, Japan

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ABSTRACT. The objectives of this study were (1) to confirm the expression of diamine oxidase (DAO) in the intestines of healthy calves immune-histologically and to clarify whether the expression of DAO in the intestines of calves with diarrhea differs from that of healthy animals, and (2) to clarify the relationship between DAO and intestinal inflammation using haptoglobin (HPT), a biomarker commonly used to evaluate inflammation. The abomasum, duodenum, jejunum and ileum, cecum, colon, and rectum of each animal were sampled at necropsy for immunohistochemical staining targeting DAO. DAO was diffusely expressed in all areas from the abomasum to the rectum in healthy calves, whereas its expression was reduced in calves with diarrhea. Four Japanese black calves with mild dehydration caused by diarrhea were included in the study to investigate the potential relationship between DAO and HPT. DAO activity was extremely low in diarrhea cases, whereas HPT levels were high. When an oral rehydration solution (ORS) was administered to these cases for three consecutive days, HPT significantly decreased logarithmically and fecal properties returned to normal on the fourth day of treatment. In contrast, DAO activities were significantly increased by oral rehydration therapy (ORT), and DAO activities were significantly negatively correlated with HPT concentrations. The present results confirmed that DAO is expressed in the epithelial cells of the intestinal mucosa in cattle, and that both the small and large intestines significantly contribute to blood DAO levels. Furthermore, DAO expression was reduced by enteritis.

KEYWORDS: calf, diarrhea, diamine oxidase, enteritis, haptoglobin

INTRODUCTION

Neonatal diarrhea remains the most common cause of death in beef and dairy calves and continues to be a major cause of economic loss in the cattle industry [4–6, 8, 21, 23, 24]. Digestive diseases cause great damage to cattle in veterinary care, and diarrhea is particularly common in calves, leading to many deaths. Although it is important to evaluate the effectiveness of treatment, difficulties are associated with performing a direct evaluation of the intestinal mucosa; therefore, subjective assessments, such as the mortality rate and fecal properties, are generally used [5]. Fukuda *et al.* [5] reported that abnormalities in plasma diamine oxidase (DAO) activity reflected the degree of intestinal mucosal disorder in calves with diarrhea. Thirty-six of 50 calves presented with diarrhea, and plasma DAO activity was significantly lower in those with severe or moderate diarrhea than in the control group. In addition, plasma DAO activity was significantly lower in the severe group than in the moderate group. Based on these findings, Fukuda *et al.* [5] suggested that plasma DAO activity reflected the degree of intestinal mucosal disorder due to diarrhea. Tsukano *et al.* [24] showed that the severity of intestinal mucosal damage was significantly lower in 32 Holstein diarrheal calves with *Cryptosporidium parvum* than in healthy calves and, thus, concluded that plasma DAO activity is a specific biomarker for *C. parvum*-associated intestinal mucosal damage in diarrheal calves.

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*Correspondence to: Suzuki K: kazuyuki@rakuno.ac.jp, The School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyoudai, Ebetsu, Hokkaido 069-5801, Japan

#These authors contributed equally to this work.

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獣医麻酔学（Veterinary Anesthesiology）

Takaharu Itami

Associate Professor

准教授　伊丹　貴晴

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Evaluation of drug distribution, sensory and motor blockade regions in canine coccygeal epidural anesthesia.
Aso R, **Itami T**, Hashimoto M, Hori A, Wei Y, Chen IY, Yamashita K.
J Vet Med Sci. in press. doi: 10.1292/jvms.25-0239

2）Evaluation of oxygen reserve index as an early warning indicator of hypoxemia in anesthetized dogs.
Hirokawa T, **Itami T**, Kato K, Minamoto Y, Chen IY, Sugita C, Endo Y, Miyasho T, Yamashita K.
J Vet Med Sci. 87: 1458-1466. 2025. doi: 10.1292/jvms.25-0197.

3）Inter-device variability in lactate measurement in dogs: comparison of blood gas analyzer and portable lactate meter.
Fujita M, **Itami T**, Kawase K, Morita T, Yamashita K.
J Vet Med Sci. 87: 697-701. 2025. doi: 10.1292/jvms.24-0489.

II. その他　＜Others＞

1）Lung injury promoted by strong inspiratory efforts and breath stacking: impact of ventilation mode.
Norisue Y, Usami S, Ito Y, Takeuchi M, Kawamura A, Nakayama R, Bunya N, Kataoka J, Endo Y, **Itami T**, Hirokawa T, Sugita C, Takeshima H, Takemoto A, Kyogoku M, Koike J, Fujitani S, Mojoli F, Miyasho T.
Intensive Care Med Exp. 13: 110. 2025. doi: 10.1186/s40635-025-00821-0.

2） Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study
Goda Y, Yamamoto S, Miyoshi K, Hanazono K, Morimura M, Sakai T, Tamura M, Deguchi T, Endo Y, Kadosawa T, Fujita M, **Itami T**, Torisu S.
Open Vet J. 15: 5739-5747. Doi: 10.5455/OVJ.2025.v15.ill.29.

Surgery

Full paper

Evaluation of drug distribution, sensory and motor blockade regions in canine coccygeal epidural anesthesia

Ryota ASO[1)], Takaharu ITAMI[1)], Masayuki HASHIMOTO[1)], Ai HORI[1)], Yixian WEI[1)], I-Ying CHEN[1)], Kazuto YAMASHITA[1)]

1) Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8591, Japan

Corresponding author: Takaharu ITAMI
Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8591, Japan
Fax/Phone: +81-11-386-4801
E-mail: t-itami@rakuno.ac.jp

RUNNING HEAD:

COCCYGEAL EPIDURAL ANESTHESIA IN DOGS

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Evaluation of drug distribution, sensory and motor blockade regions in canine coccygeal epidural anesthesia

Ryota Aso [1], Takaharu Itami [1], Masayuki Hashimoto [1], Ai Hori [1], Yixian Wei [1], I-Ying Chen [1], Kazuto Yamashita [1]

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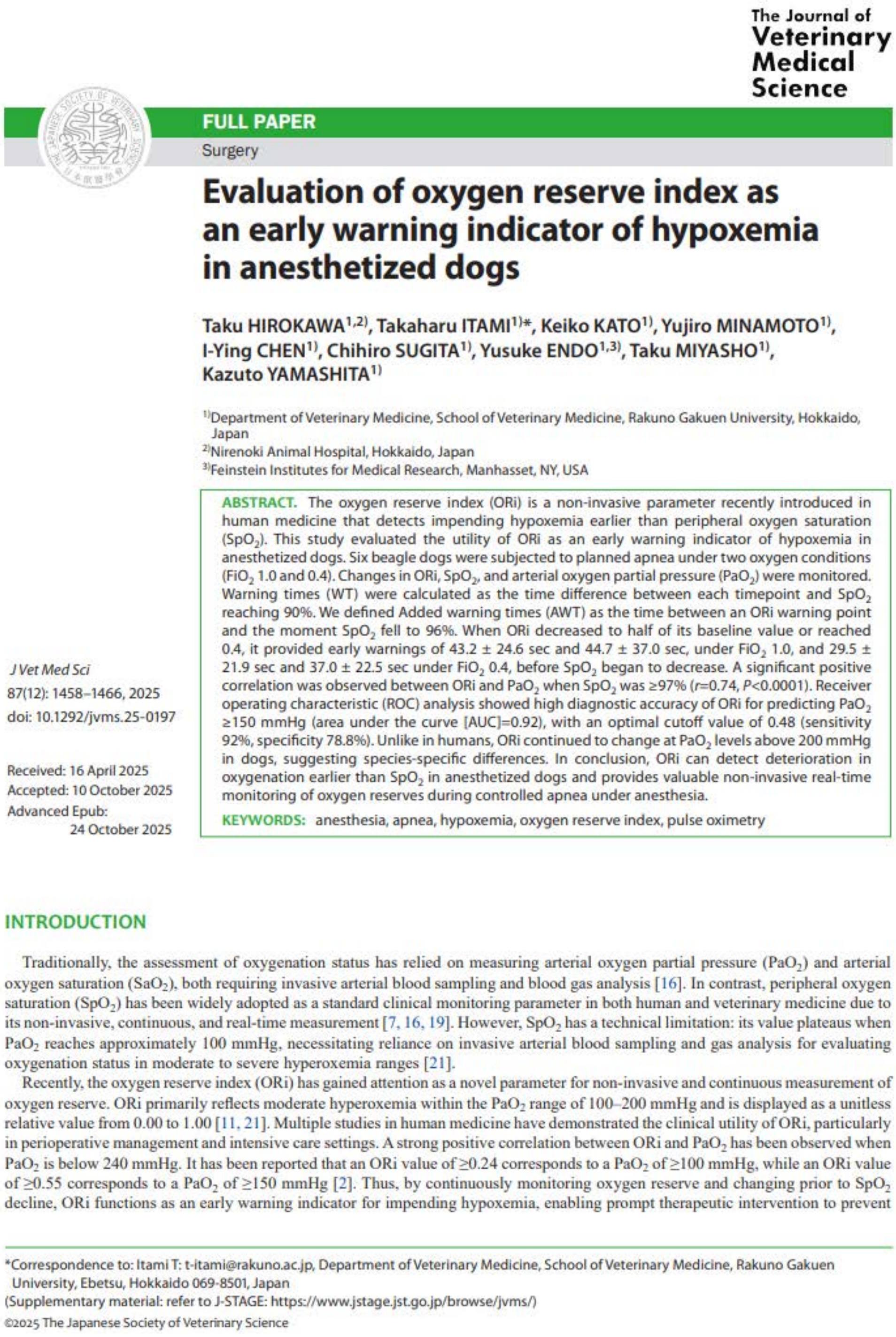

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FULL PAPER
Surgery

Evaluation of oxygen reserve index as an early warning indicator of hypoxemia in anesthetized dogs

Taku HIROKAWA[1,2], Takaharu ITAMI[1]*, Keiko KATO[1], Yujiro MINAMOTO[1], I-Ying CHEN[1], Chihiro SUGITA[1], Yusuke ENDO[1,3], Taku MIYASHO[1], Kazuto YAMASHITA[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]Nirenoki Animal Hospital, Hokkaido, Japan
[3]Feinstein Institutes for Medical Research, Manhasset, NY, USA

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ABSTRACT. The oxygen reserve index (ORi) is a non-invasive parameter recently introduced in human medicine that detects impending hypoxemia earlier than peripheral oxygen saturation (SpO_2). This study evaluated the utility of ORi as an early warning indicator of hypoxemia in anesthetized dogs. Six beagle dogs were subjected to planned apnea under two oxygen conditions (FiO_2 1.0 and 0.4). Changes in ORi, SpO_2, and arterial oxygen partial pressure (PaO_2) were monitored. Warning times (WT) were calculated as the time difference between each timepoint and SpO_2 reaching 90%. We defined Added warning times (AWT) as the time between an ORi warning point and the moment SpO_2 fell to 96%. When ORi decreased to half of its baseline value or reached 0.4, it provided early warnings of 43.2 ± 24.6 sec and 44.7 ± 37.0 sec, under FiO_2 1.0, and 29.5 ± 21.9 sec and 37.0 ± 22.5 sec under FiO_2 0.4, before SpO_2 began to decrease. A significant positive correlation was observed between ORi and PaO_2 when SpO_2 was ≥97% (r=0.74, P<0.0001). Receiver operating characteristic (ROC) analysis showed high diagnostic accuracy of ORi for predicting PaO_2 ≥150 mmHg (area under the curve [AUC]=0.92), with an optimal cutoff value of 0.48 (sensitivity 92%, specificity 78.8%). Unlike in humans, ORi continued to change at PaO_2 levels above 200 mmHg in dogs, suggesting species-specific differences. In conclusion, ORi can detect deterioration in oxygenation earlier than SpO_2 in anesthetized dogs and provides valuable non-invasive real-time monitoring of oxygen reserves during controlled apnea under anesthesia.

KEYWORDS: anesthesia, apnea, hypoxemia, oxygen reserve index, pulse oximetry

INTRODUCTION

Traditionally, the assessment of oxygenation status has relied on measuring arterial oxygen partial pressure (PaO_2) and arterial oxygen saturation (SaO_2), both requiring invasive arterial blood sampling and blood gas analysis [16]. In contrast, peripheral oxygen saturation (SpO_2) has been widely adopted as a standard clinical monitoring parameter in both human and veterinary medicine due to its non-invasive, continuous, and real-time measurement [7, 16, 19]. However, SpO_2 has a technical limitation: its value plateaus when PaO_2 reaches approximately 100 mmHg, necessitating reliance on invasive arterial blood sampling and gas analysis for evaluating oxygenation status in moderate to severe hyperoxemia ranges [21].

Recently, the oxygen reserve index (ORi) has gained attention as a novel parameter for non-invasive and continuous measurement of oxygen reserve. ORi primarily reflects moderate hyperoxemia within the PaO_2 range of 100–200 mmHg and is displayed as a unitless relative value from 0.00 to 1.00 [11, 21]. Multiple studies in human medicine have demonstrated the clinical utility of ORi, particularly in perioperative management and intensive care settings. A strong positive correlation between ORi and PaO_2 has been observed when PaO_2 is below 240 mmHg. It has been reported that an ORi value of ≥0.24 corresponds to a PaO_2 of ≥100 mmHg, while an ORi value of ≥0.55 corresponds to a PaO_2 of ≥150 mmHg [2]. Thus, by continuously monitoring oxygen reserve and changing prior to SpO_2 decline, ORi functions as an early warning indicator for impending hypoxemia, enabling prompt therapeutic intervention to prevent

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*Correspondence to: Itami T: t-itami@rakuno.ac.jp, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
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The Journal of Veterinary Medical Science

NOTE
Internal Medicine

Inter-device variability in lactate measurement in dogs: comparison of blood gas analyzer and portable lactate meter

Mayu FUJITA[1)], Takaharu ITAMI[1)]*, Kodai KAWASE[2)], Tomoya MORITA[2)], Kazuto YAMASHITA[1)]

[1)]Department of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]Sapporo Veterinary Emergency Hospital, Hokkaido, Japan

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ABSTRACT. Lactate levels are critical indicators of severe, life-threatening conditions and are widely used as prognostic markers in veterinary emergency care. Blood gas analyzers and portable lactate meters are common tools, but discrepancies between devices, especially at high lactate levels, have been reported. This study aimed to evaluate inter-device variability in canine lactate measurement. Lactate levels in 118 dogs were measured using a blood gas analyzer and a portable lactate meter. A strong correlation (R=0.906) was observed; however, the portable meter exhibited an 18% positive bias primarily characterized by additive error. These findings emphasize the need for careful device selection and the standardization of lactate measurement methods.

KEYWORDS: blood gas analyzer, canine, inter-device variability, lactate, portable lactate meter

Oxygen supply is essential for the life activities of both humans and animals. When oxygen is adequately supplied, aerobic respiration occurs, enabling efficient ATP production, with water and carbon dioxide as significant by-products. Conversely, when the balance between oxygen supply and demand is disrupted, leading to hypoxia, aerobic respiration is suppressed, and anaerobic metabolic pathways are activated. This anaerobic metabolism produces lactate as a by-product, which accumulates in the blood. Therefore, blood lactate levels serve as a widely recognized marker of oxygen deficiency in tissues and peripheral regions, reflecting hypoxic conditions and inadequate oxygenation [14]. However, recent studies have suggested that the interpretation of blood lactate levels has become more complex. Hyperlactatemia is not only caused by hypoxia but also by increased aerobic glycolysis during stress responses [12], the activation of Na^+-K^+-ATPase in skeletal muscles due to adrenergic stimulation [9], and the inhibition of glycolysis caused by hyperventilation [10]. Thus, hyperlactatemia may result from various factors, and lactate remains a valuable indicator of disease severity and mortality risk in critically ill patients.

In veterinary medicine, similar to human medicine, lactate levels have gained attention for their prognostic value and clinical utility. Recently, blood lactate levels have also been widely used as indicators of disease severity and response to treatment. In acute, life-threatening conditions in dogs, such as immune-mediated hemolytic anemia and gastric dilatation-volvulus syndrome, it has been reported that lactate levels above 4 mmol/L are associated with an increased risk of poor prognosis [5, 7]. Additionally, in cases of canine babesiosis, significant differences in lactate levels have been observed between survivors and non-survivors [13], and in cases of septic peritonitis, a high lactate clearance following treatment is correlated with improved survival rates [2].

In scientific literature on blood lactate levels, measurements are primarily conducted using blood gas analyzers, which are known for their high accuracy and calibration capabilities, ensuring reliability [15]. However, blood gas analyzers are expensive in terms of the equipment itself, maintenance, and operational costs, limiting their widespread use in veterinary practice. In contrast, portable lactate meters are more affordable and easier to use than blood gas analyzers. Consequently, in some cases, initial blood gas analysis may be performed to assess acid-base balance, followed by lactate measurement using portable meters. While inter-device discrepancies have been well-documented in human medicine, their implications for veterinary practice remain underexplored. Portable lactate meters have been reported to be useful for measuring lactate levels within the reference range and near the upper limit, but their performance in the high lactate range warrants further investigation [17]. Therefore, in situations requiring multiple lactate measurements or when high lactate levels are observed, these discrepancies may significantly impact treatment decisions.

The objective of this study was to investigate the inter-device discrepancies between blood gas analyzers and portable lactate meters in canine lactate measurement, with a particular focus on measurement errors at high lactate levels. Using cases from a veterinary

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*Correspondence to: Itami T: t-itami@rakuno.ac.jp, Department of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

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最終責任者　Takaharu Itami (Corresponding Author)

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Intensive Care Medicine
Experimental

RESEARCH ARTICLES **Open Access**

Lung injury promoted by strong inspiratory efforts and breath stacking: impact of ventilation mode

Yasuhiro Norisue[1*], Sunao Usami[2], Yukie Ito[3], Muneyuki Takeuchi[4], Atsushi Kawamura[3], Ryuichi Nakayama[5], Naofumi Bunya[6], Jun Kataoka[7], Yusuke Endo[8], Takaharu Itami[8], Taku Hirokawa[8], Chihiro Sugita[8], Hirotaka Takeshima[3,9], Airi Takemoto[3], Miyako Kyogoku[4], Junki Koike[10], Shigeki Fujitani[11], Francesco Mojoli[12] and Taku Miyasho[8]

Abstract

Background Breath stacking, particularly double triggering, is a common patient–ventilator asynchrony during strong inspiratory effort. It can cause excessive tidal volumes and high transpulmonary pressures, contributing to ventilator-induced lung injury (VILI). The mode-specific consequences of breath stacking induced by strong inspiratory effort remain unclear.

Methods In a porcine model of minimal lung injury, 17 animals were randomized to volume-controlled ventilation (VCV, $n=9$) or pressure-controlled ventilation (PCV, $n=8$). High respiratory drive was induced with continuous CO_2 inhalation, and ventilator settings were dynamically adjusted to maintain a breath stacking ratio of 40–70% of spontaneous efforts. Measurements included airway and transpulmonary pressures, driving pressures, tidal volume, esophageal pressure swings (ΔPes), stress index (SI), respiratory compliance, and histological lung injury. Risk factors for baro/volutrauma were defined by elevated plateau or driving pressures, transpulmonary pressures, or tidal volume >10 mL/kg. Atelectrauma risk was defined by SI < 0.9, negative end-expiratory transpulmonary pressure (PLexp), or vigorous effort (ΔPes > 5 cmH_2O or Pmus > 8 cmH_2O).

Results VCV animals exhibited higher respiratory rates (44.0 vs. 30.5 breaths/min, $p=0.027$), whereas PCV resulted in stronger inspiratory efforts (ΔPes 6.1 vs. 4.2 cmH_2O, $p=0.015$). During breath stacking, VCV produced larger tidal volumes and higher plateau pressures, accumulating more baro/volutrauma risk factors (median 4.0 vs. 0.0, $p<0.001$). In contrast, PCV animals developed more atelectrauma risk factors (3.0 vs. 1.0, $p=0.004$). Histological injury scores were comparable, with a non-significant trend toward greater severity in PCV.

Conclusions Breath stacking under strong inspiratory drive can promote lung injury through distinct mechanisms depending on ventilation mode. VCV was associated with the risk of overdistension, whereas PCV involved vigorous inspiratory effort and potential atelectrauma. Double triggering should be recognized as a clinical warning sign, prompting careful assessment of respiratory drive, inspiratory effort, and ventilator settings.

Keywords Breath stacking, Spontaneous effort, Ventilator-induced lung injury, Volume-controlled ventilation, Pressure-controlled ventilation, Patient–ventilator interaction, Atelectrauma, P-SILI, Porcine model

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*Correspondence:
Yasuhiro Norisue
norisue.yasuhiro@gmail.com
Full list of author information is available at the end of the article

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Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study

Yoshimichi Goda[1*], Shushi Yamamoto[1*], Kenjiro Miyoshi[1], Kiwamu Hanazono[1],
Mai Morimura[1], Toshikazu Sakai[1], Masahiro Tamura[1], Tatsuya Deguchi[1], Yoshifumi Endo[1,2],
Tsuyoshi Kadosawa[1,3], Mayu Fujita[1], Takaharu Itami[1] and Shidow Torisu[1]*

[1]*Department of Companion Animal Clinical Sciences, School of Veterinary Medicine,*
Rakuno Gakuen University, Ebetsu, Japan
[2]*Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, Japan*
[3]*Japan Small Animal Medical Center, Tokorozawa-Shi, Japan*
[*]*Both authors are equally contributed to this work*

ABSTRACT

Background: Recently, the usefulness of triple-phase contrast-enhanced computed tomography (CT) scans for liver tumors in canines has been reported. However, detailed information on the CT findings of cholangiocarcinoma (CCA) and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) remains limited.

Aim: This study aimed to retrospectively evaluate triple-phase contrast-enhanced CT findings in canine CCA and cHCC-CCA using objective parameters, such as CT values and morphological characteristics.

Methods: This study included eight dogs that underwent triple-phase contrast-enhanced CT scans and surgical removal and were pathologically diagnosed. Three CCA cases and five cHCC-CCA cases were analyzed. Parameters included CT values and mass sizes, CT values and lymph node sizes, and rim enhancement. "Rim enhancement" was defined as the contrast enhancement effect at the liver parenchyma–mass boundary.

Results: CT values for CCA were as follows: pre-contrast: 43.9 ± 3.6 Hounsfield units (HUs); arterial phase: 76.9 ± 22.5 HU; portal phase: 98.8 ± 37.7 HU; equilibrium phase: 90.9 ± 27.1 HU. For cHCC-CCA, the mean CT values were as follows: pre-contrast: 50.7 ± 6.1 HU; arterial phase: 80.2 ± 19.2 HU; portal phase: 95.0 ± 21.3 HU; equilibrium phase: 86.1 ± 13.4 HU. The peak contrast enhancement for both CCA and cHCC-CCA was in the portal phase. Rim enhancement appeared in the arterial phase in all CCA cases, whereas it appeared in the portal phase in 80% of cHCC-CCA cases, indicating a significant difference.

Conclusion: Rim enhancement may help distinguish between CCA and cHCC-CCA. However, this study has a limited number of cases, and future large-scale, multicenter studies are warranted.

Keywords: Canine, Cholangiocarcinoma, Combined hepatocellular-cholangiocarcinoma, Computed tomography, Dog.

Introduction

Primary hepatic tumors in canines are rare, accounting for 0.6%–1.3% of all canine tumors (Patnaik *et al.*, 1981). The most common malignant liver tumor is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma (CCA) (Liptak *et al.*, 2019). Unlike in humans, the cause of HCC in dogs remains unknown, and the risk of occurrence based on breed or sex is unclear. However, there are reports indicating a higher incidence in male dogs. Infection with trematodes has been reported as a risk factor for the occurrence of CCA, but cases have also been reported outside the infection area, and the relationship remains unclear (Liptak *et al.*, 2019). Combined hepatocellular-cholangiocarcinoma (cHCC-CCA), which shows the histological features of both HCC and CCA, has also been reported (Patnaik *et al.*, 1980; Trigo *et al.*, 1982; Terai *et al.*, 2022). It has been reported that among malignant canine hepatic tumors, the incidence of HCC is 77%, CCA is 9%–41%, and HCC-cCCA is 4.6%, respectively (Liptak *et al.*, 2019; Terai *et al.*, 2022). The diagnosis of HCC and CCA involves blood tests, X-ray, and ultrasonography. As advanced examinations, triple-phase contrast-enhanced computed tomography (CT) and magnetic

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***Corresponding Author:** Shidow Torisu. Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan. Email: *s-torisu@rakuno.ac.jp*

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生産動物内科（Large Animal Internal Medicine）

Ryotaro Miura

Associate Professor

准教授　三浦　亮太朗

I. 筆頭または責任著者　＜First or Corresponding Author＞

1) Factors Associated with the Occurrence of the First-Follicular-Wave Dominant Follicle on the Ovary Ipsilateral to the Corpus Luteum in Dairy Cattle.
   Miura R, Matsui M.
   Animals 15: 2253-2253. 2025. https://doi.org/10.3390/ani15152253.
2) Combined Effects of Human Chorionic Gonadotropin and Intravaginal Progesterone Device Treatment in the Early Luteal Phase After Artificial Insemination on Conception Rate in Lactating Dairy Cows.
   Miura R, Izumi T, Wada Y, Hagita Y, Iga K, Sobu N, Hirase A, Osamu Yoneyama O, Miyake YI, Tajima T, Ajito T, Haneda S, Matsui M.
   Dairy 6: 26-26. 2025. https://doi.org/10.3390/dairy6030026.
3) Effects of large follicle numbers at the onset of the short-term timed artificial insemination protocol in lactating dairy cows.
   Bandai K, Chiba E, Ogata T, Tsuchiya Y, Ajito T, **Miura R**.
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II. その他　＜Others＞

1) Differences in the Gut Microbiota in Long-Term Infertile Holstein Repeat Breeder Cows and Healthy Fertile Holstein Cows.
   Kitagawa Y, Oishi S, Koshimizu K, **Miura R**, Hamano S, Iwata H, Shirasuna K. Animals 15: 2637. 2025.
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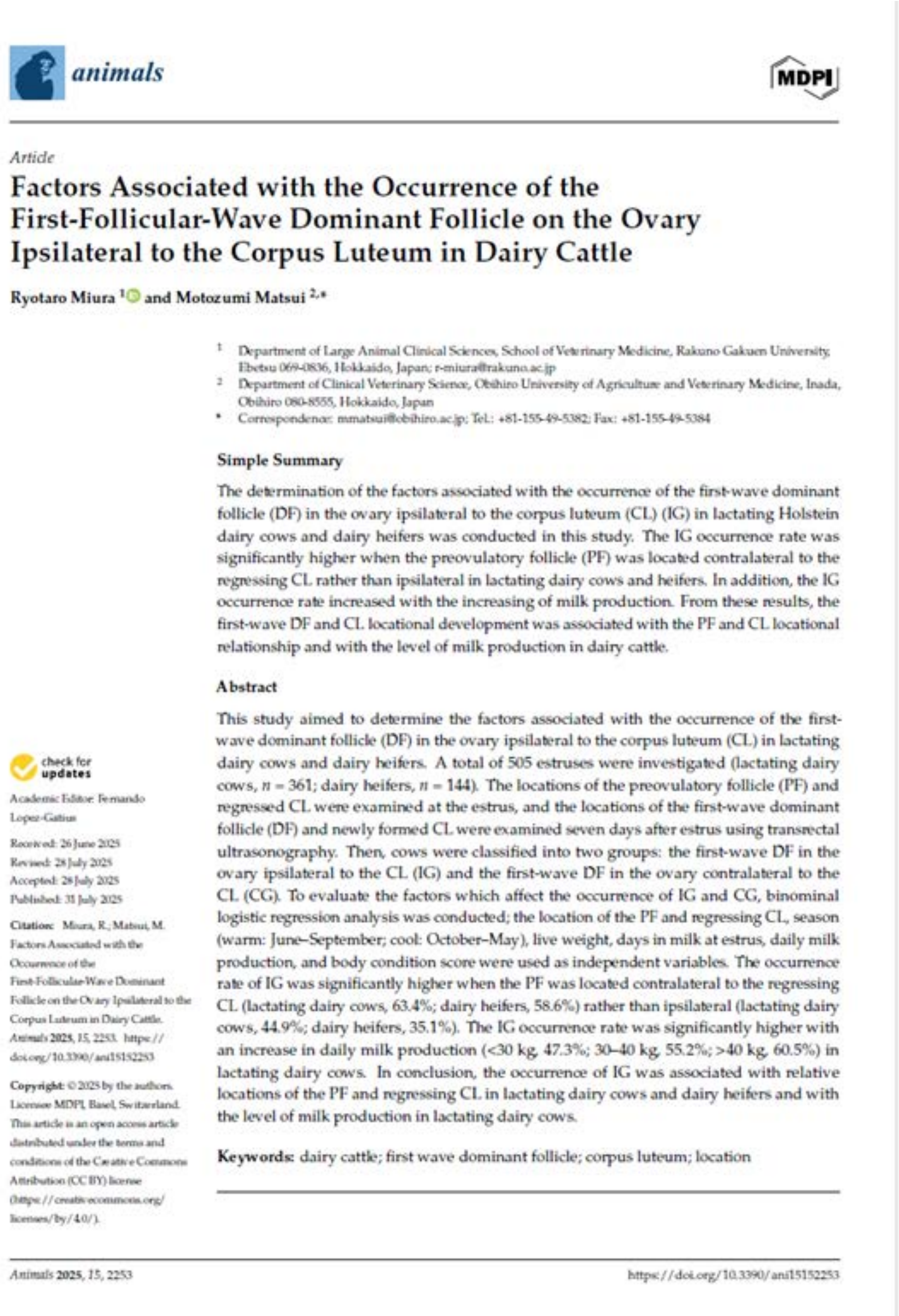
2) Effects of Oxidative Stress and Antioxidant Activity in Plasma and Uterine Fluid During Early Postpartum on Subsequent Reproductive Performance of Japanese Black Cows.
Hagita Y, **Miura R**, Shirasuna K, Tadaharu Ajito, Matsumoto H. Animals 15: 767-767. 2025. https://doi.org/10.3390/ani15060767.



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Article

Factors Associated with the Occurrence of the First-Follicular-Wave Dominant Follicle on the Ovary Ipsilateral to the Corpus Luteum in Dairy Cattle

Ryotaro Miura [1] and Motozumi Matsui [2,*]

[1] Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-0836, Hokkaido, Japan; r-miura@rakuno.ac.jp
[2] Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro 080-8555, Hokkaido, Japan
* Correspondence: mmatsui@obihiro.ac.jp; Tel.: +81-155-49-5382; Fax: +81-155-49-5384

Simple Summary

The determination of the factors associated with the occurrence of the first-wave dominant follicle (DF) in the ovary ipsilateral to the corpus luteum (CL) (IG) in lactating Holstein dairy cows and dairy heifers was conducted in this study. The IG occurrence rate was significantly higher when the preovulatory follicle (PF) was located contralateral to the regressing CL rather than ipsilateral in lactating dairy cows and heifers. In addition, the IG occurrence rate increased with the increasing of milk production. From these results, the first-wave DF and CL locational development was associated with the PF and CL locational relationship and with the level of milk production in dairy cattle.

Abstract

This study aimed to determine the factors associated with the occurrence of the first-wave dominant follicle (DF) in the ovary ipsilateral to the corpus luteum (CL) in lactating dairy cows and dairy heifers. A total of 505 estruses were investigated (lactating dairy cows, $n = 361$; dairy heifers, $n = 144$). The locations of the preovulatory follicle (PF) and regressed CL were examined at the estrus, and the locations of the first-wave dominant follicle (DF) and newly formed CL were examined seven days after estrus using transrectal ultrasonography. Then, cows were classified into two groups: the first-wave DF in the ovary ipsilateral to the CL (IG) and the first-wave DF in the ovary contralateral to the CL (CG). To evaluate the factors which affect the occurrence of IG and CG, binominal logistic regression analysis was conducted; the location of the PF and regressing CL, season (warm: June–September; cool: October–May), live weight, days in milk at estrus, daily milk production, and body condition score were used as independent variables. The occurrence rate of IG was significantly higher when the PF was located contralateral to the regressing CL (lactating dairy cows, 63.4%; dairy heifers, 58.6%) rather than ipsilateral (lactating dairy cows, 44.9%; dairy heifers, 35.1%). The IG occurrence rate was significantly higher with an increase in daily milk production (<30 kg, 47.3%; 30–40 kg, 55.2%; >40 kg, 60.5%) in lactating dairy cows. In conclusion, the occurrence of IG was associated with relative locations of the PF and regressing CL in lactating dairy cows and dairy heifers and with the level of milk production in lactating dairy cows.

Keywords: dairy cattle; first wave dominant follicle; corpus luteum; location

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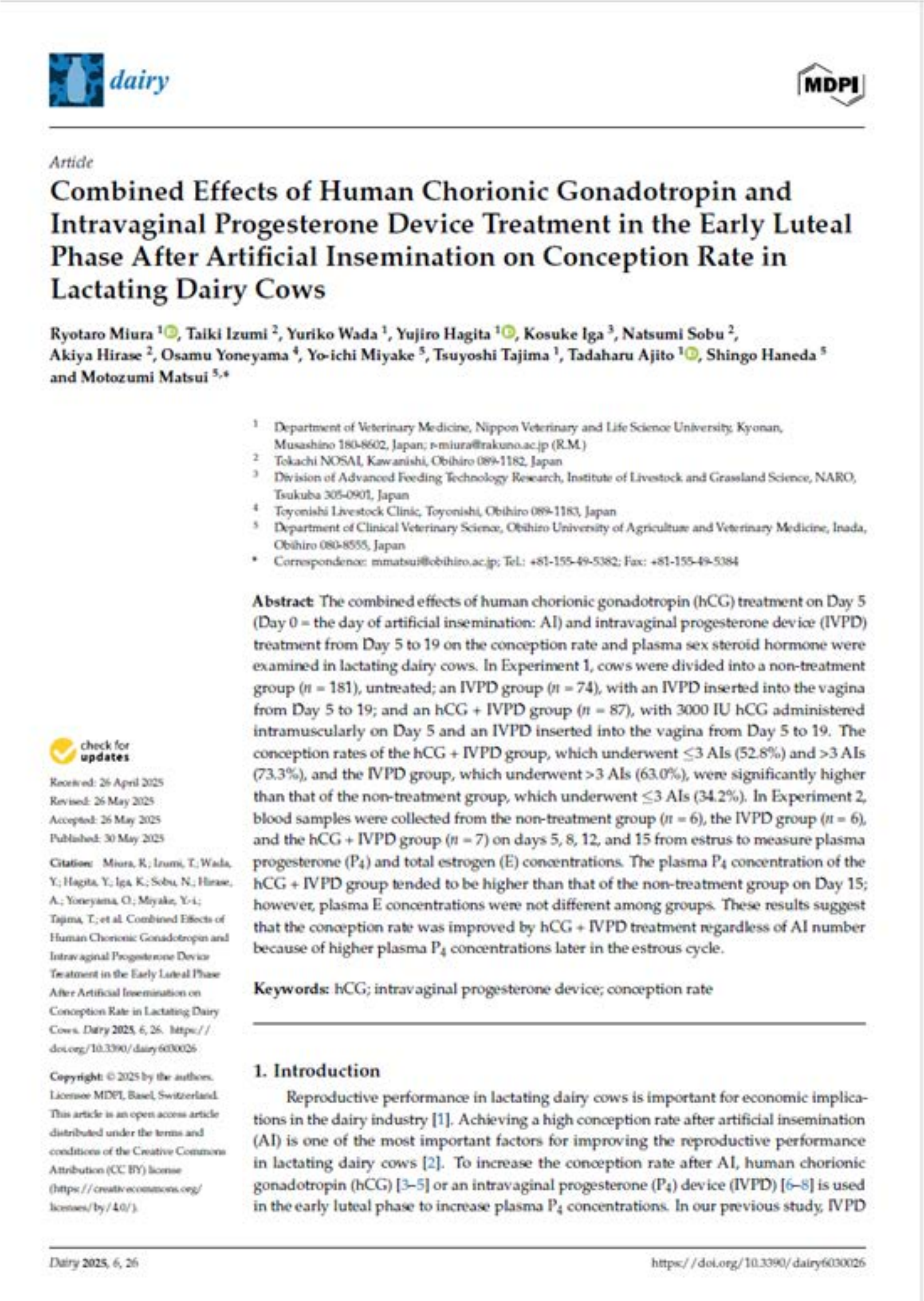

dairy

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Article

Combined Effects of Human Chorionic Gonadotropin and Intravaginal Progesterone Device Treatment in the Early Luteal Phase After Artificial Insemination on Conception Rate in Lactating Dairy Cows

Ryotaro Miura [1], Taiki Izumi [2], Yuriko Wada [1], Yujiro Hagita [1], Kosuke Iga [3], Natsumi Sobu [2], Akiya Hirase [2], Osamu Yoneyama [4], Yo-ichi Miyake [5], Tsuyoshi Tajima [1], Tadaharu Ajito [1], Shingo Haneda [5] and Motozumi Matsui [5,*]

[1] Department of Veterinary Medicine, Nippon Veterinary and Life Science University, Kyonan, Musashino 180-8602, Japan; r-miura@rakuno.ac.jp (R.M.)
[2] Tokachi NOSAI, Kawanishi, Obihiro 089-1182, Japan
[3] Division of Advanced Feeding Technology Research, Institute of Livestock and Grassland Science, NARO, Tsukuba 305-0901, Japan
[4] Toyonishi Livestock Clinic, Toyonishi, Obihiro 089-1183, Japan
[5] Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro 080-8555, Japan
* Correspondence: mmatsui@obihiro.ac.jp; Tel.: +81-155-49-5382; Fax: +81-155-49-5384

Abstract: The combined effects of human chorionic gonadotropin (hCG) treatment on Day 5 (Day 0 = the day of artificial insemination: AI) and intravaginal progesterone device (IVPD) treatment from Day 5 to 19 on the conception rate and plasma sex steroid hormone were examined in lactating dairy cows. In Experiment 1, cows were divided into a non-treatment group (n = 181), untreated; an IVPD group (n = 74), with an IVPD inserted into the vagina from Day 5 to 19; and an hCG + IVPD group (n = 87), with 3000 IU hCG administered intramuscularly on Day 5 and an IVPD inserted into the vagina from Day 5 to 19. The conception rates of the hCG + IVPD group, which underwent ≤3 AIs (52.8%) and >3 AIs (73.3%), and the IVPD group, which underwent >3 AIs (63.0%), were significantly higher than that of the non-treatment group, which underwent ≤3 AIs (34.2%). In Experiment 2, blood samples were collected from the non-treatment group (n = 6), the IVPD group (n = 6), and the hCG + IVPD group (n = 7) on days 5, 8, 12, and 15 from estrus to measure plasma progesterone (P_4) and total estrogen (E) concentrations. The plasma P_4 concentration of the hCG + IVPD group tended to be higher than that of the non-treatment group on Day 15; however, plasma E concentrations were not different among groups. These results suggest that the conception rate was improved by hCG + IVPD treatment regardless of AI number because of higher plasma P_4 concentrations later in the estrous cycle.

Keywords: hCG; intravaginal progesterone device; conception rate

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1. Introduction

Reproductive performance in lactating dairy cows is important for economic implications in the dairy industry [1]. Achieving a high conception rate after artificial insemination (AI) is one of the most important factors for improving the reproductive performance in lactating dairy cows [2]. To increase the conception rate after AI, human chorionic gonadotropin (hCG) [3–5] or an intravaginal progesterone (P_4) device (IVPD) [6–8] is used in the early luteal phase to increase plasma P_4 concentrations. In our previous study, IVPD

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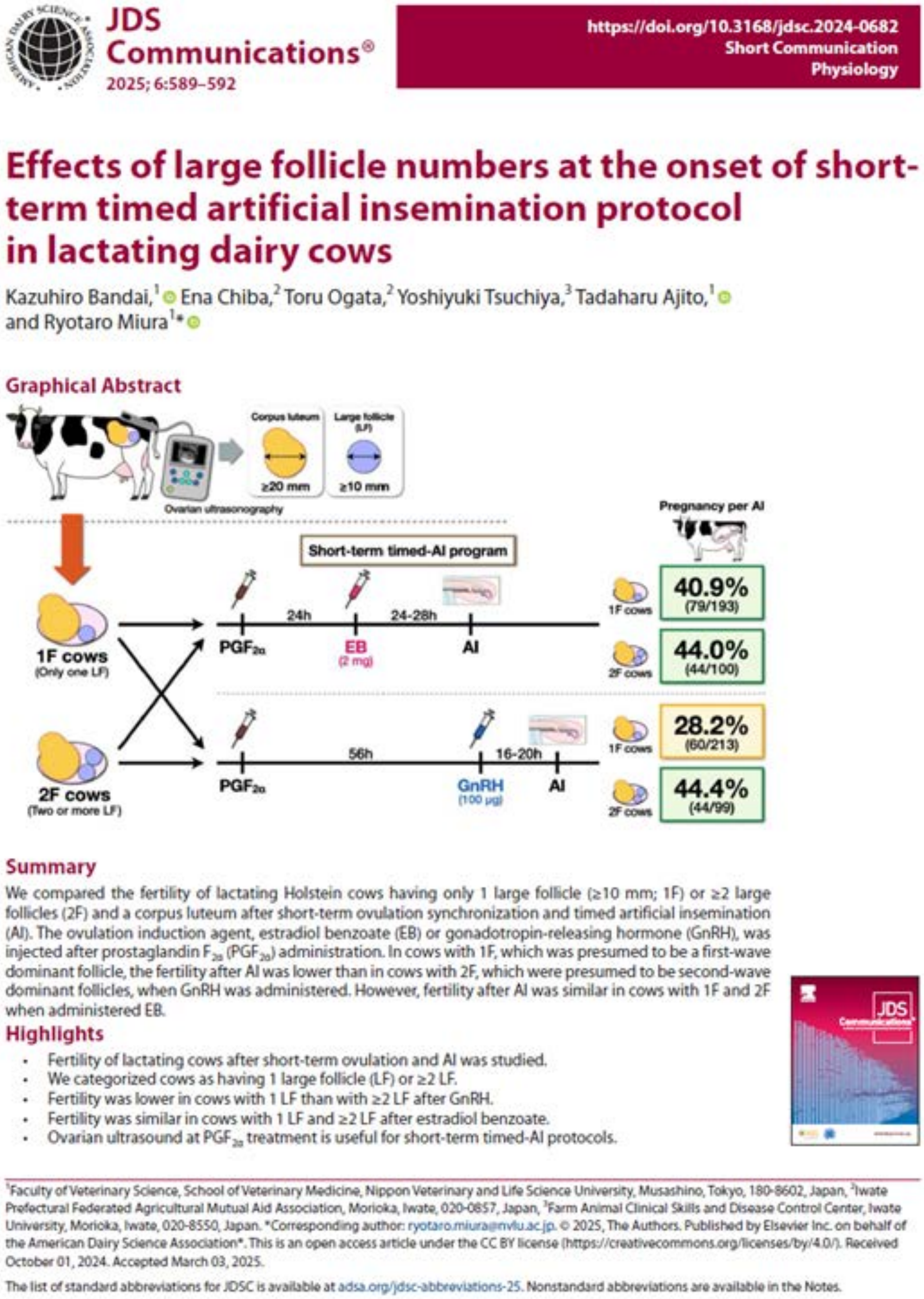

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Short Communication
Physiology

Effects of large follicle numbers at the onset of short-term timed artificial insemination protocol in lactating dairy cows

Kazuhiro Bandai,[1] Ena Chiba,[2] Toru Ogata,[2] Yoshiyuki Tsuchiya,[3] Tadaharu Ajito,[1]
and Ryotaro Miura[1]*

Graphical Abstract

Summary

We compared the fertility of lactating Holstein cows having only 1 large follicle (≥10 mm; 1F) or ≥2 large follicles (2F) and a corpus luteum after short-term ovulation synchronization and timed artificial insemination (AI). The ovulation induction agent, estradiol benzoate (EB) or gonadotropin-releasing hormone (GnRH), was injected after prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration. In cows with 1F, which was presumed to be a first-wave dominant follicle, the fertility after AI was lower than in cows with 2F, which were presumed to be second-wave dominant follicles, when GnRH was administered. However, fertility after AI was similar in cows with 1F and 2F when administered EB.

Highlights

- Fertility of lactating cows after short-term ovulation and AI was studied.
- We categorized cows as having 1 large follicle (LF) or ≥2 LF.
- Fertility was lower in cows with 1 LF than with ≥2 LF after GnRH.
- Fertility was similar in cows with 1 LF and ≥2 LF after estradiol benzoate.
- Ovarian ultrasound at $PGF_{2\alpha}$ treatment is useful for short-term timed-AI protocols.

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[1]Faculty of Veterinary Science, School of Veterinary Medicine, Nippon Veterinary and Life Science University, Musashino, Tokyo, 180-8602, Japan, [2]Iwate Prefectural Federated Agricultural Mutual Aid Association, Morioka, Iwate, 020-0857, Japan, [3]Farm Animal Clinical Skills and Disease Control Center, Iwate University, Morioka, Iwate, 020-8550, Japan. *Corresponding author: ryotaro.miura@nvlu.ac.jp. Received October 01, 2024. Accepted March 03, 2025.

The list of standard abbreviations for JDSC is available at adsa.org/jdsc-abbreviations-25. Nonstandard abbreviations are available in the Notes.

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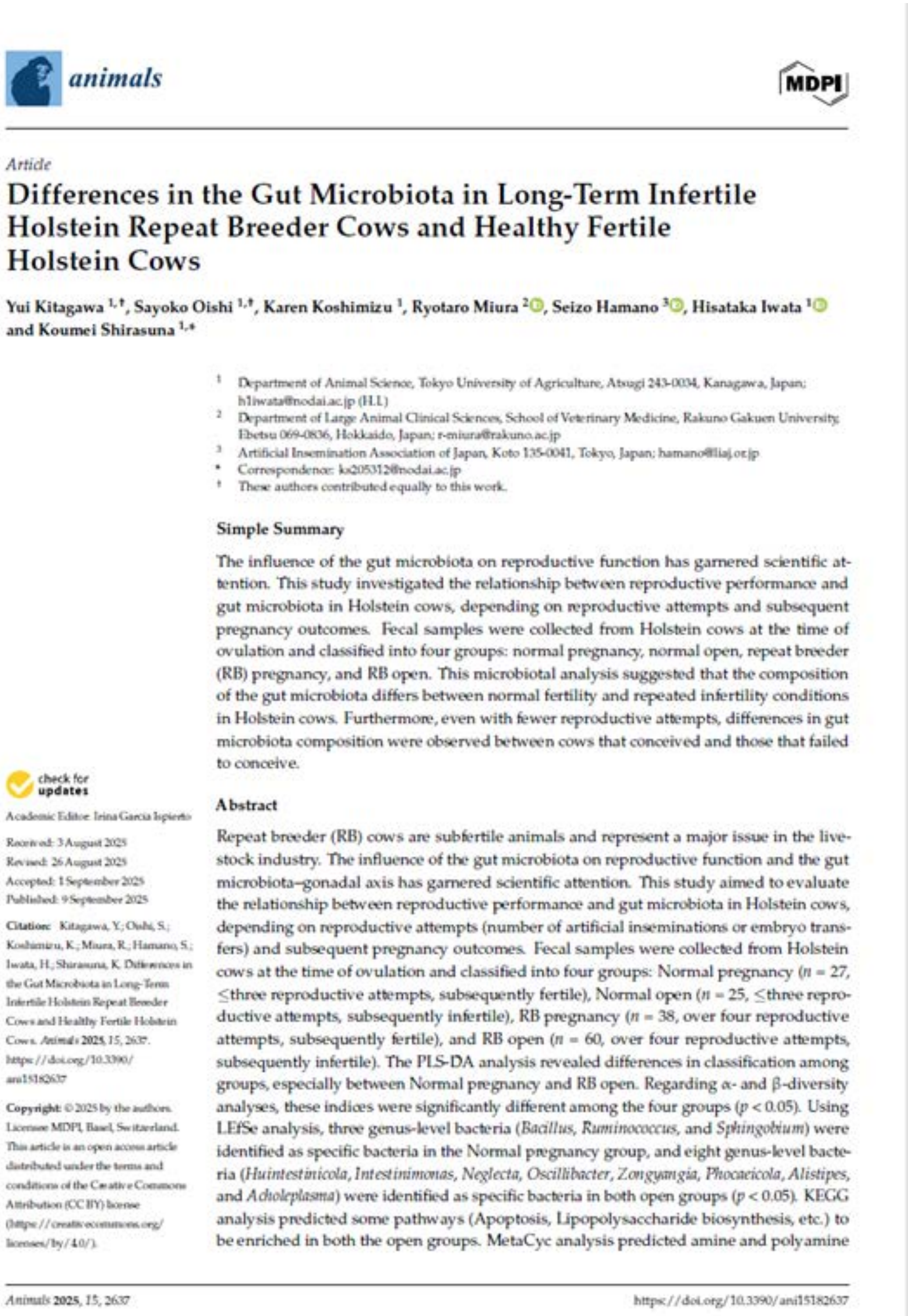

animals

Article

Differences in the Gut Microbiota in Long-Term Infertile Holstein Repeat Breeder Cows and Healthy Fertile Holstein Cows

Yui Kitagawa [1,†], Sayoko Oishi [1,†], Karen Koshimizu [1], Ryotaro Miura [2], Seizo Hamano [3], Hisataka Iwata [1]
and Koumei Shirasuna [1,*]

[1] Department of Animal Science, Tokyo University of Agriculture, Atsugi 243-0034, Kanagawa, Japan; h1iwata@nodai.ac.jp (H.I.)
[2] Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-0836, Hokkaido, Japan; r-miura@rakuno.ac.jp
[3] Artificial Insemination Association of Japan, Koto 135-0041, Tokyo, Japan; hamano@liaj.or.jp
* Correspondence: ks205312@nodai.ac.jp
† These authors contributed equally to this work.

Simple Summary

The influence of the gut microbiota on reproductive function has garnered scientific attention. This study investigated the relationship between reproductive performance and gut microbiota in Holstein cows, depending on reproductive attempts and subsequent pregnancy outcomes. Fecal samples were collected from Holstein cows at the time of ovulation and classified into four groups: normal pregnancy, normal open, repeat breeder (RB) pregnancy, and RB open. This microbiotal analysis suggested that the composition of the gut microbiota differs between normal fertility and repeated infertility conditions in Holstein cows. Furthermore, even with fewer reproductive attempts, differences in gut microbiota composition were observed between cows that conceived and those that failed to conceive.

Abstract

Repeat breeder (RB) cows are subfertile animals and represent a major issue in the livestock industry. The influence of the gut microbiota on reproductive function and the gut microbiota–gonadal axis has garnered scientific attention. This study aimed to evaluate the relationship between reproductive performance and gut microbiota in Holstein cows, depending on reproductive attempts (number of artificial inseminations or embryo transfers) and subsequent pregnancy outcomes. Fecal samples were collected from Holstein cows at the time of ovulation and classified into four groups: Normal pregnancy ($n = 27$, $\leq$three reproductive attempts, subsequently fertile), Normal open ($n = 25$, $\leq$three reproductive attempts, subsequently infertile), RB pregnancy ($n = 38$, over four reproductive attempts, subsequently fertile), and RB open ($n = 60$, over four reproductive attempts, subsequently infertile). The PLS-DA analysis revealed differences in classification among groups, especially between Normal pregnancy and RB open. Regarding α- and β-diversity analyses, these indices were significantly different among the four groups ($p < 0.05$). Using LEfSe analysis, three genus-level bacteria (*Bacillus*, *Ruminococcus*, and *Sphingobium*) were identified as specific bacteria in the Normal pregnancy group, and eight genus-level bacteria (*Huintestinicola*, *Intestinimonas*, *Neglecta*, *Oscillibacter*, *Zongyangia*, *Phocaeicola*, *Alistipes*, and *Acholeplasma*) were identified as specific bacteria in both open groups ($p < 0.05$). KEGG analysis predicted some pathways (Apoptosis, Lipopolysaccharide biosynthesis, etc.) to be enriched in both the open groups. MetaCyc analysis predicted amine and polyamine

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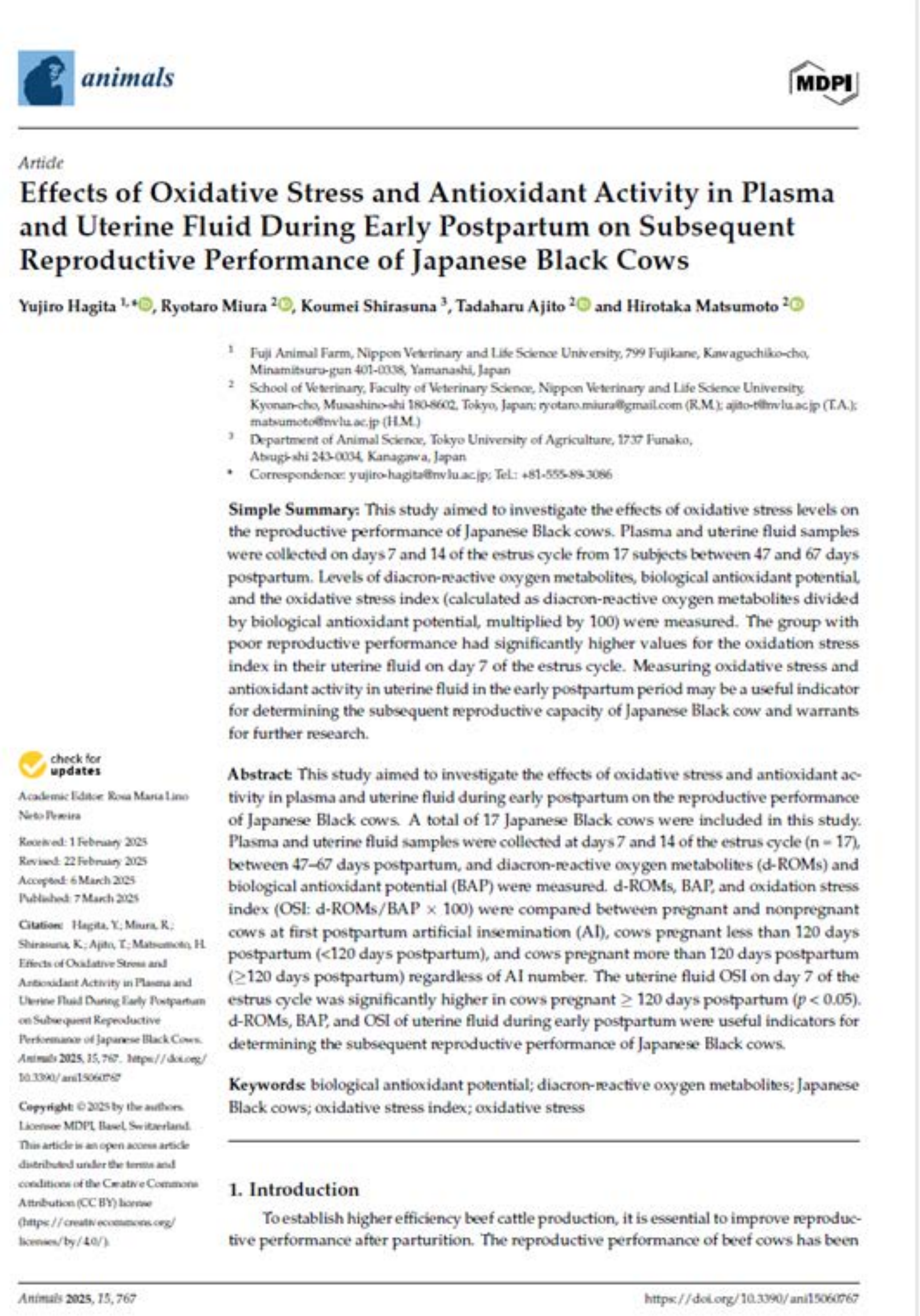
最終責任者　Shirasuna Koumei (Corresponding Author)



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Article

Effects of Oxidative Stress and Antioxidant Activity in Plasma and Uterine Fluid During Early Postpartum on Subsequent Reproductive Performance of Japanese Black Cows

Yujiro Hagita [1,*], **Ryotaro Miura** [2], **Koumei Shirasuna** [3], **Tadaharu Ajito** [2] **and Hirotaka Matsumoto** [2]

[1] Fuji Animal Farm, Nippon Veterinary and Life Science University, 799 Fujikane, Kawaguchiko-cho, Minamitsuru-gun 401-0338, Yamanashi, Japan
[2] School of Veterinary, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Kyonan-cho, Musashino-shi 180-8602, Tokyo, Japan; ryotaro.miura@gmail.com (R.M.); ajito-t@nvlu.ac.jp (T.A.); matsumoto@nvlu.ac.jp (H.M.)
[3] Department of Animal Science, Tokyo University of Agriculture, 1737 Funako, Atsugi-shi 243-0034, Kanagawa, Japan
* Correspondence: yujiro-hagita@nvlu.ac.jp; Tel.: +81-555-89-3086

Simple Summary: This study aimed to investigate the effects of oxidative stress levels on the reproductive performance of Japanese Black cows. Plasma and uterine fluid samples were collected on days 7 and 14 of the estrus cycle from 17 subjects between 47 and 67 days postpartum. Levels of diacron-reactive oxygen metabolites, biological antioxidant potential, and the oxidative stress index (calculated as diacron-reactive oxygen metabolites divided by biological antioxidant potential, multiplied by 100) were measured. The group with poor reproductive performance had significantly higher values for the oxidation stress index in their uterine fluid on day 7 of the estrus cycle. Measuring oxidative stress and antioxidant activity in uterine fluid in the early postpartum period may be a useful indicator for determining the subsequent reproductive capacity of Japanese Black cow and warrants for further research.

Abstract: This study aimed to investigate the effects of oxidative stress and antioxidant activity in plasma and uterine fluid during early postpartum on the reproductive performance of Japanese Black cows. A total of 17 Japanese Black cows were included in this study. Plasma and uterine fluid samples were collected at days 7 and 14 of the estrus cycle ($n = 17$), between 47–67 days postpartum, and diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) were measured. d-ROMs, BAP, and oxidation stress index (OSI: d-ROMs/BAP × 100) were compared between pregnant and nonpregnant cows at first postpartum artificial insemination (AI), cows pregnant less than 120 days postpartum (<120 days postpartum), and cows pregnant more than 120 days postpartum (≥120 days postpartum) regardless of AI number. The uterine fluid OSI on day 7 of the estrus cycle was significantly higher in cows pregnant ≥ 120 days postpartum ($p < 0.05$). d-ROMs, BAP, and OSI of uterine fluid during early postpartum were useful indicators for determining the subsequent reproductive performance of Japanese Black cows.

Keywords: biological antioxidant potential; diacron-reactive oxygen metabolites; Japanese Black cows; oxidative stress index; oxidative stress

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1. Introduction

To establish higher efficiency beef cattle production, it is essential to improve reproductive performance after parturition. The reproductive performance of beef cows has been

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共同著者　Ryotaro Miura
最終責任者　Hagita Yujiro (Corresponding Author)

Taku Miyasho

Associate Professor

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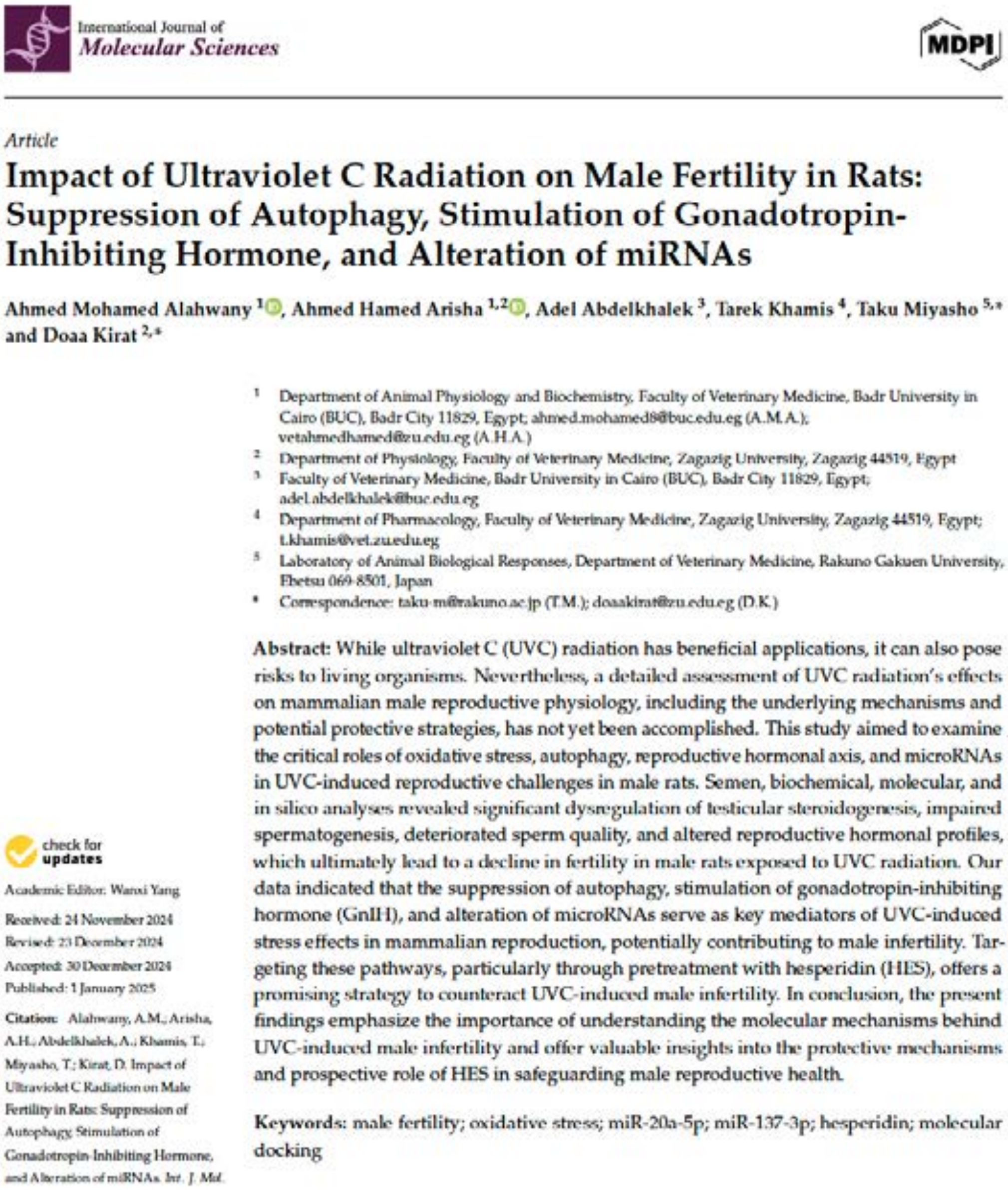
I. 筆頭または責任著者　＜First or Corresponding Author＞

1)　Impact of Ultraviolet C Radiation on Male Fertility in Rats: Suppression of Autophagy, Stimulation of Gonadotropin-Inhibiting Hormone, and Alteration of miRNAs.
Alahwany AM, Arisha AH, Abdelkhalek A, Khamis T, **Miyasho T**, Kirat D.
Int J Mol Sci. 26: 316. 2025. doi: 10.3390/ijms26010316.

II. その他　＜Others＞

1)　Lung injury promoted by strong inspiratory efforts and breath stacking: impact of ventilation mode.
Norisue Y, Usami S, Ito Y, Takeuchi M, Kawamura A, Nakayama R, Bunya N, Kataoka J, Endo Y, Itami T, Hirokawa T, Sugita C, Takeshima H, Takemoto A, Kyogoku M, Koike J, Fujitani S, Mojoli F, **Miyasho T**.
Intensive Care Med Exp. 13: 110. 2025. doi: 10.1186/s40635-025-00821-0.

2)　Evaluation of oxygen reserve index as an early warning indicator of hypoxemia in anesthetized dogs.
Hirokawa T, Itami T, Kato K, Minamoto Y, Chen IY, Sugita C, Endo Y, **Miyasho T**, Yamashita K.
J Vet Med Sci. 87:1458-1466. 2025. doi: 10.1292/jvms.25-0197.



International Journal of
Molecular Sciences

MDPI

Article

Impact of Ultraviolet C Radiation on Male Fertility in Rats: Suppression of Autophagy, Stimulation of Gonadotropin-Inhibiting Hormone, and Alteration of miRNAs

Ahmed Mohamed Alahwany [1], Ahmed Hamed Arisha [1,2], Adel Abdelkhalek [3], Tarek Khamis [4], Taku Miyasho [5,*] and Doaa Kirat [2,*]

[1] Department of Animal Physiology and Biochemistry, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Badr City 11829, Egypt; ahmed.mohamed8@buc.edu.eg (A.M.A.); vetahmedhamed@zu.edu.eg (A.H.A.)
[2] Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt
[3] Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Badr City 11829, Egypt; adel.abdelkhalek@buc.edu.eg
[4] Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt; t.khamis@vet.zu.edu.eg
[5] Laboratory of Animal Biological Responses, Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
* Correspondence: taku-m@rakuno.ac.jp (T.M.); doaakirat@zu.edu.eg (D.K.)

Abstract: While ultraviolet C (UVC) radiation has beneficial applications, it can also pose risks to living organisms. Nevertheless, a detailed assessment of UVC radiation's effects on mammalian male reproductive physiology, including the underlying mechanisms and potential protective strategies, has not yet been accomplished. This study aimed to examine the critical roles of oxidative stress, autophagy, reproductive hormonal axis, and microRNAs in UVC-induced reproductive challenges in male rats. Semen, biochemical, molecular, and in silico analyses revealed significant dysregulation of testicular steroidogenesis, impaired spermatogenesis, deteriorated sperm quality, and altered reproductive hormonal profiles, which ultimately lead to a decline in fertility in male rats exposed to UVC radiation. Our data indicated that the suppression of autophagy, stimulation of gonadotropin-inhibiting hormone (GnIH), and alteration of microRNAs serve as key mediators of UVC-induced stress effects in mammalian reproduction, potentially contributing to male infertility. Targeting these pathways, particularly through pretreatment with hesperidin (HES), offers a promising strategy to counteract UVC-induced male infertility. In conclusion, the present findings emphasize the importance of understanding the molecular mechanisms behind UVC-induced male infertility and offer valuable insights into the protective mechanisms and prospective role of HES in safeguarding male reproductive health.

Keywords: male fertility; oxidative stress; miR-20a-5p; miR-137-3p; hesperidin; molecular docking

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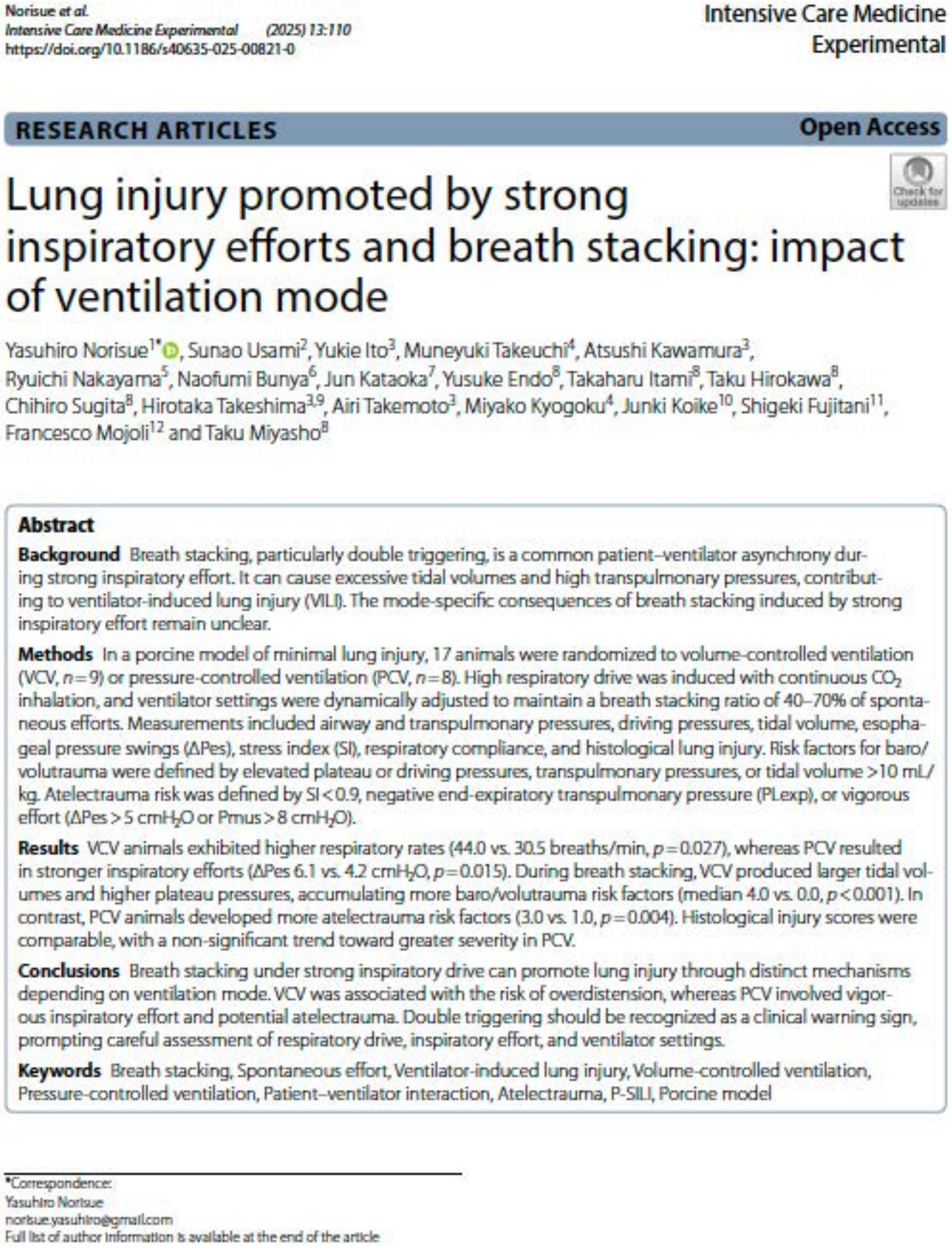

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Intensive Care Medicine
Experimental

RESEARCH ARTICLES Open Access

Lung injury promoted by strong inspiratory efforts and breath stacking: impact of ventilation mode

Yasuhiro Norisue[1*], Sunao Usami[2], Yukie Ito[3], Muneyuki Takeuchi[4], Atsushi Kawamura[3], Ryuichi Nakayama[5], Naofumi Bunya[6], Jun Kataoka[7], Yusuke Endo[8], Takaharu Itami[8], Taku Hirokawa[8], Chihiro Sugita[8], Hirotaka Takeshima[3,9], Airi Takemoto[3], Miyako Kyogoku[4], Junki Koike[10], Shigeki Fujitani[11], Francesco Mojoli[12] and Taku Miyasho[8]

Abstract

Background Breath stacking, particularly double triggering, is a common patient–ventilator asynchrony during strong inspiratory effort. It can cause excessive tidal volumes and high transpulmonary pressures, contributing to ventilator-induced lung injury (VILI). The mode-specific consequences of breath stacking induced by strong inspiratory effort remain unclear.

Methods In a porcine model of minimal lung injury, 17 animals were randomized to volume-controlled ventilation (VCV, $n=9$) or pressure-controlled ventilation (PCV, $n=8$). High respiratory drive was induced with continuous CO_2 inhalation, and ventilator settings were dynamically adjusted to maintain a breath stacking ratio of 40–70% of spontaneous efforts. Measurements included airway and transpulmonary pressures, driving pressures, tidal volume, esophageal pressure swings (ΔPes), stress index (SI), respiratory compliance, and histological lung injury. Risk factors for baro/volutrauma were defined by elevated plateau or driving pressures, transpulmonary pressures, or tidal volume >10 mL/kg. Atelectrauma risk was defined by $SI < 0.9$, negative end-expiratory transpulmonary pressure (PLexp), or vigorous effort ($\Delta Pes > 5$ cmH_2O or $Pmus > 8$ cmH_2O).

Results VCV animals exhibited higher respiratory rates (44.0 vs. 30.5 breaths/min, $p=0.027$), whereas PCV resulted in stronger inspiratory efforts (ΔPes 6.1 vs. 4.2 cmH_2O, $p=0.015$). During breath stacking, VCV produced larger tidal volumes and higher plateau pressures, accumulating more baro/volutrauma risk factors (median 4.0 vs. 0.0, $p<0.001$). In contrast, PCV animals developed more atelectrauma risk factors (3.0 vs. 1.0, $p=0.004$). Histological injury scores were comparable, with a non-significant trend toward greater severity in PCV.

Conclusions Breath stacking under strong inspiratory drive can promote lung injury through distinct mechanisms depending on ventilation mode. VCV was associated with the risk of overdistension, whereas PCV involved vigorous inspiratory effort and potential atelectrauma. Double triggering should be recognized as a clinical warning sign, prompting careful assessment of respiratory drive, inspiratory effort, and ventilator settings.

Keywords Breath stacking, Spontaneous effort, Ventilator-induced lung injury, Volume-controlled ventilation, Pressure-controlled ventilation, Patient–ventilator interaction, Atelectrauma, P-SILI, Porcine model

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*Correspondence:
Yasuhiro Norisue
norisue.yasuhiro@gmail.com
Full list of author information is available at the end of the article

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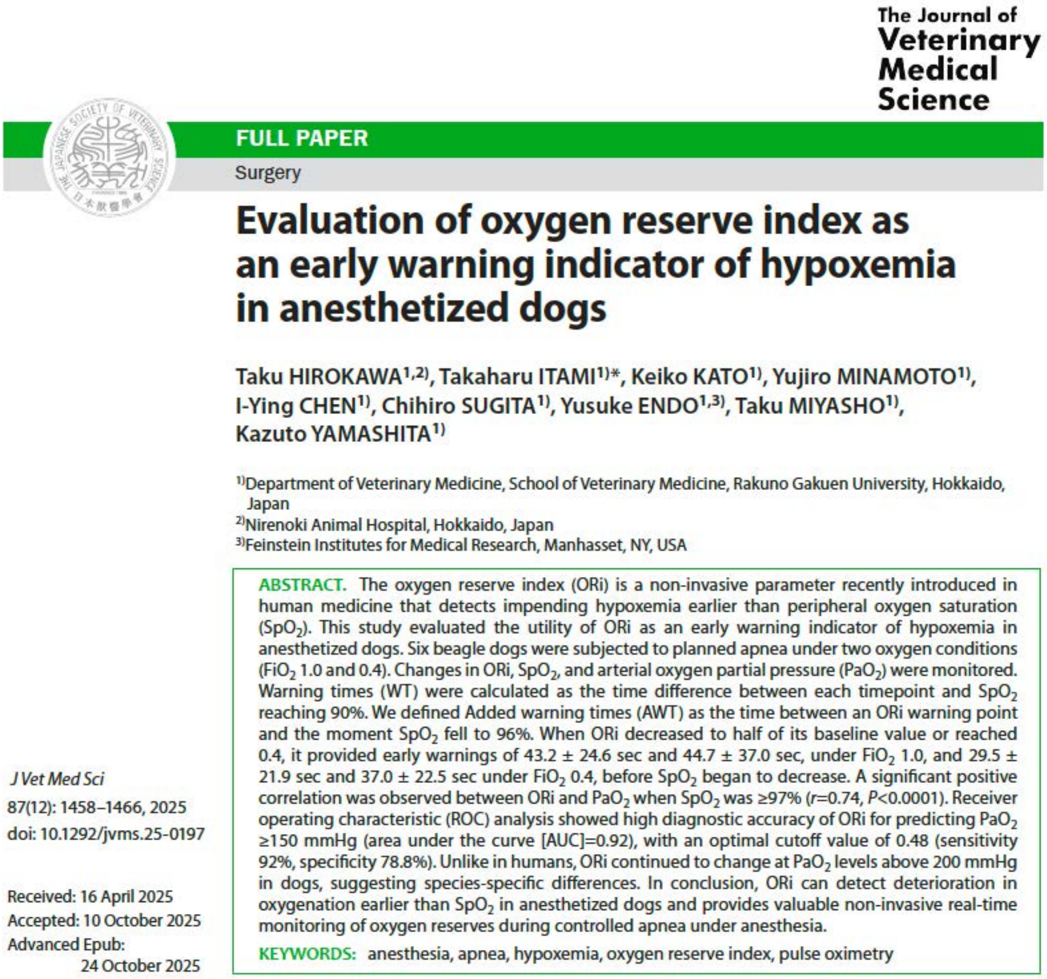

The Journal of Veterinary Medical Science

FULL PAPER

Surgery

Evaluation of oxygen reserve index as an early warning indicator of hypoxemia in anesthetized dogs

Taku HIROKAWA[1,2], Takaharu ITAMI[1]*, Keiko KATO[1], Yujiro MINAMOTO[1], I-Ying CHEN[1], Chihiro SUGITA[1], Yusuke ENDO[1,3], Taku MIYASHO[1], Kazuto YAMASHITA[1]

[1]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]Nirenoki Animal Hospital, Hokkaido, Japan
[3]Feinstein Institutes for Medical Research, Manhasset, NY, USA

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ABSTRACT. The oxygen reserve index (ORi) is a non-invasive parameter recently introduced in human medicine that detects impending hypoxemia earlier than peripheral oxygen saturation (SpO_2). This study evaluated the utility of ORi as an early warning indicator of hypoxemia in anesthetized dogs. Six beagle dogs were subjected to planned apnea under two oxygen conditions (FiO_2 1.0 and 0.4). Changes in ORi, SpO_2, and arterial oxygen partial pressure (PaO_2) were monitored. Warning times (WT) were calculated as the time difference between each timepoint and SpO_2 reaching 90%. We defined Added warning times (AWT) as the time between an ORi warning point and the moment SpO_2 fell to 96%. When ORi decreased to half of its baseline value or reached 0.4, it provided early warnings of 43.2 ± 24.6 sec and 44.7 ± 37.0 sec, under FiO_2 1.0, and 29.5 ± 21.9 sec and 37.0 ± 22.5 sec under FiO_2 0.4, before SpO_2 began to decrease. A significant positive correlation was observed between ORi and PaO_2 when SpO_2 was ≥97% (r=0.74, P<0.0001). Receiver operating characteristic (ROC) analysis showed high diagnostic accuracy of ORi for predicting PaO_2 ≥150 mmHg (area under the curve [AUC]=0.92), with an optimal cutoff value of 0.48 (sensitivity 92%, specificity 78.8%). Unlike in humans, ORi continued to change at PaO_2 levels above 200 mmHg in dogs, suggesting species-specific differences. In conclusion, ORi can detect deterioration in oxygenation earlier than SpO_2 in anesthetized dogs and provides valuable non-invasive real-time monitoring of oxygen reserves during controlled apnea under anesthesia.

KEYWORDS: anesthesia, apnea, hypoxemia, oxygen reserve index, pulse oximetry

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最終責任者　Takaharu Itami (Corresponding Author)

獣医薬理学（Veterinary Pharmacology）

Tatsuro Nakamura

Associate Professor

准教授　中村　達朗

I. 筆頭または責任著者　＜First or Corresponding Author＞

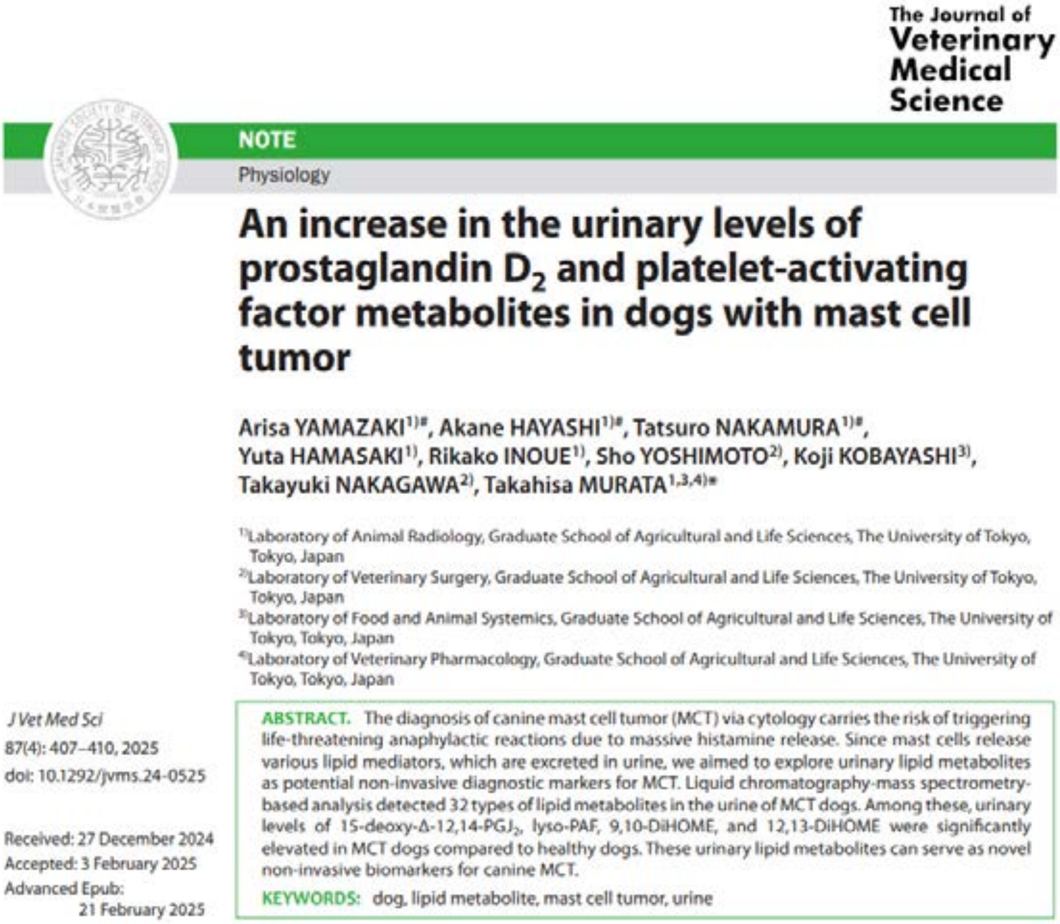
1）An increase in the urinary levels of prostaglandin D2 and platelet-activating factor metabolites in dogs with mast cell tumor. Yamazaki A, Hayashi A, **Nakamura T**, Hamasaki Y, Inoue R, Yoshimoto S, Kobayashi K, Nakagawa T, Murata T.
J Vet Med Sci. 87:407-410. 2025. doi: 10.1292/jvms.24-0525.

II. その他　＜Others＞

2）Urinary lipid production in dogs with urothelial carcinoma. Hayashi A, Maeda S, Yamazaki A, **Nakamura T**, Goto-Koshino Y, Yonezawa T, Kobayashi K, Murata T.
Vet J. 2025. 313:106373. 2025. doi: 10.1016/j.tvjl.2025.106373.

3）Skin-Derived PGD2 Promotes Antigen-Specific IgE Production via CRTH2 Signaling. Kida M, **Nakamura T**, Maeda S, Nagata N, Enomoto H, Murata T.
Allergy. 80:2674-2677. 2025. doi: 10.1111/all.16635.

4）Enhancement of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity by lipopolysaccharide in developing zebrafish lacking canonical pattern recognition pathways.
Kitamura H, Tanaka K, Wang H, Nakamura, T Kobayashi M, Teraoka H.
Comp Biochem Physiol C Toxicol Pharmacol. 297:110261. 2025.
doi:10.1016/j.cbpc.



The Journal of
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Science

NOTE
Physiology

An increase in the urinary levels of prostaglandin D_2 and platelet-activating factor metabolites in dogs with mast cell tumor

Arisa YAMAZAKI[1)#], Akane HAYASHI[1)#], Tatsuro NAKAMURA[1)#],
Yuta HAMASAKI[1)], Rikako INOUE[1)], Sho YOSHIMOTO[2)], Koji KOBAYASHI[3)],
Takayuki NAKAGAWA[2)], Takahisa MURATA[1,3,4)*]

[1)] Laboratory of Animal Radiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[2)] Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[3)] Laboratory of Food and Animal Systemics, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan
[4)] Laboratory of Veterinary Pharmacology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

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ABSTRACT. The diagnosis of canine mast cell tumor (MCT) via cytology carries the risk of triggering life-threatening anaphylactic reactions due to massive histamine release. Since mast cells release various lipid mediators, which are excreted in urine, we aimed to explore urinary lipid metabolites as potential non-invasive diagnostic markers for MCT. Liquid chromatography-mass spectrometry-based analysis detected 32 types of lipid metabolites in the urine of MCT dogs. Among these, urinary levels of 15-deoxy-Δ-12,14-PGJ_2, lyso-PAF, 9,10-DiHOME, and 12,13-DiHOME were significantly elevated in MCT dogs compared to healthy dogs. These urinary lipid metabolites can serve as novel non-invasive biomarkers for canine MCT.

KEYWORDS: dog, lipid metabolite, mast cell tumor, urine

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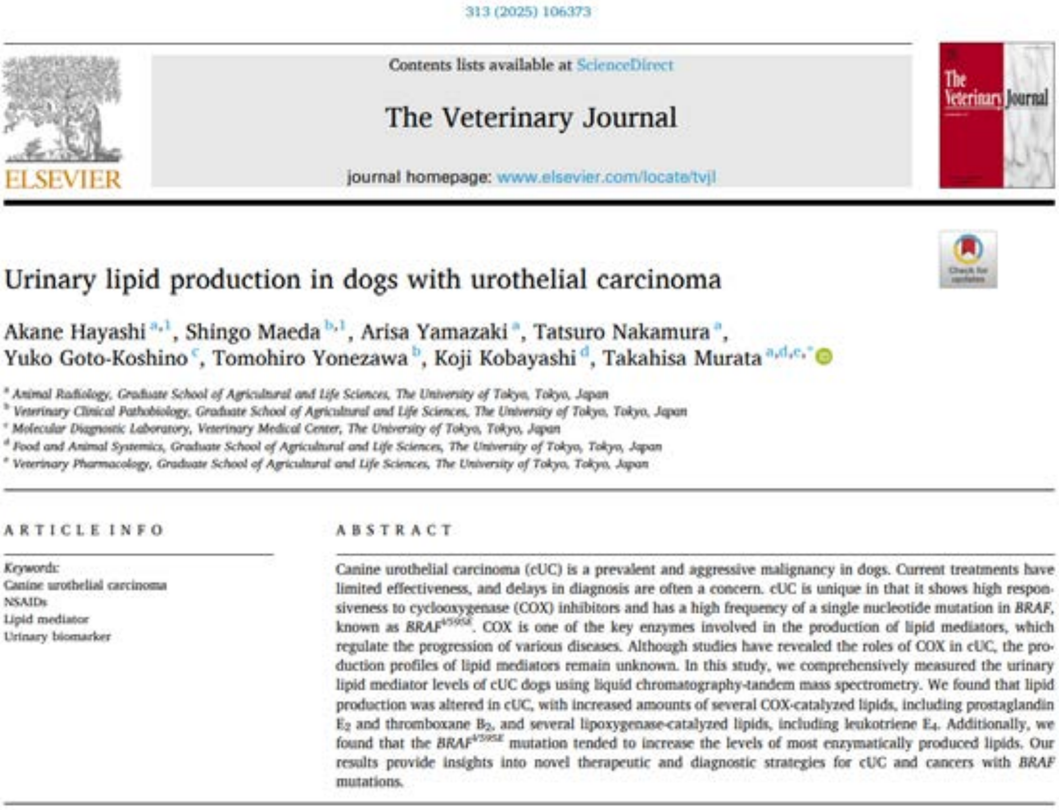

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Urinary lipid production in dogs with urothelial carcinoma

Akane Hayashi [a,1], Shingo Maeda [b,1], Arisa Yamazaki [a], Tatsuro Nakamura [a],
Yuko Goto-Koshino [c], Tomohiro Yonezawa [b], Koji Kobayashi [d], Takahisa Murata [a,d,e,*]

[a] *Animal Radiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
[b] *Veterinary Clinical Pathobiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
[c] *Molecular Diagnostic Laboratory, Veterinary Medical Center, The University of Tokyo, Tokyo, Japan*
[d] *Food and Animal Systemics, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
[e] *Veterinary Pharmacology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*

ARTICLE INFO

Keywords:
Canine urothelial carcinoma
NSAIDs
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ABSTRACT

Canine urothelial carcinoma (cUC) is a prevalent and aggressive malignancy in dogs. Current treatments have limited effectiveness, and delays in diagnosis are often a concern. cUC is unique in that it shows high responsiveness to cyclooxygenase (COX) inhibitors and has a high frequency of a single nucleotide mutation in *BRAF*, known as *BRAF*^{V595E}. COX is one of the key enzymes involved in the production of lipid mediators, which regulate the progression of various diseases. Although studies have revealed the roles of COX in cUC, the production profiles of lipid mediators remain unknown. In this study, we comprehensively measured the urinary lipid mediator levels of cUC dogs using liquid chromatography-tandem mass spectrometry. We found that lipid production was altered in cUC, with increased amounts of several COX-catalyzed lipids, including prostaglandin E_2 and thromboxane B_2, and several lipoxygenase-catalyzed lipids, including leukotriene E_4. Additionally, we found that the *BRAF*^{V595E} mutation tended to increase the levels of most enzymatically produced lipids. Our results provide insights into novel therapeutic and diagnostic strategies for cUC and cancers with *BRAF* mutations.

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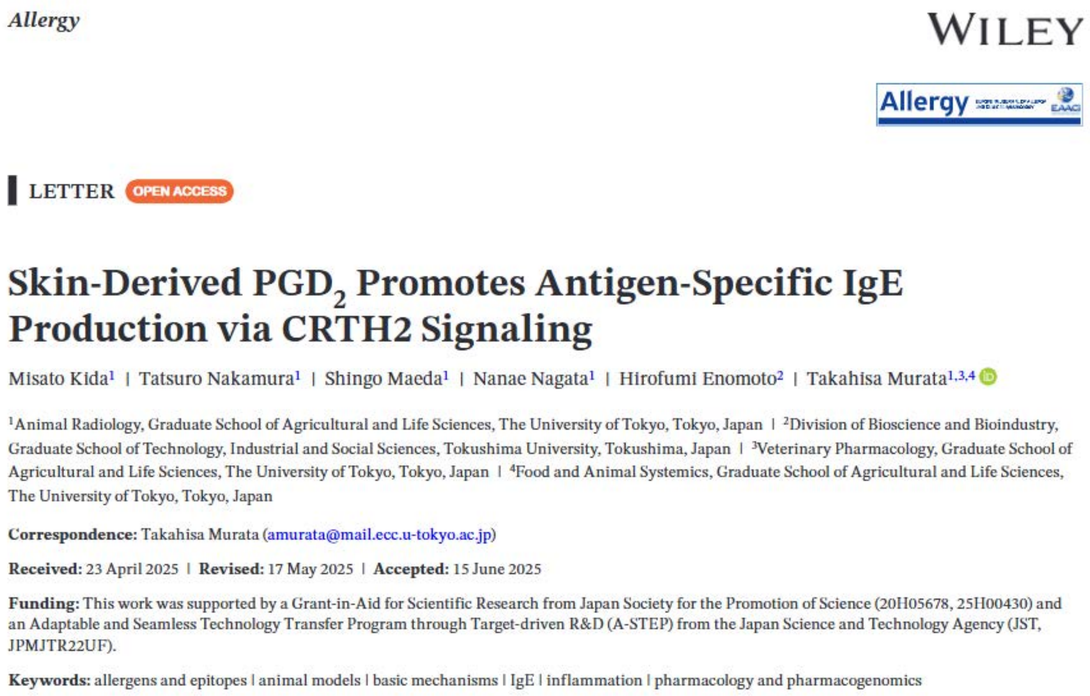

Allergy

WILEY

Allergy

LETTER OPEN ACCESS

Skin-Derived PGD_2 Promotes Antigen-Specific IgE Production via CRTH2 Signaling

Misato Kida[1] | Tatsuro Nakamura[1] | Shingo Maeda[1] | Nanae Nagata[1] | Hirofumi Enomoto[2] | Takahisa Murata[1,3,4]

[1]Animal Radiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan | [2]Division of Bioscience and Bioindustry, Graduate School of Technology, Industrial and Social Sciences, Tokushima University, Tokushima, Japan | [3]Veterinary Pharmacology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan | [4]Food and Animal Systemics, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

Correspondence: Takahisa Murata (amurata@mail.ecc.u-tokyo.ac.jp)

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Keywords: allergens and epitopes | animal models | basic mechanisms | IgE | inflammation | pharmacology and pharmacogenomics

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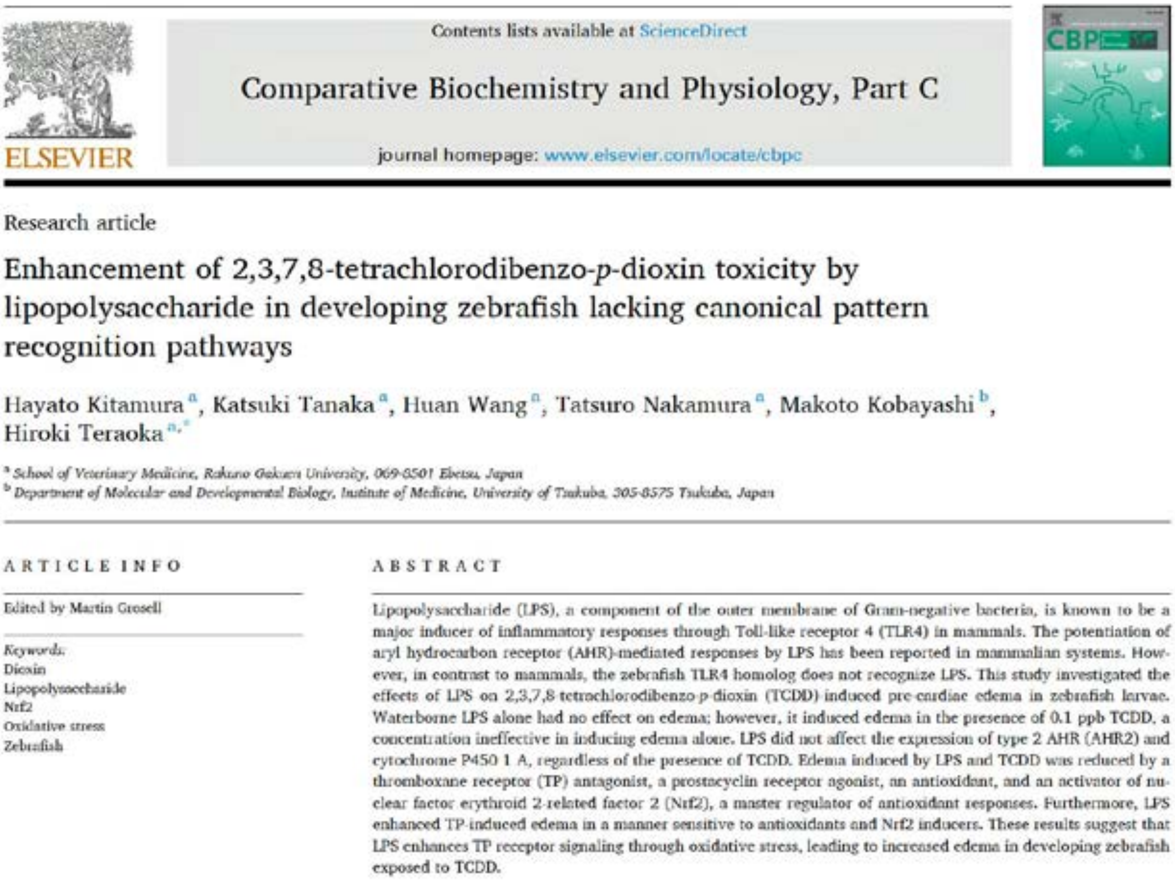

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Research article

Enhancement of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity by lipopolysaccharide in developing zebrafish lacking canonical pattern recognition pathways

Hayato Kitamura[a], Katsuki Tanaka[a], Huan Wang[a], Tatsuro Nakamura[a], Makoto Kobayashi[b], Hiroki Teraoka[a,*]

[a] *School of Veterinary Medicine, Rakuno Gakuen University, 069-8501 Ebetsu, Japan*
[b] *Department of Molecular and Developmental Biology, Institute of Medicine, University of Tsukuba, 305-8575 Tsukuba, Japan*

A R T I C L E I N F O

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A B S T R A C T

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is known to be a major inducer of inflammatory responses through Toll-like receptor 4 (TLR4) in mammals. The potentiation of aryl hydrocarbon receptor (AHR)-mediated responses by LPS has been reported in mammalian systems. However, in contrast to mammals, the zebrafish TLR4 homolog does not recognize LPS. This study investigated the effects of LPS on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced pre-cardiac edema in zebrafish larvae. Waterborne LPS alone had no effect on edema; however, it induced edema in the presence of 0.1 ppb TCDD, a concentration ineffective in inducing edema alone. LPS did not affect the expression of type 2 AHR (AHR2) and cytochrome P450 1 A, regardless of the presence of TCDD. Edema induced by LPS and TCDD was reduced by a thromboxane receptor (TP) antagonist, a prostacyclin receptor agonist, an antioxidant, and an activator of nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of antioxidant responses. Furthermore, LPS enhanced TP-induced edema in a manner sensitive to antioxidants and Nrf2 inducers. These results suggest that LPS enhances TP receptor signaling through oxidative stress, leading to increased edema in developing zebrafish exposed to TCDD.

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最終責任者　Hiroki Teraoka (Corresponding Author)

獣医臨床病理学（Veterinary Clinicopathology）

Minoru Okamoto

Associate Professor

准教授　岡本　実

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他　＜Others＞

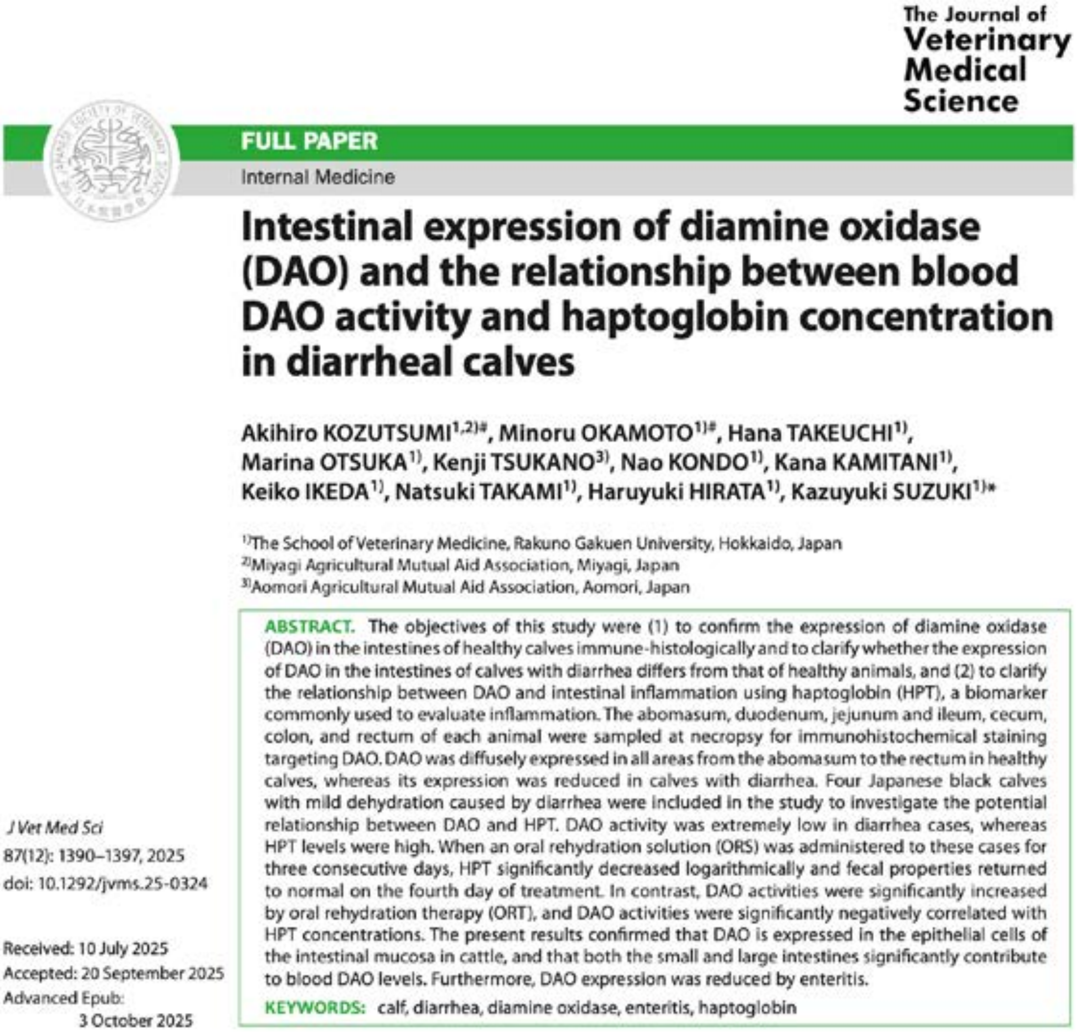
1）Intestinal expression of diamine oxidase (DAO) and the relationship between blood DAO activity and haptoglobin concentration in diarrheal calves.
Kozutsumi A, **Okamoto M**, Takeuchi H, Otsuka M, Tsukano K, Kondo N, Kamitani K, Ikeda K, Takami N, Hirata H, Suzuki K.
J Vet Med Sci. 87:1390-1397. 2025. doi: 10.1292/jvms.25-0324.

2）A simple, inexpensive, and rapid method for measuring calcium concentrations in colostrum to predict postpartum milk fever in cows using a commercially available water quality test device.
Kondo N, Maeda M, Murotsuki R, Ryoha T, Otsuka M, Tsukano K, Kozutsumi H, Ikeda K, Kamitani K, **Okamoto M**, Hirata H, Suzuki K.
J Vet Med Sci. 87:1186-1193. 2025. doi: 10.1292/jvms.25-0298.

3）Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (Choloepus didactylus).
Murakami M, Iwasa A, **Okamoto M**, Ihizaki T, Suzuki K, Hirata H.
J Vet Med Sci. 87:972-975. 2025doi: 10.1292/jvms.25-0063.

4）Validation of point-of-care devices measuring calcium (iCa), potassium (K^+), and sodium (Na^+) concentrations in whole blood of cattle and horses using general-purpose water quality testing equipment.
Kondo N, Iwasa A, **Okamoto M**, Tsukano K, Hirata H, Suzuki K.
Act Vet Brno. 94:3-7. 2025. doi: org/10.2754/avb202594010003

Sci. 87:972-975. 2025doi: 10.1292/jvms.25-0063.



The Journal of
Veterinary
Medical
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FULL PAPER

Internal Medicine

Intestinal expression of diamine oxidase (DAO) and the relationship between blood DAO activity and haptoglobin concentration in diarrheal calves

Akihiro KOZUTSUMI1,2)#, Minoru OKAMOTO1)#, Hana TAKEUCHI1), Marina OTSUKA1), Kenji TSUKANO3), Nao KONDO1), Kana KAMITANI1), Keiko IKEDA1), Natsuki TAKAMI1), Haruyuki HIRATA1), Kazuyuki SUZUKI1)*

1)The School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
2)Miyagi Agricultural Mutual Aid Association, Miyagi, Japan
3)Aomori Agricultural Mutual Aid Association, Aomori, Japan

ABSTRACT. The objectives of this study were (1) to confirm the expression of diamine oxidase (DAO) in the intestines of healthy calves immune-histologically and to clarify whether the expression of DAO in the intestines of calves with diarrhea differs from that of healthy animals, and (2) to clarify the relationship between DAO and intestinal inflammation using haptoglobin (HPT), a biomarker commonly used to evaluate inflammation. The abomasum, duodenum, jejunum and ileum, cecum, colon, and rectum of each animal were sampled at necropsy for immunohistochemical staining targeting DAO. DAO was diffusely expressed in all areas from the abomasum to the rectum in healthy calves, whereas its expression was reduced in calves with diarrhea. Four Japanese black calves with mild dehydration caused by diarrhea were included in the study to investigate the potential relationship between DAO and HPT. DAO activity was extremely low in diarrhea cases, whereas HPT levels were high. When an oral rehydration solution (ORS) was administered to these cases for three consecutive days, HPT significantly decreased logarithmically and fecal properties returned to normal on the fourth day of treatment. In contrast, DAO activities were significantly increased by oral rehydration therapy (ORT), and DAO activities were significantly negatively correlated with HPT concentrations. The present results confirmed that DAO is expressed in the epithelial cells of the intestinal mucosa in cattle, and that both the small and large intestines significantly contribute to blood DAO levels. Furthermore, DAO expression was reduced by enteritis.

KEYWORDS: calf, diarrhea, diamine oxidase, enteritis, haptoglobin

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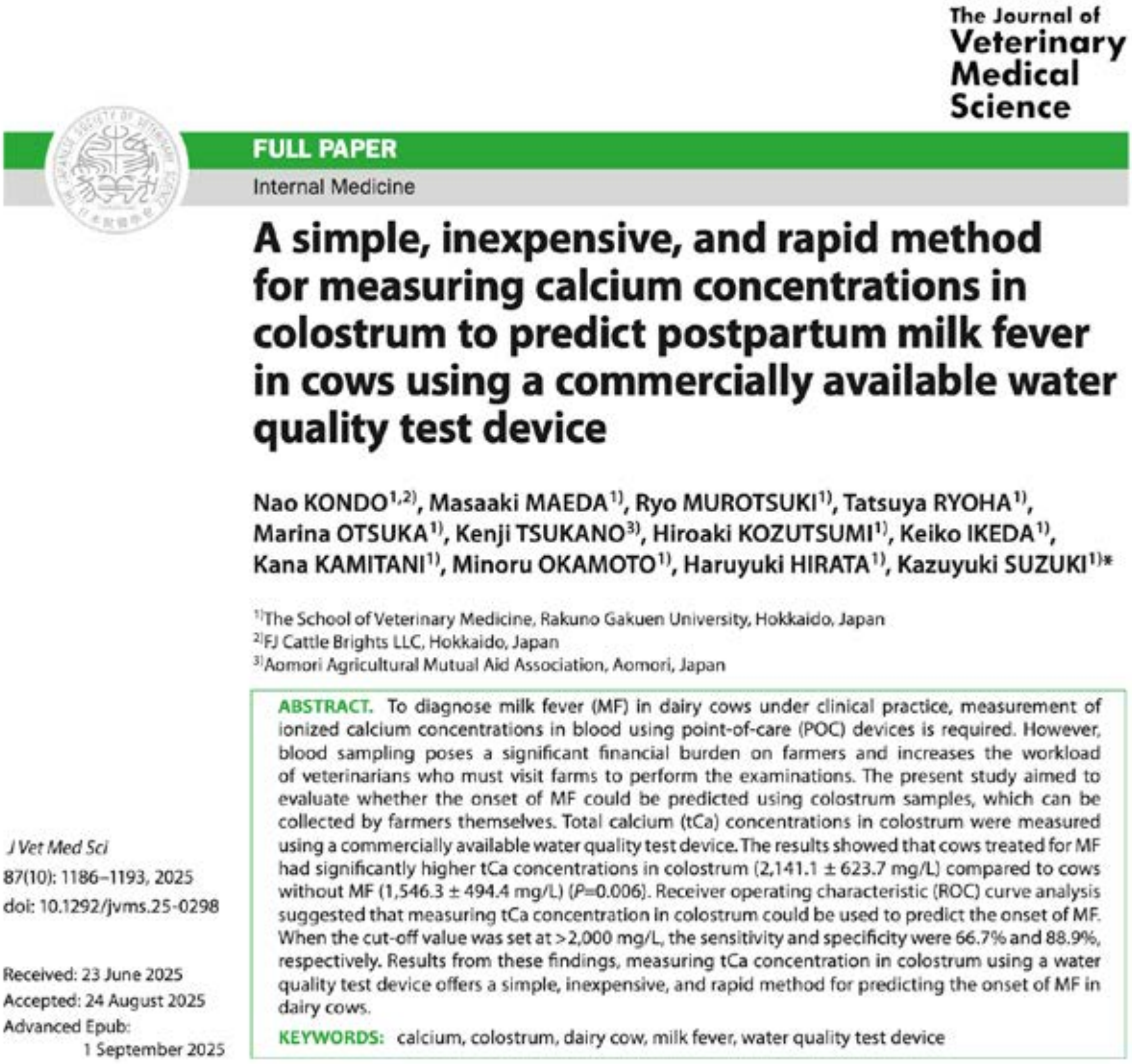

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Medical
Science

FULL PAPER
Internal Medicine

A simple, inexpensive, and rapid method for measuring calcium concentrations in colostrum to predict postpartum milk fever in cows using a commercially available water quality test device

Nao KONDO[1,2], Masaaki MAEDA[1], Ryo MUROTSUKI[1], Tatsuya RYOHA[1], Marina OTSUKA[1], Kenji TSUKANO[3], Hiroaki KOZUTSUMI[1], Keiko IKEDA[1], Kana KAMITANI[1], Minoru OKAMOTO[1], Haruyuki HIRATA[1], Kazuyuki SUZUKI[1]*

[1]The School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]FJ Cattle Brights LLC, Hokkaido, Japan
[3]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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ABSTRACT. To diagnose milk fever (MF) in dairy cows under clinical practice, measurement of ionized calcium concentrations in blood using point-of-care (POC) devices is required. However, blood sampling poses a significant financial burden on farmers and increases the workload of veterinarians who must visit farms to perform the examinations. The present study aimed to evaluate whether the onset of MF could be predicted using colostrum samples, which can be collected by farmers themselves. Total calcium (tCa) concentrations in colostrum were measured using a commercially available water quality test device. The results showed that cows treated for MF had significantly higher tCa concentrations in colostrum (2,141.1 ± 623.7 mg/L) compared to cows without MF (1,546.3 ± 494.4 mg/L) (P=0.006). Receiver operating characteristic (ROC) curve analysis suggested that measuring tCa concentration in colostrum could be used to predict the onset of MF. When the cut-off value was set at >2,000 mg/L, the sensitivity and specificity were 66.7% and 88.9%, respectively. Results from these findings, measuring tCa concentration in colostrum using a water quality test device offers a simple, inexpensive, and rapid method for predicting the onset of MF in dairy cows.

KEYWORDS: calcium, colostrum, dairy cow, milk fever, water quality test device

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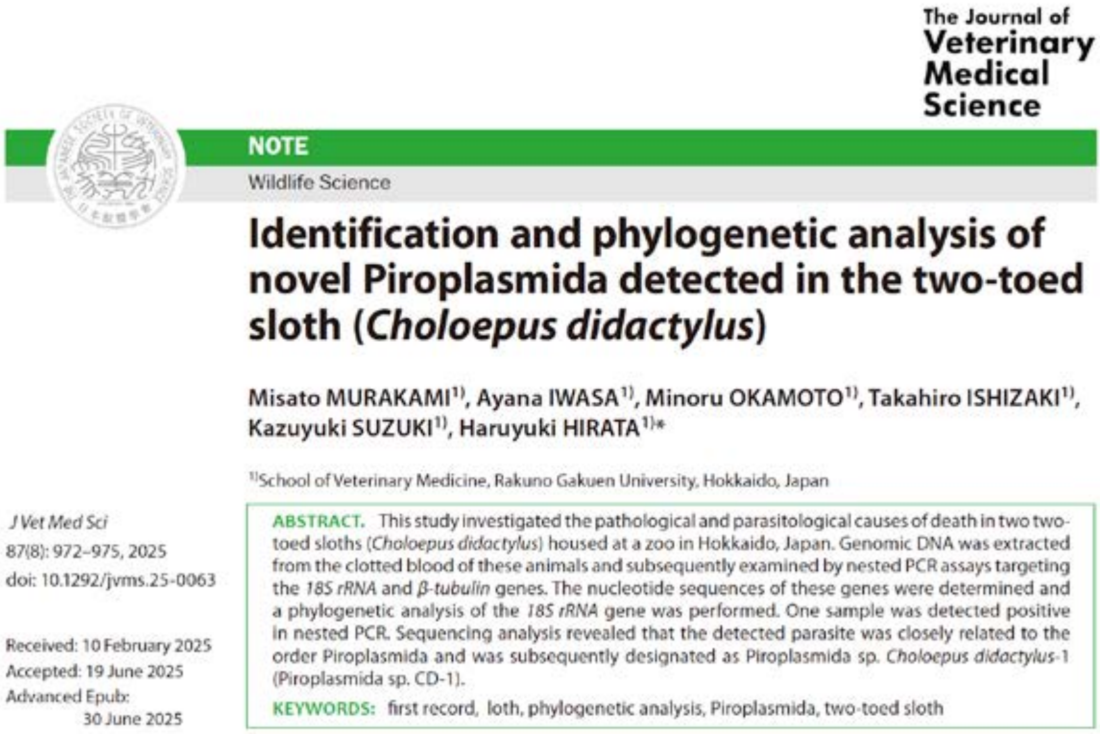

The Journal of Veterinary Medical Science

NOTE

Wildlife Science

Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (*Choloepus didactylus*)

Misato MURAKAMI[1)], Ayana IWASA[1)], Minoru OKAMOTO[1)], Takahiro ISHIZAKI[1)], Kazuyuki SUZUKI[1)], Haruyuki HIRATA[1)]*

[1)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. This study investigated the pathological and parasitological causes of death in two two-toed sloths (*Choloepus didactylus*) housed at a zoo in Hokkaido, Japan. Genomic DNA was extracted from the clotted blood of these animals and subsequently examined by nested PCR assays targeting the *18S rRNA* and *β-tubulin* genes. The nucleotide sequences of these genes were determined and a phylogenetic analysis of the *18S rRNA* gene was performed. One sample was detected positive in nested PCR. Sequencing analysis revealed that the detected parasite was closely related to the order Piroplasmida and was subsequently designated as Piroplasmida sp. *Choloepus didactylus*-1 (Piroplasmida sp. CD-1).

KEYWORDS: first record, loth, phylogenetic analysis, Piroplasmida, two-toed sloth

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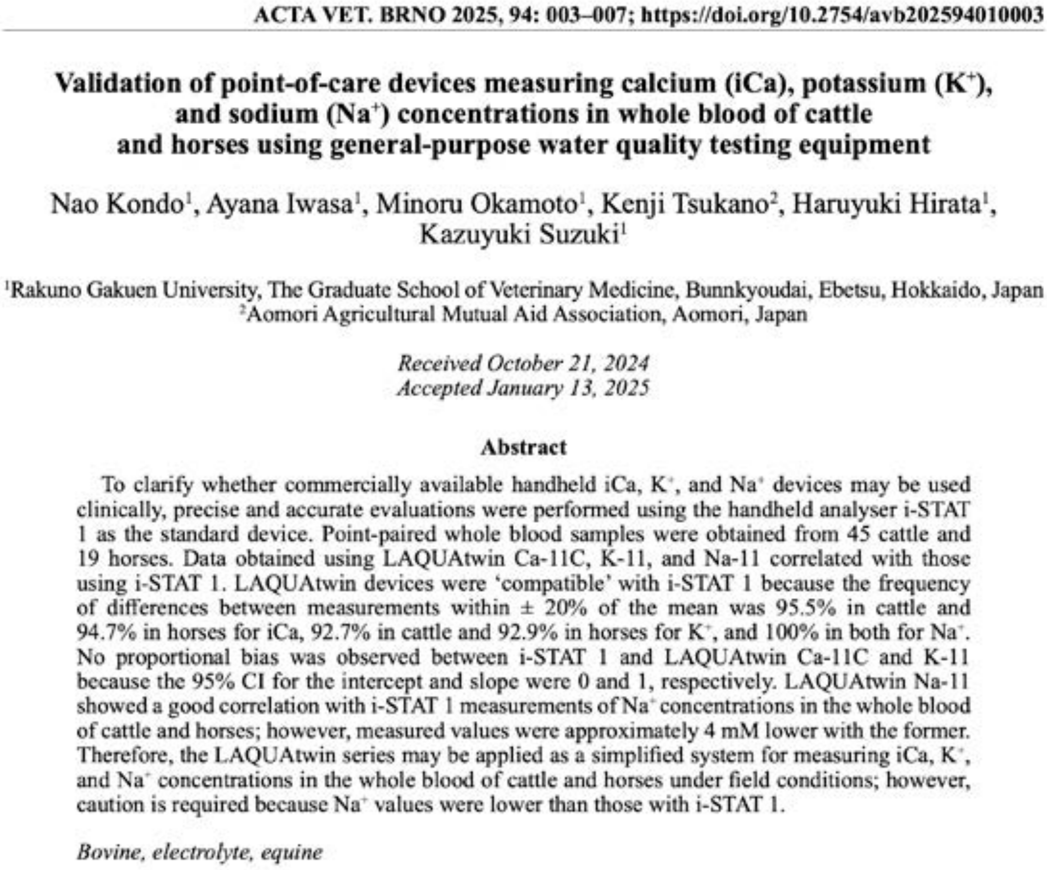

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Validation of point-of-care devices measuring calcium (iCa), potassium (K^+), and sodium (Na^+) concentrations in whole blood of cattle and horses using general-purpose water quality testing equipment

Nao Kondo[1], Ayana Iwasa[1], Minoru Okamoto[1], Kenji Tsukano[2], Haruyuki Hirata[1], Kazuyuki Suzuki[1]

[1]Rakuno Gakuen University, The Graduate School of Veterinary Medicine, Bunnkyoudai, Ebetsu, Hokkaido, Japan
[2]Aomori Agricultural Mutual Aid Association, Aomori, Japan

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Abstract

To clarify whether commercially available handheld iCa, K^+, and Na^+ devices may be used clinically, precise and accurate evaluations were performed using the handheld analyser i-STAT 1 as the standard device. Point-paired whole blood samples were obtained from 45 cattle and 19 horses. Data obtained using LAQUAtwin Ca-11C, K-11, and Na-11 correlated with those using i-STAT 1. LAQUAtwin devices were 'compatible' with i-STAT 1 because the frequency of differences between measurements within ± 20% of the mean was 95.5% in cattle and 94.7% in horses for iCa, 92.7% in cattle and 92.9% in horses for K^+, and 100% in both for Na^+. No proportional bias was observed between i-STAT 1 and LAQUAtwin Ca-11C and K-11 because the 95% CI for the intercept and slope were 0 and 1, respectively. LAQUAtwin Na-11 showed a good correlation with i-STAT 1 measurements of Na^+ concentrations in the whole blood of cattle and horses; however, measured values were approximately 4 mM lower with the former. Therefore, the LAQUAtwin series may be applied as a simplified system for measuring iCa, K^+, and Na^+ concentrations in the whole blood of cattle and horses under field conditions; however, caution is required because Na^+ values were lower than those with i-STAT 1.

Bovine, electrolyte, equine

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疾患モデル学（Laboratory of Disease Models）

Keisuke Shimada

Associate professor

准教授　嶋田　圭祐

I. 筆頭または責任著者　＜First or Corresponding Author＞

1） Loss of CCDC188 causes male infertility with defects in the sperm head–neck connection in mice.
Qiu, Y, **Shimada, K**, Yamamoto K, Ikawa M. *Biol. Reprod.* 112, 169-178. 2025. doi: 10.1093/biolre/ioae137.

2） The absence of both RIBC1 and RIBC2 induces decreased sperm motility and litter size in male mice.
Katsuma K, **Shimada, K**, Tonai S, Mashiko D, Iida-Norita R, Kaneda Y, Miyata H, Ikawa, M.
Andrology. doi: 10.1111/andr.70045. Epub 2025 Apr 23.

3） Compensation for X-linked *Pdha1* silencing by *Pdha2* is essential for meiotic double-strand break repair in spermatogenesis.
Pan, C., **Shimada, K.**, Chang, H.-Y., Wang, H., Ikawa, M., 2025. *Development*. 152:dev204683. doi: 10.1242/dev.204683.

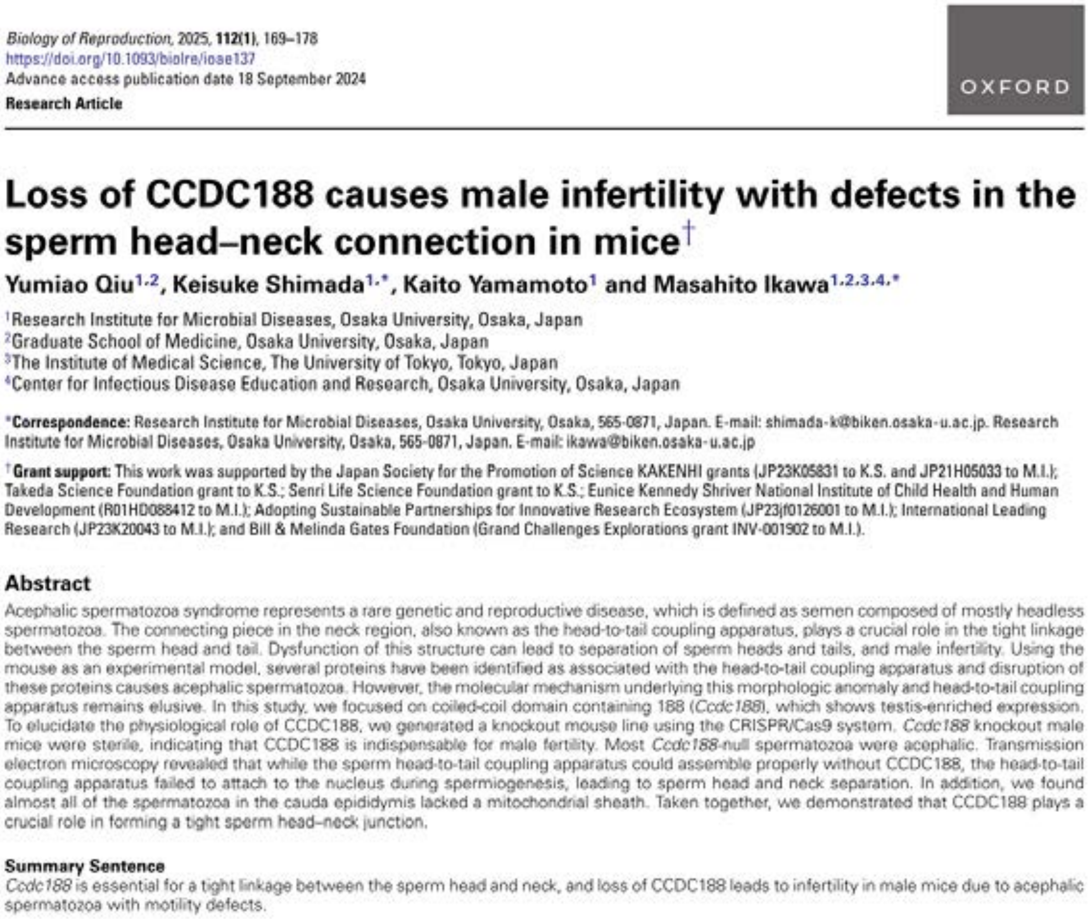
4） CRISPR/Cas9-mediated genome-editing reveals 10 testis-enriched Genes and one non-testis-enriched gene are dispensable for male fecundity in mice.
Qiu, Y., **Shimada, K.**, Ikawa, M.
Andrology. 2025 Nov 10. doi: 10.1111/andr.70144. Online ahead of print.

II. その他　＜Others＞

1） TEX38 localizes ZDHHC19 to the plasma membrane and regulates sperm head morphogenesis in mice.
Kaneda Y, Lu Y, Sun J, **Shimada, K**, Emori C, Noda T, Koyano T, Matsuyama M, Miyata H, Ikawa M.
Proc Natl Acad Sci U S A. 122:e2417943122.
doi: 10.1073/pnas.2417943122.

2） Mouse genome engineering uncovers 18 genes dispensable for male reproduction.
Chang HY, Lu Y, Yamamoto K, Sun J, **Shimada K**, Hiradate Y, Fujihara Y, Ikawa M.
Andrology. 2025 Jun 26. doi: 10.1111/andr.70088.　Online ahead of print.

3） Proximity labeling of axonemal protein CFAP91 identifies EFCAB5 that regulates sperm motility.
Wang H, **Shimada, K**, Pham AH, Oyama Y, Kamoshita M, Kobayashi H, Oura S, Yabuta N, Ikawa M, Miyata H.
Nat. Commun. 16:8238. doi: 10.1038/s41467-025-63705-7.



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Research Article

Loss of CCDC188 causes male infertility with defects in the sperm head–neck connection in mice†

Yumiao Qiu[1,2], Keisuke Shimada[1,*], Kaito Yamamoto[1] and Masahito Ikawa[1,2,3,4,*]

[1]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan
[2]Graduate School of Medicine, Osaka University, Osaka, Japan
[3]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[4]Center for Infectious Disease Education and Research, Osaka University, Osaka, Japan

[*]**Correspondence:** Research Institute for Microbial Diseases, Osaka University, Osaka, 565-0871, Japan. E-mail: shimada-k@biken.osaka-u.ac.jp. Research Institute for Microbial Diseases, Osaka University, Osaka, 565-0871, Japan. E-mail: ikawa@biken.osaka-u.ac.jp

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Abstract

Acephalic spermatozoa syndrome represents a rare genetic and reproductive disease, which is defined as semen composed of mostly headless spermatozoa. The connecting piece in the neck region, also known as the head-to-tail coupling apparatus, plays a crucial role in the tight linkage between the sperm head and tail. Dysfunction of this structure can lead to separation of sperm heads and tails, and male infertility. Using the mouse as an experimental model, several proteins have been identified as associated with the head-to-tail coupling apparatus and disruption of these proteins causes acephalic spermatozoa. However, the molecular mechanism underlying this morphologic anomaly and head-to-tail coupling apparatus remains elusive. In this study, we focused on coiled-coil domain containing 188 (*Ccdc188*), which shows testis-enriched expression. To elucidate the physiological role of CCDC188, we generated a knockout mouse line using the CRISPR/Cas9 system. *Ccdc188* knockout male mice were sterile, indicating that CCDC188 is indispensable for male fertility. Most *Ccdc188*-null spermatozoa were acephalic. Transmission electron microscopy revealed that while the sperm head-to-tail coupling apparatus could assemble properly without CCDC188, the head-to-tail coupling apparatus failed to attach to the nucleus during spermiogenesis, leading to sperm head and neck separation. In addition, we found almost all of the spermatozoa in the cauda epididymis lacked a mitochondrial sheath. Taken together, we demonstrated that CCDC188 plays a crucial role in forming a tight sperm head–neck junction.

Summary Sentence

Ccdc188 is essential for a tight linkage between the sperm head and neck, and loss of CCDC188 leads to infertility in male mice due to acephalic spermatozoa with motility defects.

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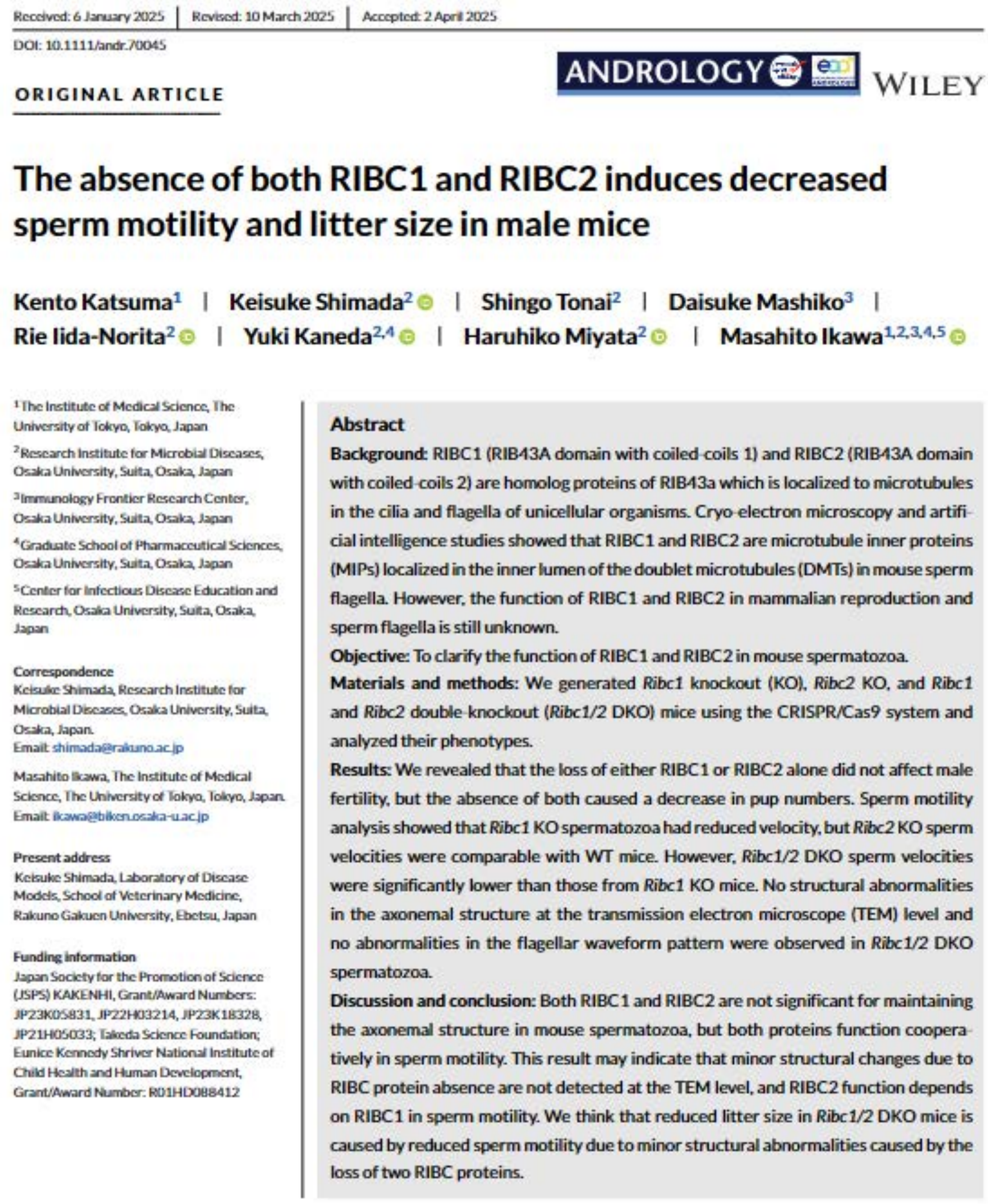
最終責任者　Keisuke Shimada and Masahito Ikawa (Correspondence)



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ANDROLOGY WILEY

ORIGINAL ARTICLE

The absence of both RIBC1 and RIBC2 induces decreased sperm motility and litter size in male mice

Kento Katsuma[1] | Keisuke Shimada[2] | Shingo Tonai[2] | Daisuke Mashiko[3] | Rie Iida-Norita[2] | Yuki Kaneda[2,4] | Haruhiko Miyata[2] | Masahito Ikawa[1,2,3,4,5]

[1] The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[2] Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan
[3] Immunology Frontier Research Center, Osaka University, Suita, Osaka, Japan
[4] Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan
[5] Center for Infectious Disease Education and Research, Osaka University, Suita, Osaka, Japan

Correspondence
Keisuke Shimada, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan.
Email: shimada@rakuno.ac.jp

Masahito Ikawa, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
Email: ikawa@biken.osaka-u.ac.jp

Present address
Keisuke Shimada, Laboratory of Disease Models, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

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Abstract

Background: RIBC1 (RIB43A domain with coiled-coils 1) and RIBC2 (RIB43A domain with coiled-coils 2) are homolog proteins of RIB43a which is localized to microtubules in the cilia and flagella of unicellular organisms. Cryo-electron microscopy and artificial intelligence studies showed that RIBC1 and RIBC2 are microtubule inner proteins (MIPs) localized in the inner lumen of the doublet microtubules (DMTs) in mouse sperm flagella. However, the function of RIBC1 and RIBC2 in mammalian reproduction and sperm flagella is still unknown.

Objective: To clarify the function of RIBC1 and RIBC2 in mouse spermatozoa.

Materials and methods: We generated *Ribc1* knockout (KO), *Ribc2* KO, and *Ribc1* and *Ribc2* double-knockout (*Ribc1/2* DKO) mice using the CRISPR/Cas9 system and analyzed their phenotypes.

Results: We revealed that the loss of either RIBC1 or RIBC2 alone did not affect male fertility, but the absence of both caused a decrease in pup numbers. Sperm motility analysis showed that *Ribc1* KO spermatozoa had reduced velocity, but *Ribc2* KO sperm velocities were comparable with WT mice. However, *Ribc1/2* DKO sperm velocities were significantly lower than those from *Ribc1* KO mice. No structural abnormalities in the axonemal structure at the transmission electron microscope (TEM) level and no abnormalities in the flagellar waveform pattern were observed in *Ribc1/2* DKO spermatozoa.

Discussion and conclusion: Both RIBC1 and RIBC2 are not significant for maintaining the axonemal structure in mouse spermatozoa, but both proteins function cooperatively in sperm motility. This result may indicate that minor structural changes due to RIBC protein absence are not detected at the TEM level, and RIBC2 function depends on RIBC1 in sperm motility. We think that reduced litter size in *Ribc1/2* DKO mice is caused by reduced sperm motility due to minor structural abnormalities caused by the loss of two RIBC proteins.

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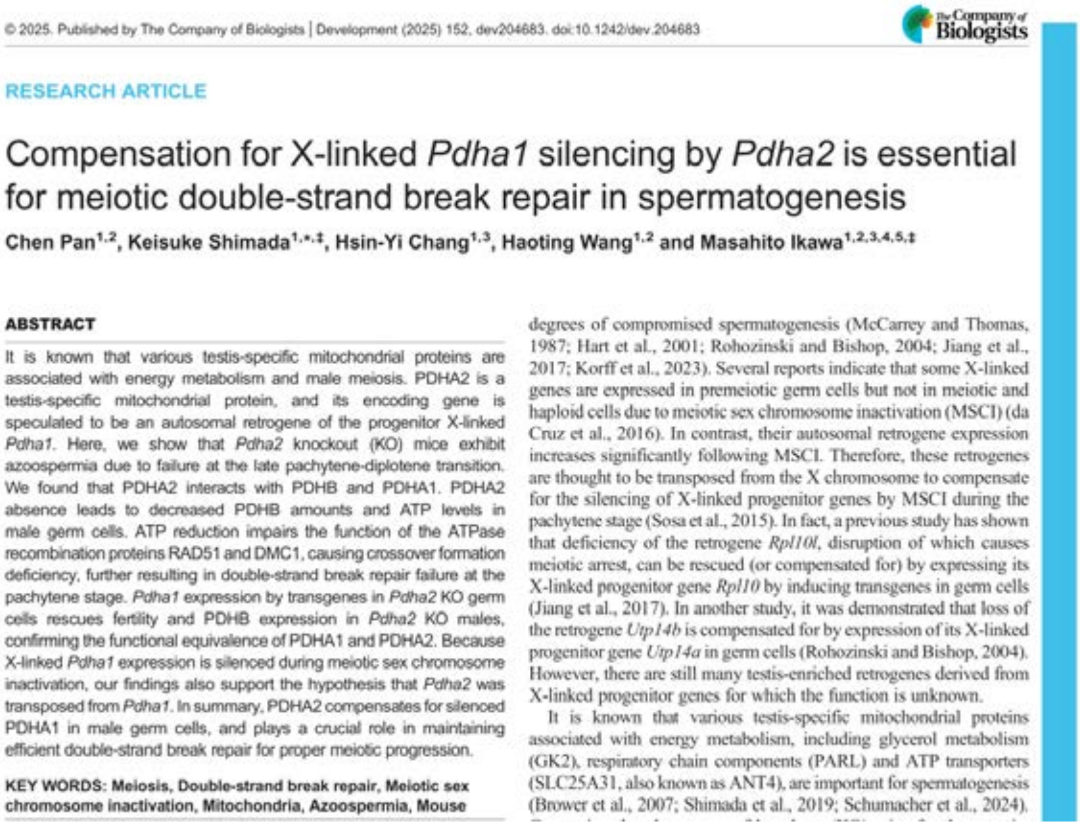
© 2025. Published by The Company of Biologists | Development (2025) 152, dev204683. doi:10.1242/dev.204683

RESEARCH ARTICLE

Compensation for X-linked *Pdha1* silencing by *Pdha2* is essential for meiotic double-strand break repair in spermatogenesis

Chen Pan[1,2], Keisuke Shimada[1,*,‡], Hsin-Yi Chang[1,3], Haoting Wang[1,2] and Masahito Ikawa[1,2,3,4,5,‡]

ABSTRACT

It is known that various testis-specific mitochondrial proteins are associated with energy metabolism and male meiosis. PDHA2 is a testis-specific mitochondrial protein, and its encoding gene is speculated to be an autosomal retrogene of the progenitor X-linked *Pdha1*. Here, we show that *Pdha2* knockout (KO) mice exhibit azoospermia due to failure at the late pachytene-diplotene transition. We found that PDHA2 interacts with PDHB and PDHA1. PDHA2 absence leads to decreased PDHB amounts and ATP levels in male germ cells. ATP reduction impairs the function of the ATPase recombination proteins RAD51 and DMC1, causing crossover formation deficiency, further resulting in double-strand break repair failure at the pachytene stage. *Pdha1* expression by transgenes in *Pdha2* KO germ cells rescues fertility and PDHB expression in *Pdha2* KO males, confirming the functional equivalence of PDHA1 and PDHA2. Because X-linked *Pdha1* expression is silenced during meiotic sex chromosome inactivation, our findings also support the hypothesis that *Pdha2* was transposed from *Pdha1*. In summary, PDHA2 compensates for silenced PDHA1 in male germ cells, and plays a crucial role in maintaining efficient double-strand break repair for proper meiotic progression.

KEY WORDS: Meiosis, Double-strand break repair, Meiotic sex chromosome inactivation, Mitochondria, Azoospermia, Mouse

degrees of compromised spermatogenesis (McCarrey and Thomas, 1987; Hart et al., 2001; Rohozinski and Bishop, 2004; Jiang et al., 2017; Korff et al., 2023). Several reports indicate that some X-linked genes are expressed in premeiotic germ cells but not in meiotic and haploid cells due to meiotic sex chromosome inactivation (MSCI) (da Cruz et al., 2016). In contrast, their autosomal retrogene expression increases significantly following MSCI. Therefore, these retrogenes are thought to be transposed from the X chromosome to compensate for the silencing of X-linked progenitor genes by MSCI during the pachytene stage (Sosa et al., 2015). In fact, a previous study has shown that deficiency of the retrogene *Rpl10l*, disruption of which causes meiotic arrest, can be rescued (or compensated for) by expressing its X-linked progenitor gene *Rpl10* by inducing transgenes in germ cells (Jiang et al., 2017). In another study, it was demonstrated that loss of the retrogene *Utp14b* is compensated for by expression of its X-linked progenitor gene *Utp14a* in germ cells (Rohozinski and Bishop, 2004). However, there are still many testis-enriched retrogenes derived from X-linked progenitor genes for which the function is unknown.

It is known that various testis-specific mitochondrial proteins associated with energy metabolism, including glycerol metabolism (GK2), respiratory chain components (PARL) and ATP transporters (SLC25A31, also known as ANT4), are important for spermatogenesis (Brower et al., 2007; Shimada et al., 2019; Schumacher et al., 2024).


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[1]Department of Experimental Genome Research, Research Institute for Microbial Diseases, The University of Osaka, Osaka 5650871, Japan. [2]Department of Experimental Genome Research, Graduate School of Pharmaceutical Sciences, The University of Osaka, Osaka 5650871, Japan. [3]Department of Experimental Genome Research, Graduate School of Medicine, The University of Osaka, Osaka 5650871, Japan. [4]Regulation of Host Defense Team, Center for Infectious Disease Education and Research, The University of Osaka, Osaka 5650871, Japan. [5]Laboratory of Reproductive Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo 1088639, Japan.
*Present address: Laboratory of Disease Models, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 0698501, Japan.

‡Authors for correspondence (shimada@rakuno.ac.jp; ikawa@biken.osaka-u.ac.jp)

K.S., 0000-0003-3739-7163; M.I., 0000-0001-9859-6217

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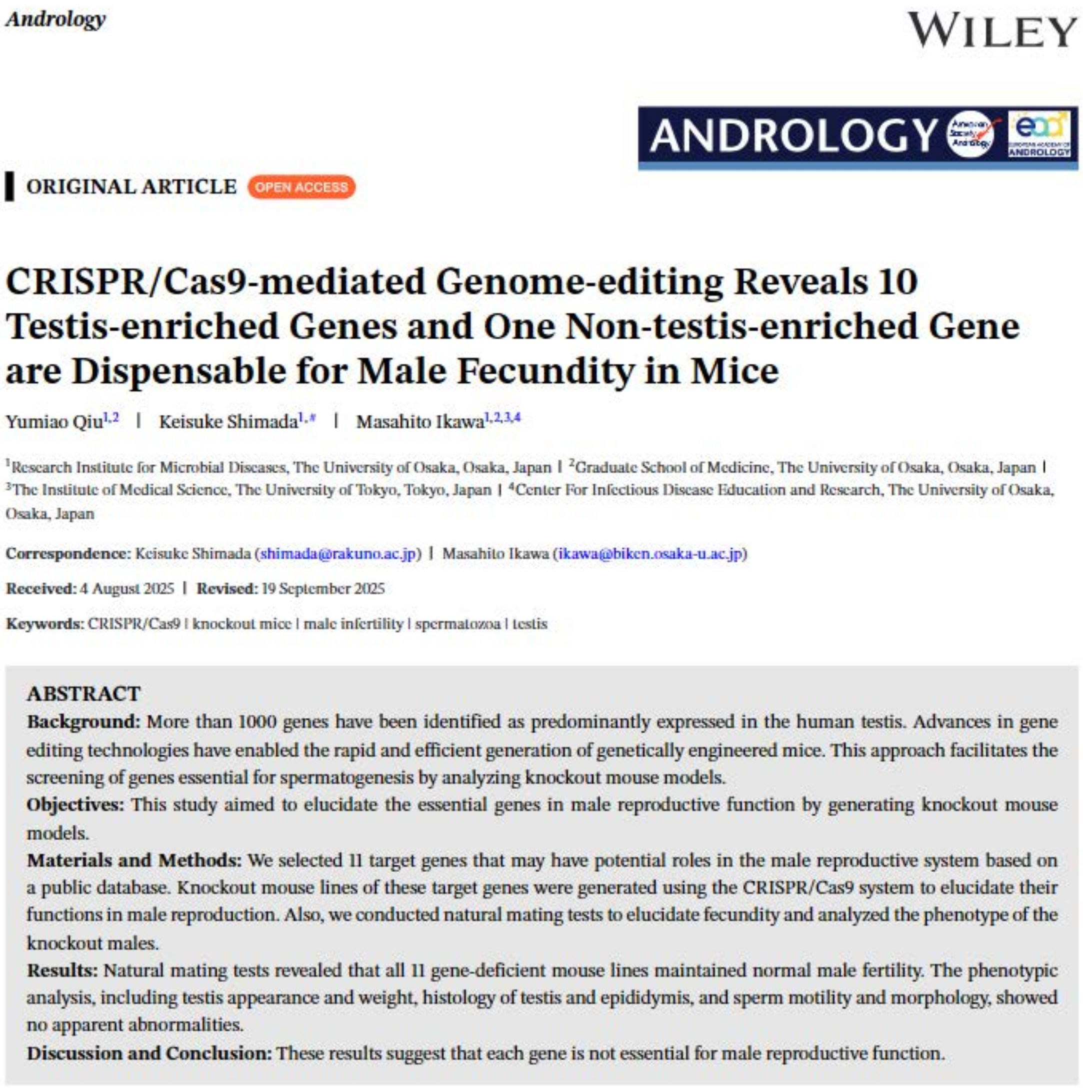

Andrology

WILEY

ANDROLOGY

ORIGINAL ARTICLE OPEN ACCESS

CRISPR/Cas9-mediated Genome-editing Reveals 10 Testis-enriched Genes and One Non-testis-enriched Gene are Dispensable for Male Fecundity in Mice

Yumiao Qiu[1,2] | Keisuke Shimada[1,*] | Masahito Ikawa[1,2,3,4]

[1]Research Institute for Microbial Diseases, The University of Osaka, Osaka, Japan | [2]Graduate School of Medicine, The University of Osaka, Osaka, Japan | [3]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan | [4]Center For Infectious Disease Education and Research, The University of Osaka, Osaka, Japan

Correspondence: Keisuke Shimada (shimada@rakuno.ac.jp) | Masahito Ikawa (ikawa@biken.osaka-u.ac.jp)

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Keywords: CRISPR/Cas9 | knockout mice | male infertility | spermatozoa | testis

ABSTRACT

Background: More than 1000 genes have been identified as predominantly expressed in the human testis. Advances in gene editing technologies have enabled the rapid and efficient generation of genetically engineered mice. This approach facilitates the screening of genes essential for spermatogenesis by analyzing knockout mouse models.

Objectives: This study aimed to elucidate the essential genes in male reproductive function by generating knockout mouse models.

Materials and Methods: We selected 11 target genes that may have potential roles in the male reproductive system based on a public database. Knockout mouse lines of these target genes were generated using the CRISPR/Cas9 system to elucidate their functions in male reproduction. Also, we conducted natural mating tests to elucidate fecundity and analyzed the phenotype of the knockout males.

Results: Natural mating tests revealed that all 11 gene-deficient mouse lines maintained normal male fertility. The phenotypic analysis, including testis appearance and weight, histology of testis and epididymis, and sperm motility and morphology, showed no apparent abnormalities.

Discussion and Conclusion: These results suggest that each gene is not essential for male reproductive function.

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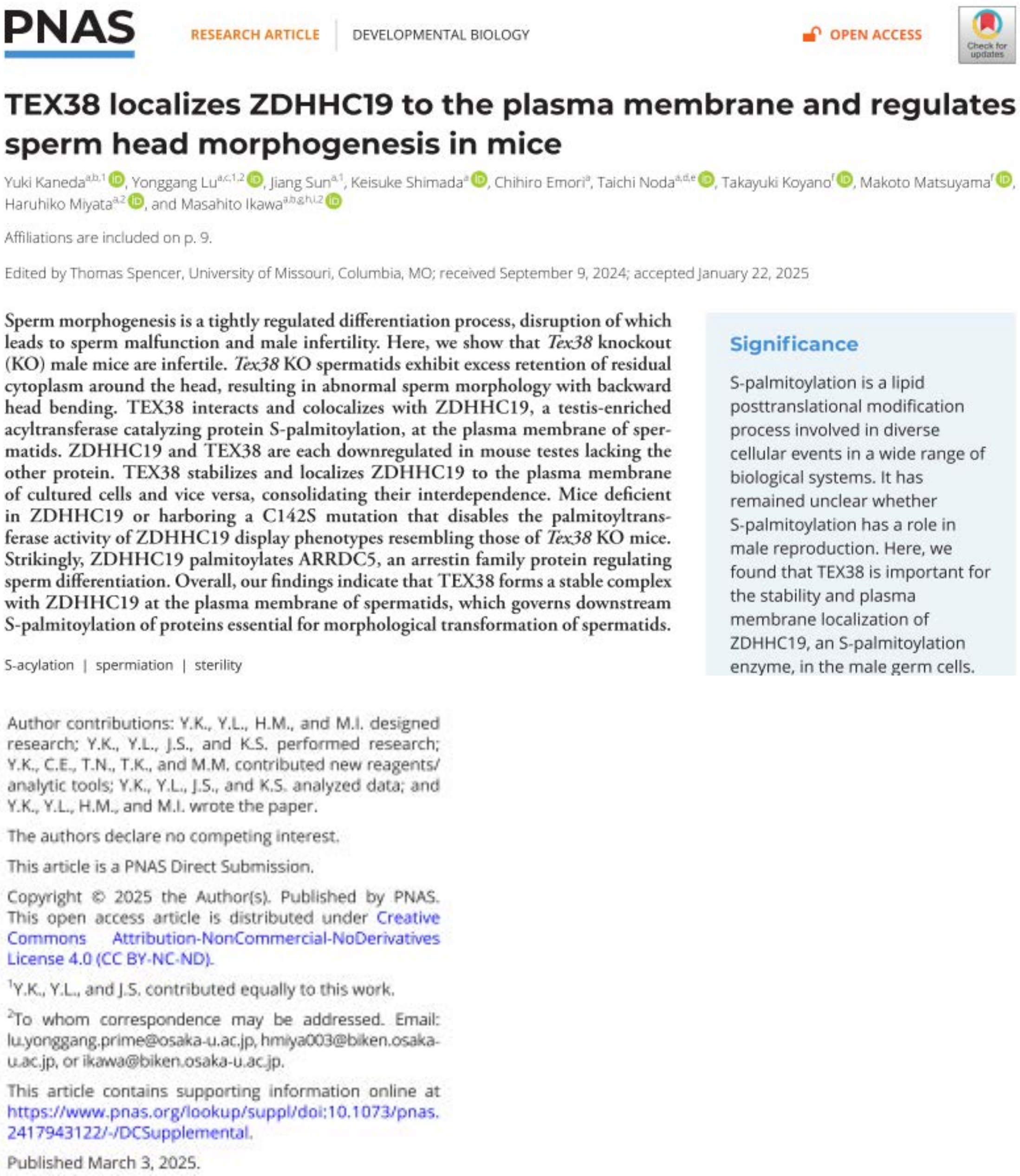

PNAS RESEARCH ARTICLE | DEVELOPMENTAL BIOLOGY OPEN ACCESS

TEX38 localizes ZDHHC19 to the plasma membrane and regulates sperm head morphogenesis in mice

Yuki Kaneda[a,b,1], Yonggang Lu[a,c,1,2], Jiang Sun[a,1], Keisuke Shimada[a], Chihiro Emori[a], Taichi Noda[a,d,e], Takayuki Koyano[f], Makoto Matsuyama[f], Haruhiko Miyata[a,2], and Masahito Ikawa[a,b,g,h,i,2]

Affiliations are included on p. 9.

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Sperm morphogenesis is a tightly regulated differentiation process, disruption of which leads to sperm malfunction and male infertility. Here, we show that *Tex38* knockout (KO) male mice are infertile. *Tex38* KO spermatids exhibit excess retention of residual cytoplasm around the head, resulting in abnormal sperm morphology with backward head bending. TEX38 interacts and colocalizes with ZDHHC19, a testis-enriched acyltransferase catalyzing protein S-palmitoylation, at the plasma membrane of spermatids. ZDHHC19 and TEX38 are each downregulated in mouse testes lacking the other protein. TEX38 stabilizes and localizes ZDHHC19 to the plasma membrane of cultured cells and vice versa, consolidating their interdependence. Mice deficient in ZDHHC19 or harboring a C142S mutation that disables the palmitoyltransferase activity of ZDHHC19 display phenotypes resembling those of *Tex38* KO mice. Strikingly, ZDHHC19 palmitoylates ARRDC5, an arrestin family protein regulating sperm differentiation. Overall, our findings indicate that TEX38 forms a stable complex with ZDHHC19 at the plasma membrane of spermatids, which governs downstream S-palmitoylation of proteins essential for morphological transformation of spermatids.

S-acylation | spermiation | sterility

Significance

S-palmitoylation is a lipid posttranslational modification process involved in diverse cellular events in a wide range of biological systems. It has remained unclear whether S-palmitoylation has a role in male reproduction. Here, we found that TEX38 is important for the stability and plasma membrane localization of ZDHHC19, an S-palmitoylation enzyme, in the male germ cells.

Author contributions: Y.K., Y.L., H.M., and M.I. designed research; Y.K., Y.L., J.S., and K.S. performed research; Y.K., C.E., T.N., T.K., and M.M. contributed new reagents/analytic tools; Y.K., Y.L., J.S., and K.S. analyzed data; and Y.K., Y.L., H.M., and M.I. wrote the paper.

The authors declare no competing interest.

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[1] Y.K., Y.L., and J.S. contributed equally to this work.

[2] To whom correspondence may be addressed. Email: lu.yonggang.prime@osaka-u.ac.jp, hmiya003@biken.osaka-u.ac.jp, or ikawa@biken.osaka-u.ac.jp.

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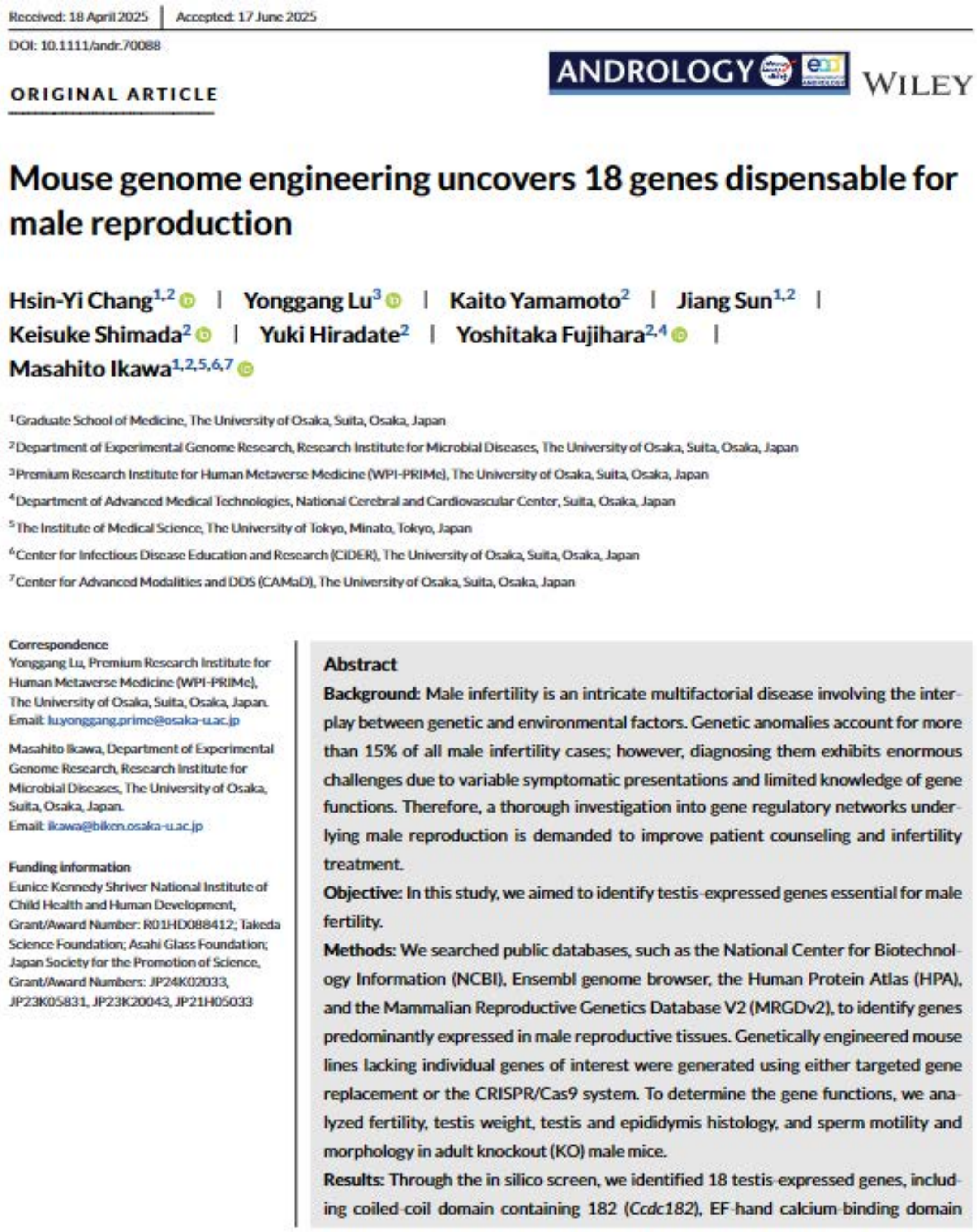

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ANDROLOGY
WILEY

ORIGINAL ARTICLE

Mouse genome engineering uncovers 18 genes dispensable for male reproduction

Hsin-Yi Chang[1,2] | Yonggang Lu[3] | Kaito Yamamoto[2] | Jiang Sun[1,2] | Keisuke Shimada[2] | Yuki Hiradate[2] | Yoshitaka Fujihara[2,4] | Masahito Ikawa[1,2,5,6,7]

[1] Graduate School of Medicine, The University of Osaka, Suita, Osaka, Japan
[2] Department of Experimental Genome Research, Research Institute for Microbial Diseases, The University of Osaka, Suita, Osaka, Japan
[3] Premium Research Institute for Human Metaverse Medicine (WPI-PRIMe), The University of Osaka, Suita, Osaka, Japan
[4] Department of Advanced Medical Technologies, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan
[5] The Institute of Medical Science, The University of Tokyo, Minato, Tokyo, Japan
[6] Center for Infectious Disease Education and Research (CiDER), The University of Osaka, Suita, Osaka, Japan
[7] Center for Advanced Modalities and DDS (CAMaD), The University of Osaka, Suita, Osaka, Japan

Correspondence
Yonggang Lu, Premium Research Institute for Human Metaverse Medicine (WPI-PRIMe), The University of Osaka, Suita, Osaka, Japan.
Email: lu.yonggang.prime@osaka-u.ac.jp

Masahito Ikawa, Department of Experimental Genome Research, Research Institute for Microbial Diseases, The University of Osaka, Suita, Osaka, Japan.
Email: ikawa@biken.osaka-u.ac.jp

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Abstract

Background: Male infertility is an intricate multifactorial disease involving the interplay between genetic and environmental factors. Genetic anomalies account for more than 15% of all male infertility cases; however, diagnosing them exhibits enormous challenges due to variable symptomatic presentations and limited knowledge of gene functions. Therefore, a thorough investigation into gene regulatory networks underlying male reproduction is demanded to improve patient counseling and infertility treatment.

Objective: In this study, we aimed to identify testis-expressed genes essential for male fertility.

Methods: We searched public databases, such as the National Center for Biotechnology Information (NCBI), Ensembl genome browser, the Human Protein Atlas (HPA), and the Mammalian Reproductive Genetics Database V2 (MRGDv2), to identify genes predominantly expressed in male reproductive tissues. Genetically engineered mouse lines lacking individual genes of interest were generated using either targeted gene replacement or the CRISPR/Cas9 system. To determine the gene functions, we analyzed fertility, testis weight, testis and epididymis histology, and sperm motility and morphology in adult knockout (KO) male mice.

Results: Through the in silico screen, we identified 18 testis-expressed genes, including coiled-coil domain containing 182 (*Ccdc182*), EF-hand calcium-binding domain

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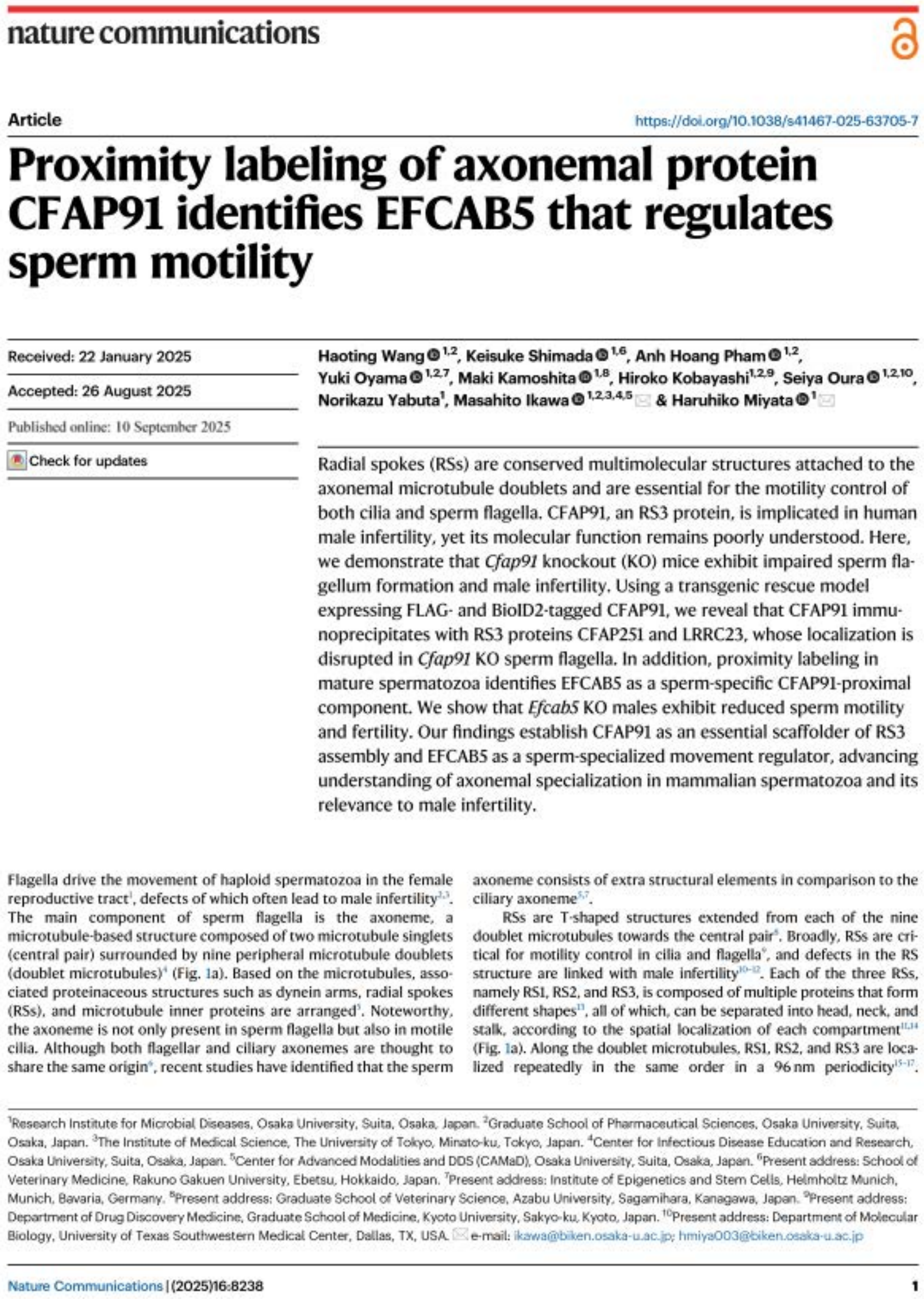
最終責任者　Yonggang Lu and Masahito Ikawa (Correspondence)



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Proximity labeling of axonemal protein CFAP91 identifies EFCAB5 that regulates sperm motility

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Haoting Wang[1,2], Keisuke Shimada[1,6], Anh Hoang Pham[1,2], Yuki Oyama[1,2,7], Maki Kamoshita[1,8], Hiroko Kobayashi[1,2,9], Seiya Oura[1,2,10], Norikazu Yabuta[1], Masahito Ikawa[1,2,3,4,5] ✉ & Haruhiko Miyata[1] ✉

Radial spokes (RSs) are conserved multimolecular structures attached to the axonemal microtubule doublets and are essential for the motility control of both cilia and sperm flagella. CFAP91, an RS3 protein, is implicated in human male infertility, yet its molecular function remains poorly understood. Here, we demonstrate that *Cfap91* knockout (KO) mice exhibit impaired sperm flagellum formation and male infertility. Using a transgenic rescue model expressing FLAG- and BioID2-tagged CFAP91, we reveal that CFAP91 immunoprecipitates with RS3 proteins CFAP251 and LRRC23, whose localization is disrupted in *Cfap91* KO sperm flagella. In addition, proximity labeling in mature spermatozoa identifies EFCAB5 as a sperm-specific CFAP91-proximal component. We show that *Efcab5* KO males exhibit reduced sperm motility and fertility. Our findings establish CFAP91 as an essential scaffolder of RS3 assembly and EFCAB5 as a sperm-specialized movement regulator, advancing understanding of axonemal specialization in mammalian spermatozoa and its relevance to male infertility.

Flagella drive the movement of haploid spermatozoa in the female reproductive tract[1], defects of which often lead to male infertility[2,3]. The main component of sperm flagella is the axoneme, a microtubule-based structure composed of two microtubule singlets (central pair) surrounded by nine peripheral microtubule doublets (doublet microtubules)[4] (Fig. 1a). Based on the microtubules, associated proteinaceous structures such as dynein arms, radial spokes (RSs), and microtubule inner proteins are arranged[5]. Noteworthy, the axoneme is not only present in sperm flagella but also in motile cilia. Although both flagellar and ciliary axonemes are thought to share the same origin[6], recent studies have identified that the sperm axoneme consists of extra structural elements in comparison to the ciliary axoneme[5,7].

RSs are T-shaped structures extended from each of the nine doublet microtubules towards the central pair[8]. Broadly, RSs are critical for motility control in cilia and flagella[9], and defects in the RS structure are linked with male infertility[10–12]. Each of the three RSs, namely RS1, RS2, and RS3, is composed of multiple proteins that form different shapes[13], all of which, can be separated into head, neck, and stalk, according to the spatial localization of each compartment[11,14] (Fig. 1a). Along the doublet microtubules, RS1, RS2, and RS3 are localized repeatedly in the same order in a 96 nm periodicity[15–17].

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[1] Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan. [2] Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan. [3] The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo, Japan. [4] Center for Infectious Disease Education and Research, Osaka University, Suita, Osaka, Japan. [5] Center for Advanced Modalities and DDS (CAMaD), Osaka University, Suita, Osaka, Japan. [6] Present address: School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan. [7] Present address: Institute of Epigenetics and Stem Cells, Helmholtz Munich, Munich, Bavaria, Germany. [8] Present address: Graduate School of Veterinary Science, Azabu University, Sagamihara, Kanagawa, Japan. [9] Present address: Department of Drug Discovery Medicine, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan. [10] Present address: Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA. ✉ e-mail: ikawa@biken.osaka-u.ac.jp; hmiya003@biken.osaka-u.ac.jp

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最終責任者　Masahito Ikawa and Haruhiko Miyata (Correspondence)

馬医療学（Equine Medicine）

Nao Tsuzuki

Associate Professor

准教授　都築　直

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1）Preoperative computed tomography imaging for accurate diagnosis and surgical planning in equine cranial disorders: two case reports of congenital malformations. Yamaga T, Tagami M, Takeyama A, Kato F, Suzuki T, Tagami M, **Tsuzuki N**. *J Equine Sci.* 36: 33-37. 2025.
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II. その他＜Others＞

1）Growth pattern analysis of the internal cranial structure in the male Hokkaido brown bear (Ursus arctos yesoensis) by computed tomography. Itakura R, **Tsuzuki N**, Kayano M, Sasaki M. *J Vet Med Sci.* in press.
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—Note—

Preoperative computed tomography imaging for accurate diagnosis and surgical planning in equine cranial disorders: two case reports of congenital malformations

Takashi YAMAGA[1], **Masaaki TAGAMI**[1], **Akiko TAKEYAMA**[2], **Fumiki KATO**[1],
Tsukasa SUZUKI[1], **Masayuki TAGAMI**[1] and **Nao TSUZUKI**[3]*
[1]Shadai Horse Clinic, Hokkaido 059-1365, Japan
[2]Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine,
Hokkaido 080-8555, Japan
[3]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan

Computed tomography (CT) offers high-resolution, three-dimensional imaging, making it particularly valuable for assessing complex structures, such as the head, especially when conventional radiography and endoscopy are insufficient for a definitive diagnosis. Herein, we present two cases of equine cranial disorders resulting from congenital malformations. In case 1, which had a dentigerous cyst, CT images confirmed the location of the ectopic tooth within the skull and a detailed fistula tube. In case 2, which had temporohyoid osteoarthropathy, CT examination revealed that the stylohyoid bone was malformed, and the inner ear was presumed to be filled with soft tissue with calcification. The information obtained by CT was invaluable, as it allowed for accurate diagnosis and precise surgical planning.

Keywords: *congenital malformation, computed tomography imaging, dentigerous cyst, horse*

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Computed tomography (CT) is a diagnostic imaging method wherein an object is scanned using X-rays and the results are computationally processed to obtain tomographic images. CT has been widely used in veterinary medicine since 2000; moreover, it has been applied in various equine veterinary studies, such as in the diagnosis of orthopedic, neurological, and respiratory diseases [1, 4–6]. CT enables three-dimensional evaluation using tomographic images and offers high spatial resolution, making it especially useful for visualizing complex anatomical features.

The equine skull is a complex structure comprising numerous overlapping bones. Additionally, several of these bone structures have similar X-ray transparency characteristics, making imaging using contrast challenging. Therefore, cranial disorders are often difficult to diagnose using X-ray, as it has a two-dimensional evaluation capacity and limited soft tissue contrast resolution [5]. The skull can be examined using an endoscope; however, evaluation is limited to only the nasal passage, pharynx, larynx, and guttural pouch. Thus, CT evaluation has been deemed useful in cases where sufficient information cannot be obtained by X-ray or endoscopy alone [5] and is particularly beneficial in diagnosing sinus cysts, dental diseases, and other intricate cranial conditions [1, 2, 5, 7].

Case 1: The first case was of a 4-month-old male Thoroughbred foal admitted to the Shadai Horse Clinic for the drainage of pus under the left ear. No other symptoms, such as lameness or neurological symptoms, were observed. An ectopic tooth was identified on X-ray examination (Fig. 1), leading to the diagnosis of a dentigerous cyst. However, the exact location of the ectopic tooth was unclear. Therefore, the foal was referred to the Veterinary Medical Center of Obihiro University of Agriculture and Veterinary Medicine to determine the precise location of the ectopic tooth and fistula using CT before its removal.

The foal was premedicated with 5 μg/kg intravenous

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*Corresponding author. e-mail: n-tsuzuki@rakuno.ac.jp

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最終責任者　Nao Tsuzuki (Corresponding Author)

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FULL PAPER
Anatomy

Growth pattern analysis of the internal cranial structure in the male Hokkaido brown bear (*Ursus arctos yesoensis*) by computed tomography

Raito ITAKURA1), **Nao TSUZUKI**1,2), **Mitsunori KAYANO**1), **Motoki SASAKI**1)*

1)Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan
2)Present address: Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

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ABSTRACT. Forty male brown bear heads were non-destructively analyzed by computed tomography. Skull, frontal, and cranial cavity volumes were measured, and growth curves and regression lines were constructed. The morphological dynamics of the frontal sinus were also examined. Skull volume increased rapidly from 0 to 11 years of age, then grew more slowly until 21 years, when it plateaued. The frontal sinus also increased rapidly from 0 years, reaching approximately 300 mL by 10 years, after which growth ceased. The frontal sinus-to-skull ratio rose quickly from 0 to 4 years, then increased more gradually until 10 years, and then plateaued. Morphologically, the frontal sinus developed posteriorly to cover the anterior part of the cranial cavity, extended ventrally in the anterior lateral part of the cranial cavity and the orbital region, and protruded into the zygomatic process of the frontal bone. The cranial cavity increased slowly from 0 years, with continued growth beyond 11 years at a reduced rate. The cranial cavity-to-skull ratio decreased because the skull volume significantly increased. Both the frontal sinus and cranial cavity volumes strongly correlated with skull volume, and a well-fitting regression line was obtained. This means that the volumes of the frontal sinus and cranial cavity correlate more closely with skull volume than with age. This is the first report on the non-destructive analysis of the changes in volume and morphology of the frontal sinus and cranial cavity in male brown bears.

KEYWORDS: cranial cavity, frontal sinus, paranasal sinus, quantification, three-dimensional analysis

INTRODUCTION

The brown bear (*Ursus arctos*) is a large mammal with a widespread global distribution. However, Hokkaido brown bears (*U. a. yesoensis*) live only in Hokkaido, Japan. Wild Hokkaido brown bears have been captured at ages of 34 years in females and 30 years in males, and the maximum lifespan of captive bears is reported to be 38 years [13]. The males reach sexual maturity from 2 to 4 years of age [16], and in females, the earliest parturition has been recorded at 4 years old [13]. Within this species, there is considerable variation in the size and shape of the external skull. Several studies have attributed this variability to biogeographical factors [1, 8, 9, 11, 19], and one study emphasized that differences in feeding resources under diverse environmental conditions appear to play a significant role [7]. Overall, skull variation is generally considered an adaptive response to differing environmental conditions. Rivrud *et al*. [12] noted that the evolutionary rate of head circumference size in brown bears is approximately 0.2%, which may lead to substantial changes in average trait values over hundreds of generations. Accurate assessment of the morphological changes necessitates the consideration of multiple factors such as growth and sexual dimorphism. Studies on the external skull growth of brown bears indicate that while the width of the braincase ceases to grow at an early age, the zygomatic arch and facial bones continue to develop until old age [9, 14]. Suenaga [14] hypothesized that this developmental discrepancy is closely linked to the formation of a cavity within the skull. However, current knowledge regarding morphological assessment is primarily limited to the external cranium shape, and there is a paucity of research on internal structures related to external morphological growth [2, 4, 6, 10].

The paranasal sinuses are cavities encased by bones in the skull, of which the functions remain largely unknown. Blanton and Biggs [3] proposed several possible functions, including voice resonance, humidification and warming of inhaled air, increased olfactory mucosa surface area, shock absorption, mucus secretion for nasal cavity moistening, thermal insulation, and skull weight reduction.

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*Correspondence to: Sasaki M: sasakim@obihiro.ac.jp, Obihiro University of Agriculture and Veterinary Medicine, Nishi 2-11, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

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最終責任者　Motoki Sasaki (Corresponding Author)

Leo Uchida

Associate Professor

准教授　内田　玲麻

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Detection of haemosporidian parasite lineages from wild bird carcasses collected in eastern Japan.
Eki M, Sasaki M, Muramatsu Y, Kobayashi M, Ishikawa T, **Uchida L.**
J Vet Med Sci. 87:838-842. 2025. doi: 10.1292/jvms.24-0267.

II. その他　＜Others＞

該当なし

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NOTE
Public Health

Detection of haemosporidian parasite lineages from wild bird carcasses collected in eastern Japan

Momoko EKI[1,3], Mizuki SASAKI[2], Yasukazu MURAMATSU[3], Mari KOBAYASHI[4], Takanori ISHIKAWA[5], Leo UCHIDA[3]*

[1]Department of Ecosystem Studies, School of Environmental Science, The University of Shiga Prefecture, Shiga, Japan
[2]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[3]Laboratory of Zoonotic Diseases, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[4]Department of Ocean and Fisheries Science, Faculty of Bioindustry, Tokyo University of Agriculture, Hokkaido, Japan
[5]Tochigi Prefectural Fisheries Experiment Station, Tochigi, Japan

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ABSTRACT. Avian haemosporidia are a group of vector-borne hemoparasites that are non-pathogenic to native bird hosts; however, some species/lineages cause severe disease or death in poultry and non-native birds. We investigated haemosporidian parasites in wild bird carcasses collected from four prefectures in eastern Japan. DNA was extracted from the liver, and the haemosporidian cytochrome *b* gene was amplified by nested PCR followed by direct sequencing. Of the 40 bird samples belonging to 25 species, 12 haemosporidian lineages were detected: four from *Plasmodium*, one from *Haemoproteus*, and seven from *Leucocytozoon*. Of these 12, two *Leucocytozoon* lineages were suspected to be novel. *Leucocytozoon* lineage TUMER02 was first detected in Japan. Six lineages were detected among the six novel host bird species.

KEYWORDS: cytochrome *b* gene, haemosporidia, Japan, wild bird

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*Correspondence to: Uchida L: uchidaleo@rakuno.ac.jp, Laboratory of Zoonotic Diseases, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan

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最終責任者　Leo Uchida (Corresponding Author)

獣医疫学（Veterinary Epidemiology）

Shingo Asakura

Lecturer

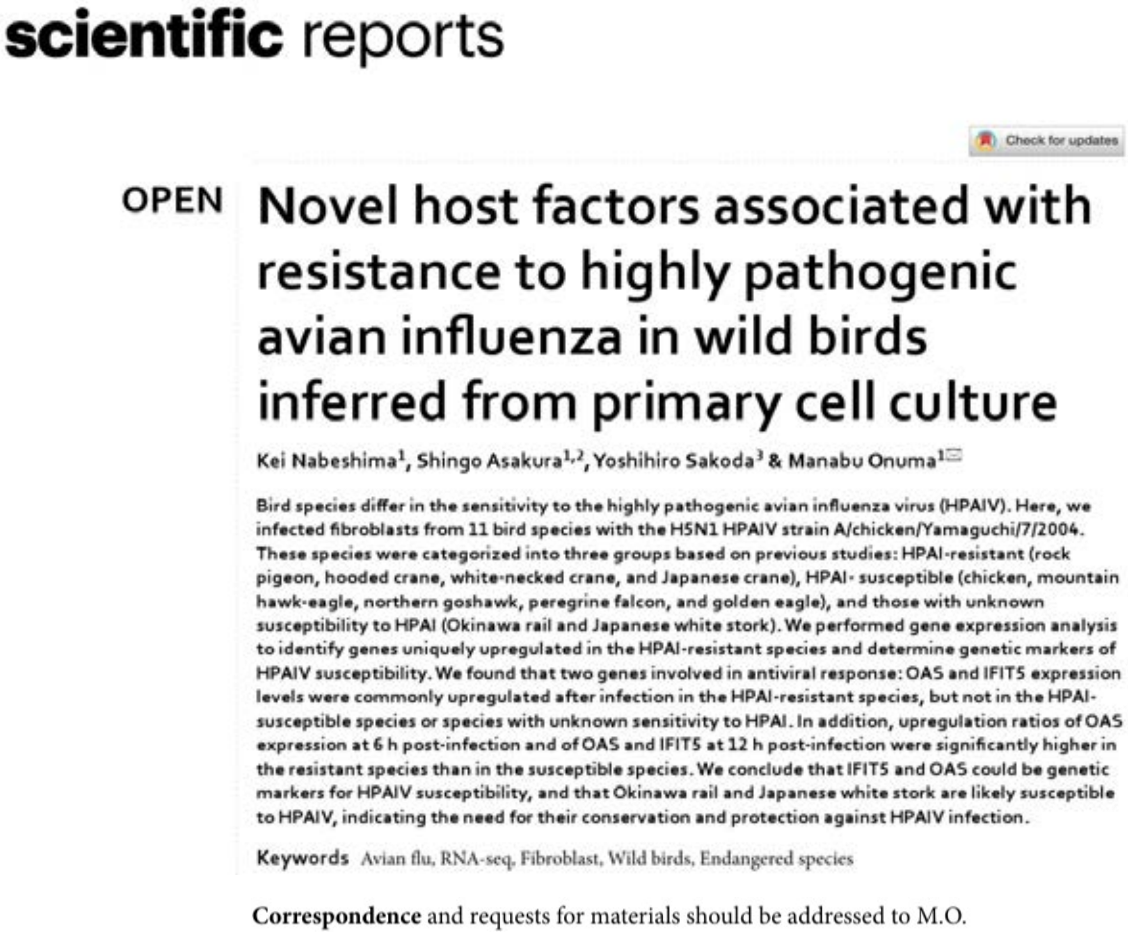
講師　浅倉　真吾

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他　＜Others＞

1）Novel host factors associated with resistance to highly pathogenic avian influenza in wild birds inferred from primary cell culture.
Nabeshima K, **Asakura S**, Sakoda Y, Onuma M.
Scientific Reports. 15:18809. doi: 10.1038/s41598-025-01316-4.



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Novel host factors associated with resistance to highly pathogenic avian influenza in wild birds inferred from primary cell culture

Kei Nabeshima[1], Shingo Asakura[1,2], Yoshihiro Sakoda[3] & Manabu Onuma[1]

Bird species differ in the sensitivity to the highly pathogenic avian influenza virus (HPAIV). Here, we infected fibroblasts from 11 bird species with the H5N1 HPAIV strain A/chicken/Yamaguchi/7/2004. These species were categorized into three groups based on previous studies: HPAI-resistant (rock pigeon, hooded crane, white-necked crane, and Japanese crane), HPAI- susceptible (chicken, mountain hawk-eagle, northern goshawk, peregrine falcon, and golden eagle), and those with unknown susceptibility to HPAI (Okinawa rail and Japanese white stork). We performed gene expression analysis to identify genes uniquely upregulated in the HPAI-resistant species and determine genetic markers of HPAIV susceptibility. We found that two genes involved in antiviral response: OAS and IFIT5 expression levels were commonly upregulated after infection in the HPAI-resistant species, but not in the HPAI-susceptible species or species with unknown sensitivity to HPAI. In addition, upregulation ratios of OAS expression at 6 h post-infection and of OAS and IFIT5 at 12 h post-infection were significantly higher in the resistant species than in the susceptible species. We conclude that IFIT5 and OAS could be genetic markers for HPAIV susceptibility, and that Okinawa rail and Japanese white stork are likely susceptible to HPAIV, indicating the need for their conservation and protection against HPAIV infection.

Keywords Avian flu, RNA-seq, Fibroblast, Wild birds, Endangered species

Correspondence and requests for materials should be addressed to M.O.

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最終責任者　Manabu Onuma (Corresponding Author)

伴侶動物内科学（Companion Animal Internal Medicine）

Tatsuya Deguchi

Lecturer

講師　出口　辰弥

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他＜Others＞

1）Clinical efficacy of anti-programmed death ligand 1 antibody HFC-L1/c4G12 in dogs with malignant tumors: an exploratory study.
Takagi S, Tagawa M, Maekawa N, Konnai S, Kagawa Y, Hosoya K, Yamauchi A, Kudo A, Kamo S, Kim S, Kinoshita R, **Deguchi T**, Owaki R, Tachibana Y, Yokokawa M, Takeuchi H, Nakamura H, Kato Y, Kanazawa S, Abe T, Furuta T, Yamamoto K, Suzuki Y, Okagawa T, Murata S, Ohashi K.
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Mol Ther Oncol. 33:200946. 2025. doi:10.1016/j.omton.2025.200946.

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FULL PAPER

Surgery

Clinical efficacy of anti-programmed death ligand 1 antibody HFC-L1/c4G12 in dogs with malignant tumors: an exploratory study

Satoshi TAKAGI[1)#], Michihito TAGAWA[2)#], Naoya MAEKAWA[3–5)#],
Satoru KONNAI[3–7)*], Yumiko KAGAWA[8)], Kenji HOSOYA[5,9)], Akinori YAMAUCHI[1)],
Ayano KUDO[1)], Shintaro KAMO[1)], Sangho KIM[5,9)], Ryohei KINOSHITA[5,9)],
Tatsuya DEGUCHI[9,10)], Ryo OWAKI[9)], Yurika TACHIBANA[9)],
Madoka YOKOKAWA[9)], Hiroto TAKEUCHI[4)], Hayato NAKAMURA[4)],
Yukinari KATO[11)], Shigeki KANAZAWA[3,12)], Tomoyuki ABE[3,12)],
Takuya FURUTA[3,12)], Keiichi YAMAMOTO[3,6,12)], Yasuhiko SUZUKI[3,6,13,14)],
Tomohiro OKAGAWA[3,4)], Shiro MURATA[3,4,7)], Kazuhiko OHASHI[3,4,7,15)]

[1)]Laboratory of Small Animal Surgery, School of Veterinary Medicine, Azabu University, Kanagawa, Japan
[2)]Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[3)]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[4)]Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[5)]Cancer Research Unit, One Health Research Center, Hokkaido University, Hokkaido, Japan
[6)]Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Hokkaido, Japan
[7)]Veterinary Research Unit, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan
[8)]North Lab, Hokkaido, Japan
[9)]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[10)]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[11)]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Miyagi, Japan
[12)]FUSO Pharmaceutical Industries, Ltd., Osaka, Japan
[13)]Division of Bioresources, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan
[14)]Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Hokkaido, Japan
[15)]International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan

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ABSTRACT. Cancer in dogs remains a major challenge in modern veterinary medicine. Immunotherapy using immune checkpoint inhibitors (ICIs) is available for various human tumor types, and recent veterinary clinical studies have shown that ICIs are a promising approach for treating canine cancers. A canine chimeric anti-PD-L1 antibody, c4G12 (HFC-L1), has been investigated for canine cancer immunotherapy; however, its clinical benefits have not been well characterized in tumors other than pulmonary metastatic (stage IV) oral malignant melanoma (OMM). To explore the efficacy and safety of HFC-L1, we conducted a clinical study in dogs with stage I–III OMM or other tumor types (n=12). HFC-L1 treatment at a dose of 5 mg/kg every 2 weeks was well tolerated, and no grade 3 or higher treatment-related adverse events were reported. Among the dogs eligible for response evaluation (n=10), a partial response was observed in one dog with squamous cell carcinoma, resulting in an objective response rate of 10%. In addition, in a dog with ceruminous cell carcinoma, clinical evidence of a tumor response was observed in metastatic lung lesions. Together, these results suggest that the HFC-L1 therapy is applicable for the treatment of various tumor types, although its clinical benefits should be further evaluated in clinical studies involving a larger number of dogs with each tumor type.

KEYWORDS: canine tumor, immune checkpoint inhibitor, immunotherapy, programmed death ligand 1 (PD-L1)

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*Correspondence to: Konnai S: konnai@vetmed.hokudai.ac.jp, Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan

#These authors contributed equally to this work.

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Article

Exploratory, Randomized, Dose-Response Study of the Anti-PD-L1 Antibody HFC-L1/c4G12 in Dogs with Pulmonary Metastatic Oral Malignant Melanoma

Kenji Hosoya [1,2,†], Sangho Kim [1,2,†], Ryohei Kinoshita [1,2,†], Naoya Maekawa [2,3,4,†], Satoru Konnai [2,3,4,5,6,*], Satoshi Takagi [7], Michihito Tagawa [8], Yumiko Kagawa [9], Tatsuya Deguchi [1,10], Ryo Owaki [1], Yurika Tachibana [1], Madoka Yokokawa [1], Hiroto Takeuchi [4], Hayato Nakamura [4], Akinori Yamauchi [7], Ayano Kudo [7], Shintaro Kamo [7], Yukinari Kato [11,*], Shigeki Kanazawa [3,12], Tomoyuki Abe [3,12], Takuya Furuta [3,12], Keiichi Yamamoto [3,5,12], Yasuhiko Suzuki [3,5,13,14], Tomohiro Okagawa [3], Shiro Murata [3,4,6] and Kazuhiko Ohashi [3,4,6,15]

[1] Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0819, Japan
[2] Cancer Research Unit, One Health Research Center, Hokkaido University, Sapporo 060-0818, Japan
[3] Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[4] Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[5] Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Sapporo 001-0021, Japan
[6] Veterinary Research Unit, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan
[7] Laboratory of Small Animal Surgery, School of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan
[8] Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan
[9] North Lab, Sapporo 003-0027, Japan
[10] Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[11] Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan
[12] FUSO Pharmaceutical Industries, Ltd., Osaka 541-0045, Japan
[13] Division of Bioresources, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan
[14] Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo 060-0808, Japan
[15] International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[*] Correspondence: konnai@vetmed.hokudai.ac.jp (S.K.); yukinari.kato.e6@tohoku.ac.jp (Y.K.)
[†] These authors contributed equally to this work.

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Simple Summary

Pulmonary metastatic oral malignant melanoma is a highly aggressive cancer in dogs, and effective systemic therapies are urgently needed. Immune checkpoint inhibitors, which help the immune system attack cancer cells, have shown promise in canine studies. This exploratory, randomized clinical study evaluated the safety and efficacy of an anti-PD-L1 antibody, HFC-L1 (also known as c4G12), in dogs with pulmonary metastatic oral malignant melanoma. Twenty-six dogs were treated with three different doses of HFC-L1 (2, 5, or 10 mg/kg every 2 weeks). The safety profiles were similar across all dose groups, and no severe treatment-related adverse events were observed. Dogs treated with higher doses (5 or 10 mg/kg) showed numerically longer overall survival compared to the 2 mg/kg group. These findings suggest that HFC-L1 therapy is well tolerated and that higher doses may provide improved clinical benefit. Although the study was exploratory in nature with a small sample size, the results support the use of 5–10 mg/kg as the preferred dose in

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Veterinary and Comparative Oncology

ORIGINAL ARTICLE

Outcomes and Prognostic Factors in Dogs With Presumed Intracranial Gliomas Treated With Definitive-Intent Intensity Modulated Radiation Therapy: 55 Cases (2014–2023)

Yohichi Fukuyama, Kenji Hosoya, Sangho Kim, Koangyong Sung, Tatsuya Deguchi, Genya Shimbo, Kazuyoshi Sasaoka, Ryouhei Kinoshita, Mitsuyoshi Takiguchi

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ABSTRACT

Radiation therapy (RT) is the treatment of choice for canine intracranial gliomas. Recently, modern advanced radiation techniques, including intensity modulated RT (IMRT) and volumetric modulated arc therapy (VMAT), have become widely available in veterinary medicine. However, the glioma-specific therapeutic outcomes of patients treated with modern RT remain unclear. This study aimed to describe survival outcomes and tumour response and to identify whether any treatment, clinical, and imaging factors were predictive of prognosis in dogs with intracranial gliomas treated with definitive-intent IMRT alone. Medical records of dogs with presumed intracranial gliomas that underwent definitive-intent IMRT were retrospectively reviewed. Fifty-five dogs were included. Amongst them, 29 and 26 underwent fractionated RT (FRT) and stereotactic RT (SRT), respectively. In the 44 dogs that underwent follow-up magnetic resonance imaging (MRI), the overall measurable response rate was 77.3%. Clinical improvement was observed in 92% of the dogs. Local tumour regrowth and drop metastases were observed in 17 (30.9%) and 10 dogs (18.2%), respectively. The median overall survival, disease-specific survival, and progression-free survival were 432, 670, and 441 days, respectively. Seven dogs (12.7%) died during RT or within 6 weeks. There was no statistically significant difference in the survival times between FRT and SRT. In the multivariate analysis, poor performance status, tumour location in the diencephalon, and fluid-attenuated inversion recovery heterogeneity were significantly associated with shorter survival times. These findings suggest that definitive-intent RT results in tumour shrinkage and prolonged survival (432 days) with minimal radiation toxicity regardless of the RT protocol used. Performance status and MRI findings can be useful for predicting prognosis.

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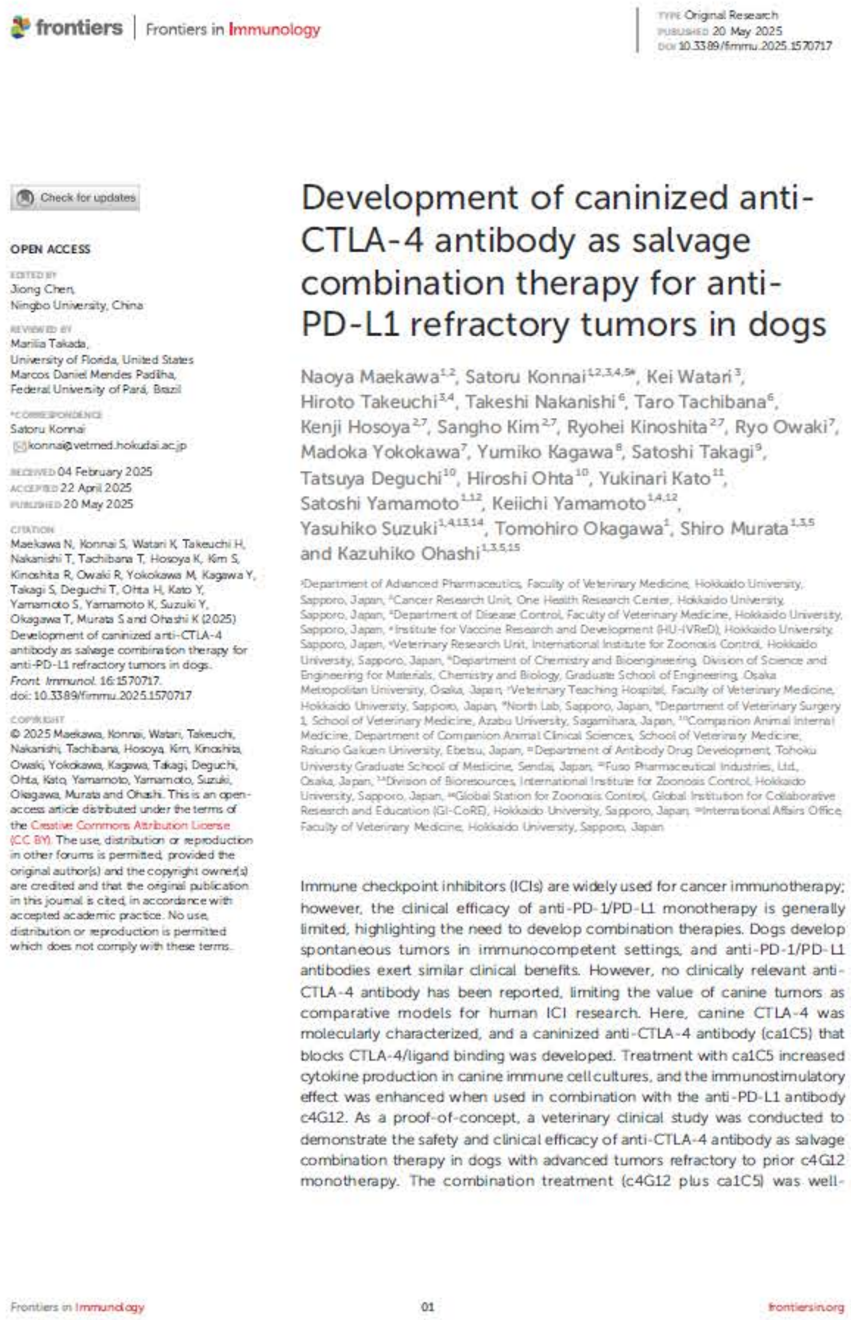

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Jiong Chen,
Ningbo University, China

REVIEWED BY
Marilia Takada,
University of Florida, United States
Marcos Daniel Mendes Padilha,
Federal University of Pará, Brazil

*CORRESPONDENCE
Satoru Konnai
✉ konnai@vetmed.hokudai.ac.jp

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Development of caninized anti-CTLA-4 antibody as salvage combination therapy for anti-PD-L1 refractory tumors in dogs

Naoya Maekawa[1,2], Satoru Konnai[1,2,3,4,5*], Kei Watari[3], Hiroto Takeuchi[3,4], Takeshi Nakanishi[6], Taro Tachibana[6], Kenji Hosoya[2,7], Sangho Kim[2,7], Ryohei Kinoshita[2,7], Ryo Owaki[7], Madoka Yokokawa[7], Yumiko Kagawa[8], Satoshi Takagi[9], Tatsuya Deguchi[10], Hiroshi Ohta[10], Yukinari Kato[11], Satoshi Yamamoto[1,12], Keiichi Yamamoto[1,4,12], Yasuhiko Suzuki[1,4,13,14], Tomohiro Okagawa[1], Shiro Murata[1,3,5] and Kazuhiko Ohashi[1,3,5,15]

[1]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [2]Cancer Research Unit, One Health Research Center, Hokkaido University, Sapporo, Japan, [3]Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [4]Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Sapporo, Japan, [5]Veterinary Research Unit, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, [6]Department of Chemistry and Bioengineering, Division of Science and Engineering for Materials, Chemistry and Biology, Graduate School of Engineering, Osaka Metropolitan University, Osaka, Japan, [7]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [8]North Lab, Sapporo, Japan, [9]Department of Veterinary Surgery 1, School of Veterinary Medicine, Azabu University, Sagamihara, Japan, [10]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, [11]Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan, [12]Fuso Pharmaceutical Industries, Ltd., Osaka, Japan, [13]Division of Bioresources, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, [14]Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo, Japan, [15]International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Immune checkpoint inhibitors (ICIs) are widely used for cancer immunotherapy; however, the clinical efficacy of anti-PD-1/PD-L1 monotherapy is generally limited, highlighting the need to develop combination therapies. Dogs develop spontaneous tumors in immunocompetent settings, and anti-PD-1/PD-L1 antibodies exert similar clinical benefits. However, no clinically relevant anti-CTLA-4 antibody has been reported, limiting the value of canine tumors as comparative models for human ICI research. Here, canine CTLA-4 was molecularly characterized, and a caninized anti-CTLA-4 antibody (ca1C5) that blocks CTLA-4/ligand binding was developed. Treatment with ca1C5 increased cytokine production in canine immune cell cultures, and the immunostimulatory effect was enhanced when used in combination with the anti-PD-L1 antibody c4G12. As a proof-of-concept, a veterinary clinical study was conducted to demonstrate the safety and clinical efficacy of anti-CTLA-4 antibody as salvage combination therapy in dogs with advanced tumors refractory to prior c4G12 monotherapy. The combination treatment (c4G12 plus ca1C5) was well-

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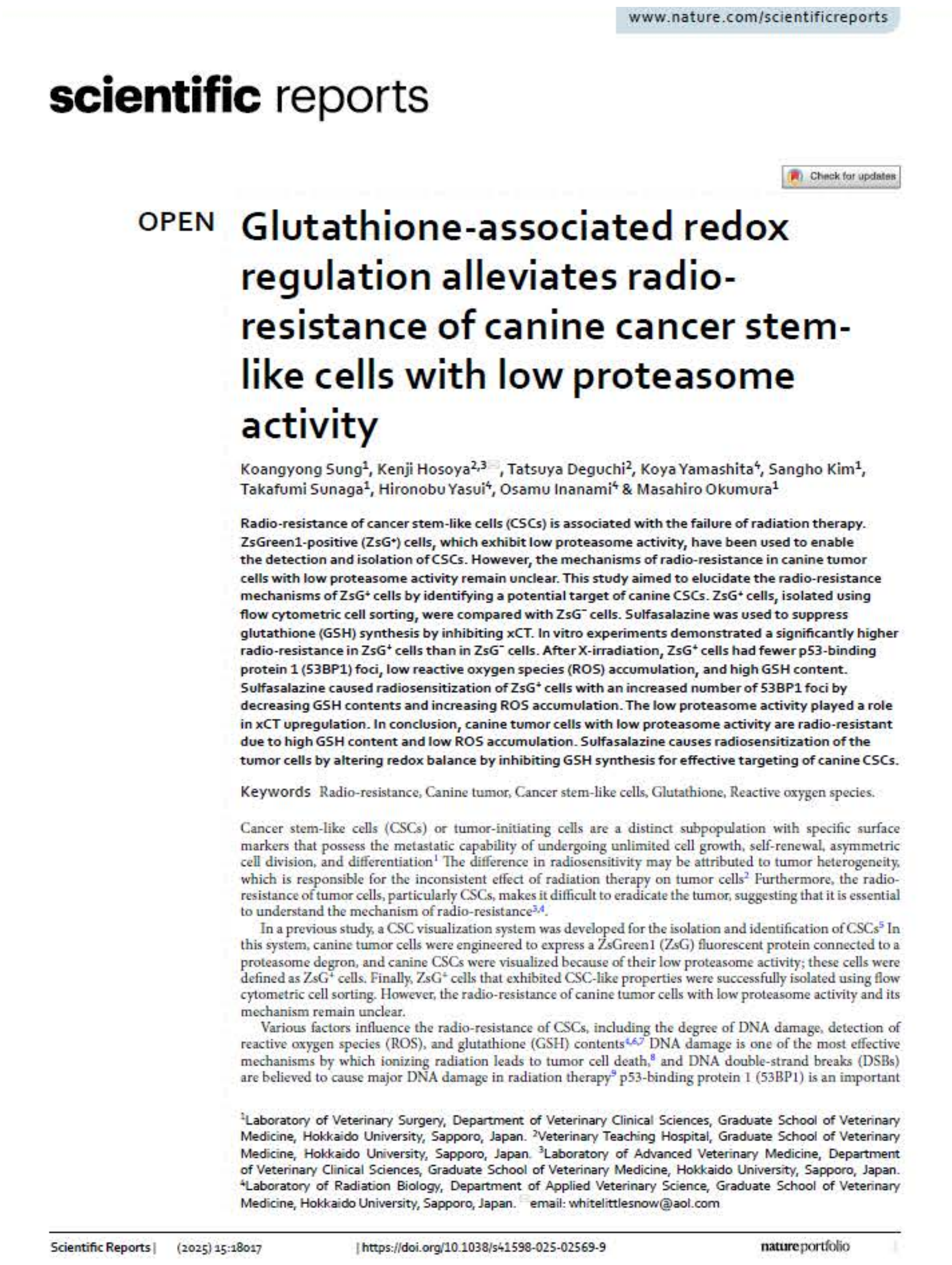

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Glutathione-associated redox regulation alleviates radio-resistance of canine cancer stem-like cells with low proteasome activity

Koangyong Sung[1], Kenji Hosoya[2,3], Tatsuya Deguchi[2], Koya Yamashita[4], Sangho Kim[1], Takafumi Sunaga[1], Hironobu Yasui[4], Osamu Inanami[4] & Masahiro Okumura[1]

Radio-resistance of cancer stem-like cells (CSCs) is associated with the failure of radiation therapy. ZsGreen1-positive (ZsG$^+$) cells, which exhibit low proteasome activity, have been used to enable the detection and isolation of CSCs. However, the mechanisms of radio-resistance in canine tumor cells with low proteasome activity remain unclear. This study aimed to elucidate the radio-resistance mechanisms of ZsG$^+$ cells by identifying a potential target of canine CSCs. ZsG$^+$ cells, isolated using flow cytometric cell sorting, were compared with ZsG$^-$ cells. Sulfasalazine was used to suppress glutathione (GSH) synthesis by inhibiting xCT. In vitro experiments demonstrated a significantly higher radio-resistance in ZsG$^+$ cells than in ZsG$^-$ cells. After X-irradiation, ZsG$^+$ cells had fewer p53-binding protein 1 (53BP1) foci, low reactive oxygen species (ROS) accumulation, and high GSH content. Sulfasalazine caused radiosensitization of ZsG$^+$ cells with an increased number of 53BP1 foci by decreasing GSH contents and increasing ROS accumulation. The low proteasome activity played a role in xCT upregulation. In conclusion, canine tumor cells with low proteasome activity are radio-resistant due to high GSH content and low ROS accumulation. Sulfasalazine causes radiosensitization of the tumor cells by altering redox balance by inhibiting GSH synthesis for effective targeting of canine CSCs.

Keywords Radio-resistance, Canine tumor, Cancer stem-like cells, Glutathione, Reactive oxygen species.

Cancer stem-like cells (CSCs) or tumor-initiating cells are a distinct subpopulation with specific surface markers that possess the metastatic capability of undergoing unlimited cell growth, self-renewal, asymmetric cell division, and differentiation[1] The difference in radiosensitivity may be attributed to tumor heterogeneity, which is responsible for the inconsistent effect of radiation therapy on tumor cells[2] Furthermore, the radio-resistance of tumor cells, particularly CSCs, makes it difficult to eradicate the tumor, suggesting that it is essential to understand the mechanism of radio-resistance[3,4].

In a previous study, a CSC visualization system was developed for the isolation and identification of CSCs[5] In this system, canine tumor cells were engineered to express a ZsGreen1 (ZsG) fluorescent protein connected to a proteasome degron, and canine CSCs were visualized because of their low proteasome activity; these cells were defined as ZsG$^+$ cells. Finally, ZsG$^+$ cells that exhibited CSC-like properties were successfully isolated using flow cytometric cell sorting. However, the radio-resistance of canine tumor cells with low proteasome activity and its mechanism remain unclear.

Various factors influence the radio-resistance of CSCs, including the degree of DNA damage, detection of reactive oxygen species (ROS), and glutathione (GSH) contents[4,6,7] DNA damage is one of the most effective mechanisms by which ionizing radiation leads to tumor cell death,[8] and DNA double-strand breaks (DSBs) are believed to cause major DNA damage in radiation therapy[9] p53-binding protein 1 (53BP1) is an important

[1]Laboratory of Veterinary Surgery, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. [2]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. [3]Laboratory of Advanced Veterinary Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. [4]Laboratory of Radiation Biology, Department of Applied Veterinary Science, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. email: whitelittlesnow@aol.com

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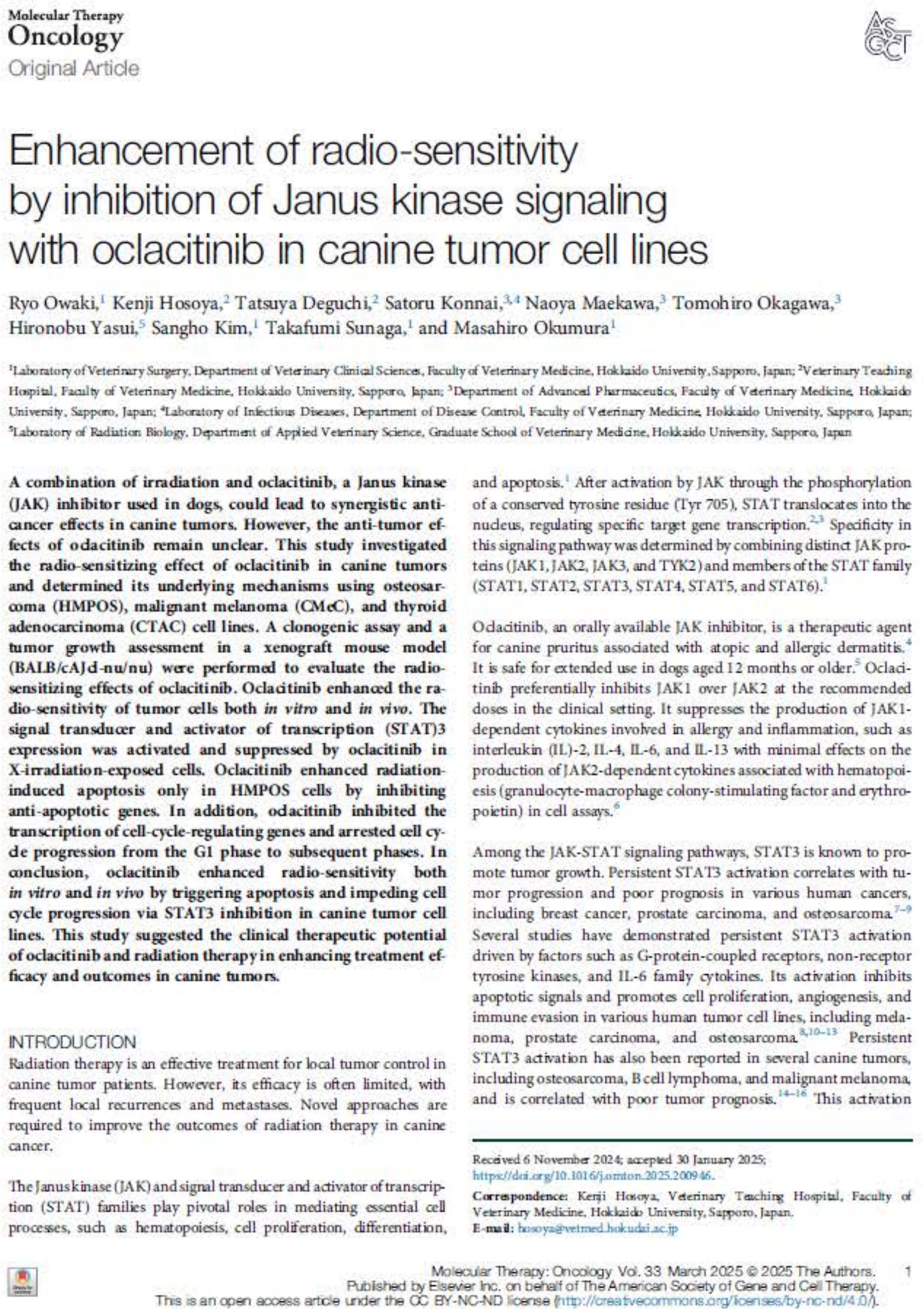

Molecular Therapy
Oncology
Original Article

Enhancement of radio-sensitivity by inhibition of Janus kinase signaling with oclacitinib in canine tumor cell lines

Ryo Owaki,[1] Kenji Hosoya,[2] Tatsuya Deguchi,[2] Satoru Konnai,[3,4] Naoya Maekawa,[3] Tomohiro Okagawa,[3] Hironobu Yasui,[5] Sangho Kim,[1] Takafumi Sunaga,[1] and Masahiro Okumura[1]

[1]Laboratory of Veterinary Surgery, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan; [2]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan; [3]Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan; [4]Laboratory of Infectious Diseases, Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan; [5]Laboratory of Radiation Biology, Department of Applied Veterinary Science, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

A combination of irradiation and oclacitinib, a Janus kinase (JAK) inhibitor used in dogs, could lead to synergistic anti-cancer effects in canine tumors. However, the anti-tumor effects of oclacitinib remain unclear. This study investigated the radio-sensitizing effect of oclacitinib in canine tumors and determined its underlying mechanisms using osteosarcoma (HMPOS), malignant melanoma (CMeC), and thyroid adenocarcinoma (CTAC) cell lines. A clonogenic assay and a tumor growth assessment in a xenograft mouse model (BALB/cAJcl-nu/nu) were performed to evaluate the radio-sensitizing effects of oclacitinib. Oclacitinib enhanced the radio-sensitivity of tumor cells both *in vitro* and *in vivo*. The signal transducer and activator of transcription (STAT)3 expression was activated and suppressed by oclacitinib in X-irradiation-exposed cells. Oclacitinib enhanced radiation-induced apoptosis only in HMPOS cells by inhibiting anti-apoptotic genes. In addition, oclacitinib inhibited the transcription of cell-cycle-regulating genes and arrested cell cycle progression from the G1 phase to subsequent phases. In conclusion, oclacitinib enhanced radio-sensitivity both *in vitro* and *in vivo* by triggering apoptosis and impeding cell cycle progression via STAT3 inhibition in canine tumor cell lines. This study suggested the clinical therapeutic potential of oclacitinib and radiation therapy in enhancing treatment efficacy and outcomes in canine tumors.

INTRODUCTION

Radiation therapy is an effective treatment for local tumor control in canine tumor patients. However, its efficacy is often limited, with frequent local recurrences and metastases. Novel approaches are required to improve the outcomes of radiation therapy in canine cancer.

The Janus kinase (JAK) and signal transducer and activator of transcription (STAT) families play pivotal roles in mediating essential cell processes, such as hematopoiesis, cell proliferation, differentiation, and apoptosis.[1] After activation by JAK through the phosphorylation of a conserved tyrosine residue (Tyr 705), STAT translocates into the nucleus, regulating specific target gene transcription.[2,3] Specificity in this signaling pathway was determined by combining distinct JAK proteins (JAK1, JAK2, JAK3, and TYK2) and members of the STAT family (STAT1, STAT2, STAT3, STAT4, STAT5, and STAT6).[1]

Oclacitinib, an orally available JAK inhibitor, is a therapeutic agent for canine pruritus associated with atopic and allergic dermatitis.[4] It is safe for extended use in dogs aged 12 months or older.[5] Oclacitinib preferentially inhibits JAK1 over JAK2 at the recommended doses in the clinical setting. It suppresses the production of JAK1-dependent cytokines involved in allergy and inflammation, such as interleukin (IL)-2, IL-4, IL-6, and IL-13 with minimal effects on the production of JAK2-dependent cytokines associated with hematopoiesis (granulocyte-macrophage colony-stimulating factor and erythropoietin) in cell assays.[6]

Among the JAK-STAT signaling pathways, STAT3 is known to promote tumor growth. Persistent STAT3 activation correlates with tumor progression and poor prognosis in various human cancers, including breast cancer, prostate carcinoma, and osteosarcoma.[7–9] Several studies have demonstrated persistent STAT3 activation driven by factors such as G-protein-coupled receptors, non-receptor tyrosine kinases, and IL-6 family cytokines. Its activation inhibits apoptotic signals and promotes cell proliferation, angiogenesis, and immune evasion in various human tumor cell lines, including melanoma, prostate carcinoma, and osteosarcoma.[8,10–13] Persistent STAT3 activation has also been reported in several canine tumors, including osteosarcoma, B cell lymphoma, and malignant melanoma, and is correlated with poor tumor prognosis.[14–16] This activation

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Correspondence: Kenji Hosoya, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan.
E-mail: hosoya@vetmed.hokudai.ac.jp

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獣医生化学（Veterinary Biochemistry）

Jumpei Fujiki

Lecturer

講師　藤木　純平

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1）Phage cocktails containing a dual-receptor phikzvirus suppress resistance evolution in *Pseudomonas aeruginosa*
Fujiki J, Tamamura K, Nakamura K, Nakamura N, Sakata Y, Kimura N, Ono S, Kojima N, Inaba-Hasegawa K, Sasaki M, Usui M, Iwasaki T, Ando H, Sawa H, Iwano H.
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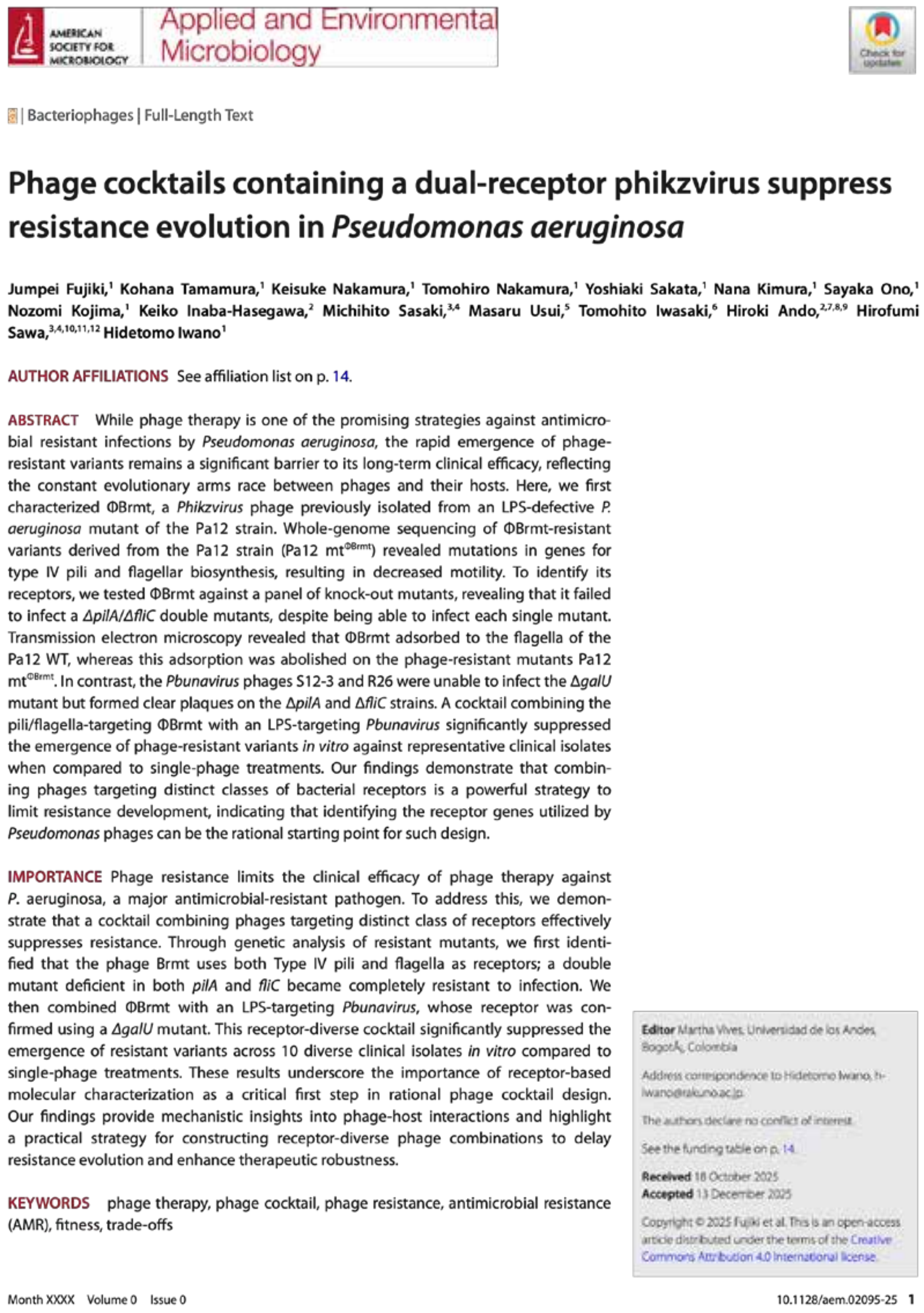

Applied and Environmental Microbiology

Bacteriophages | Full-Length Text

Phage cocktails containing a dual-receptor phikzvirus suppress resistance evolution in *Pseudomonas aeruginosa*

Jumpei Fujiki,[1] Kohana Tamamura,[1] Keisuke Nakamura,[1] Tomohiro Nakamura,[1] Yoshiaki Sakata,[1] Nana Kimura,[1] Sayaka Ono,[1] Nozomi Kojima,[1] Keiko Inaba-Hasegawa,[2] Michihito Sasaki,[3,4] Masaru Usui,[5] Tomohito Iwasaki,[6] Hiroki Ando,[2,7,8,9] Hirofumi Sawa,[3,4,10,11,12] Hidetomo Iwano[1]

AUTHOR AFFILIATIONS See affiliation list on p. 14.

ABSTRACT While phage therapy is one of the promising strategies against antimicrobial resistant infections by *Pseudomonas aeruginosa*, the rapid emergence of phage-resistant variants remains a significant barrier to its long-term clinical efficacy, reflecting the constant evolutionary arms race between phages and their hosts. Here, we first characterized ΦBrmt, a *Phikzvirus* phage previously isolated from an LPS-defective *P. aeruginosa* mutant of the Pa12 strain. Whole-genome sequencing of ΦBrmt-resistant variants derived from the Pa12 strain (Pa12 mt$^{\Phi\mathrm{Brmt}}$) revealed mutations in genes for type IV pili and flagellar biosynthesis, resulting in decreased motility. To identify its receptors, we tested ΦBrmt against a panel of knock-out mutants, revealing that it failed to infect a *ΔpilA*/*ΔfliC* double mutants, despite being able to infect each single mutant. Transmission electron microscopy revealed that ΦBrmt adsorbed to the flagella of the Pa12 WT, whereas this adsorption was abolished on the phage-resistant mutants Pa12 mt$^{\Phi\mathrm{Brmt}}$. In contrast, the *Pbunavirus* phages S12-3 and R26 were unable to infect the *ΔgalU* mutant but formed clear plaques on the *ΔpilA* and *ΔfliC* strains. A cocktail combining the pili/flagella-targeting ΦBrmt with an LPS-targeting *Pbunavirus* significantly suppressed the emergence of phage-resistant variants *in vitro* against representative clinical isolates when compared to single-phage treatments. Our findings demonstrate that combining phages targeting distinct classes of bacterial receptors is a powerful strategy to limit resistance development, indicating that identifying the receptor genes utilized by *Pseudomonas* phages can be the rational starting point for such design.

IMPORTANCE Phage resistance limits the clinical efficacy of phage therapy against *P.* aeruginosa, a major antimicrobial-resistant pathogen. To address this, we demonstrate that a cocktail combining phages targeting distinct class of receptors effectively suppresses resistance. Through genetic analysis of resistant mutants, we first identified that the phage Brmt uses both Type IV pili and flagella as receptors; a double mutant deficient in both *pilA* and *fliC* became completely resistant to infection. We then combined ΦBrmt with an LPS-targeting *Pbunavirus*, whose receptor was confirmed using a *ΔgalU* mutant. This receptor-diverse cocktail significantly suppressed the emergence of resistant variants across 10 diverse clinical isolates *in vitro* compared to single-phage treatments. These results underscore the importance of receptor-based molecular characterization as a critical first step in rational phage cocktail design. Our findings provide mechanistic insights into phage-host interactions and highlight a practical strategy for constructing receptor-diverse phage combinations to delay resistance evolution and enhance therapeutic robustness.

KEYWORDS phage therapy, phage cocktail, phage resistance, antimicrobial resistance (AMR), fitness, trade-offs

Editor Martha Vives, Universidad de los Andes, BogotÅ, Colombia

Address correspondence to Hidetomo Iwano, h-iwano@rakuno.ac.jp.

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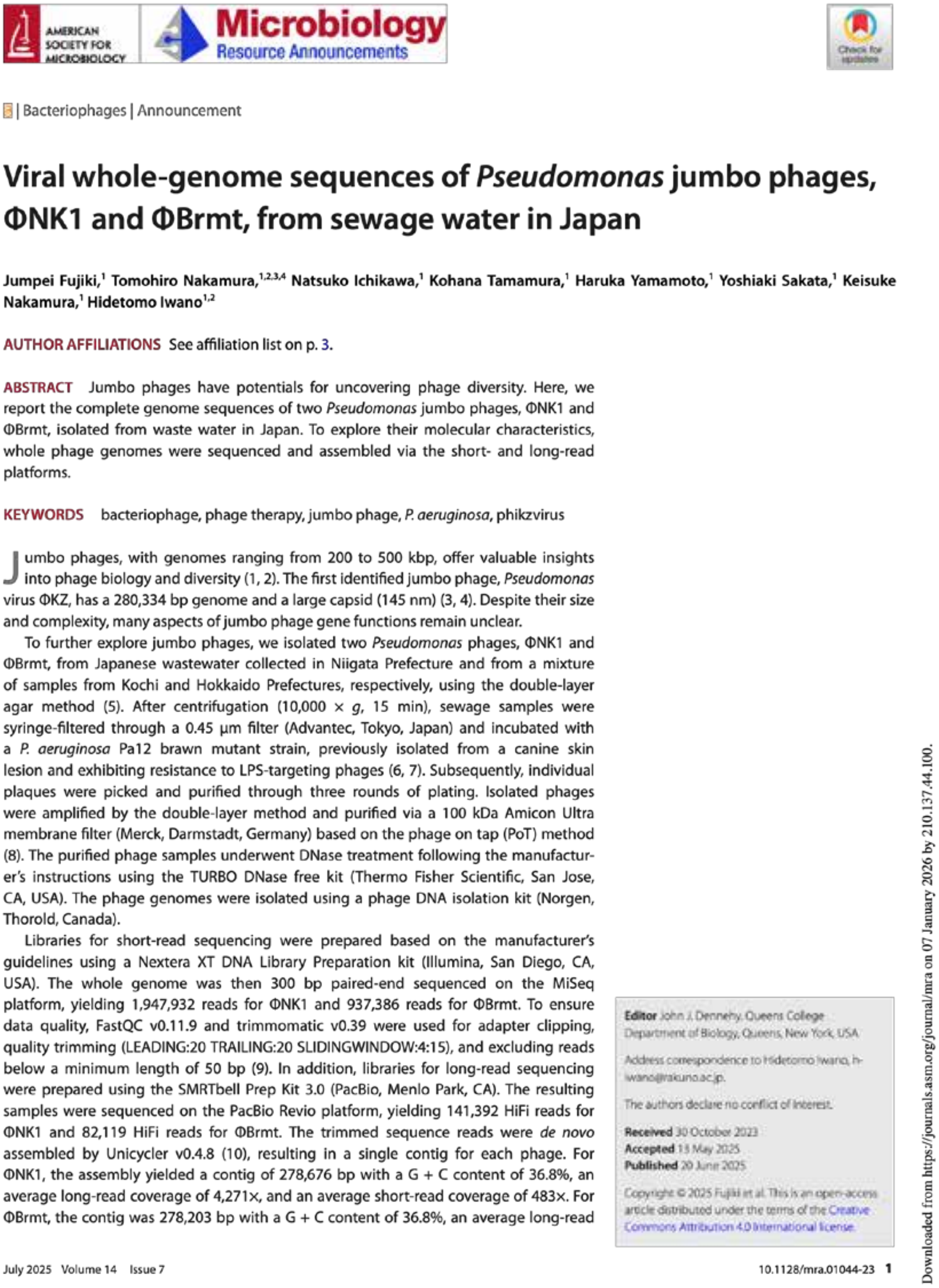
最終責任者　Hidetomo Iwano (Corresponding Author)



AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Bacteriophages | Announcement

Viral whole-genome sequences of *Pseudomonas* jumbo phages, ΦNK1 and ΦBrmt, from sewage water in Japan

Jumpei Fujiki,[1] **Tomohiro Nakamura**,[1,2,3,4] **Natsuko Ichikawa**,[1] **Kohana Tamamura**,[1] **Haruka Yamamoto**,[1] **Yoshiaki Sakata**,[1] **Keisuke Nakamura**,[1] **Hidetomo Iwano**[1,2]

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Jumbo phages have potentials for uncovering phage diversity. Here, we report the complete genome sequences of two *Pseudomonas* jumbo phages, ΦNK1 and ΦBrmt, isolated from waste water in Japan. To explore their molecular characteristics, whole phage genomes were sequenced and assembled via the short- and long-read platforms.

KEYWORDS bacteriophage, phage therapy, jumbo phage, *P. aeruginosa*, phikzvirus

Jumbo phages, with genomes ranging from 200 to 500 kbp, offer valuable insights into phage biology and diversity (1, 2). The first identified jumbo phage, *Pseudomonas* virus ΦKZ, has a 280,334 bp genome and a large capsid (145 nm) (3, 4). Despite their size and complexity, many aspects of jumbo phage gene functions remain unclear.

To further explore jumbo phages, we isolated two *Pseudomonas* phages, ΦNK1 and ΦBrmt, from Japanese wastewater collected in Niigata Prefecture and from a mixture of samples from Kochi and Hokkaido Prefectures, respectively, using the double-layer agar method (5). After centrifugation (10,000 × *g*, 15 min), sewage samples were syringe-filtered through a 0.45 µm filter (Advantec, Tokyo, Japan) and incubated with a *P. aeruginosa* Pa12 brawn mutant strain, previously isolated from a canine skin lesion and exhibiting resistance to LPS-targeting phages (6, 7). Subsequently, individual plaques were picked and purified through three rounds of plating. Isolated phages were amplified by the double-layer method and purified via a 100 kDa Amicon Ultra membrane filter (Merck, Darmstadt, Germany) based on the phage on tap (PoT) method (8). The purified phage samples underwent DNase treatment following the manufacturer's instructions using the TURBO DNase free kit (Thermo Fisher Scientific, San Jose, CA, USA). The phage genomes were isolated using a phage DNA isolation kit (Norgen, Thorold, Canada).

Libraries for short-read sequencing were prepared based on the manufacturer's guidelines using a Nextera XT DNA Library Preparation kit (Illumina, San Diego, CA, USA). The whole genome was then 300 bp paired-end sequenced on the MiSeq platform, yielding 1,947,932 reads for ΦNK1 and 937,386 reads for ΦBrmt. To ensure data quality, FastQC v0.11.9 and trimmomatic v0.39 were used for adapter clipping, quality trimming (LEADING:20 TRAILING:20 SLIDINGWINDOW:4:15), and excluding reads below a minimum length of 50 bp (9). In addition, libraries for long-read sequencing were prepared using the SMRTbell Prep Kit 3.0 (PacBio, Menlo Park, CA). The resulting samples were sequenced on the PacBio Revio platform, yielding 141,392 HiFi reads for ΦNK1 and 82,119 HiFi reads for ΦBrmt. The trimmed sequence reads were *de novo* assembled by Unicycler v0.4.8 (10), resulting in a single contig for each phage. For ΦNK1, the assembly yielded a contig of 278,676 bp with a G + C content of 36.8%, an average long-read coverage of 4,271×, and an average short-read coverage of 483×. For ΦBrmt, the contig was 278,203 bp with a G + C content of 36.8%, an average long-read

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Address correspondence to Hidetomo Iwano, h-iwano@rakuno.ac.jp.

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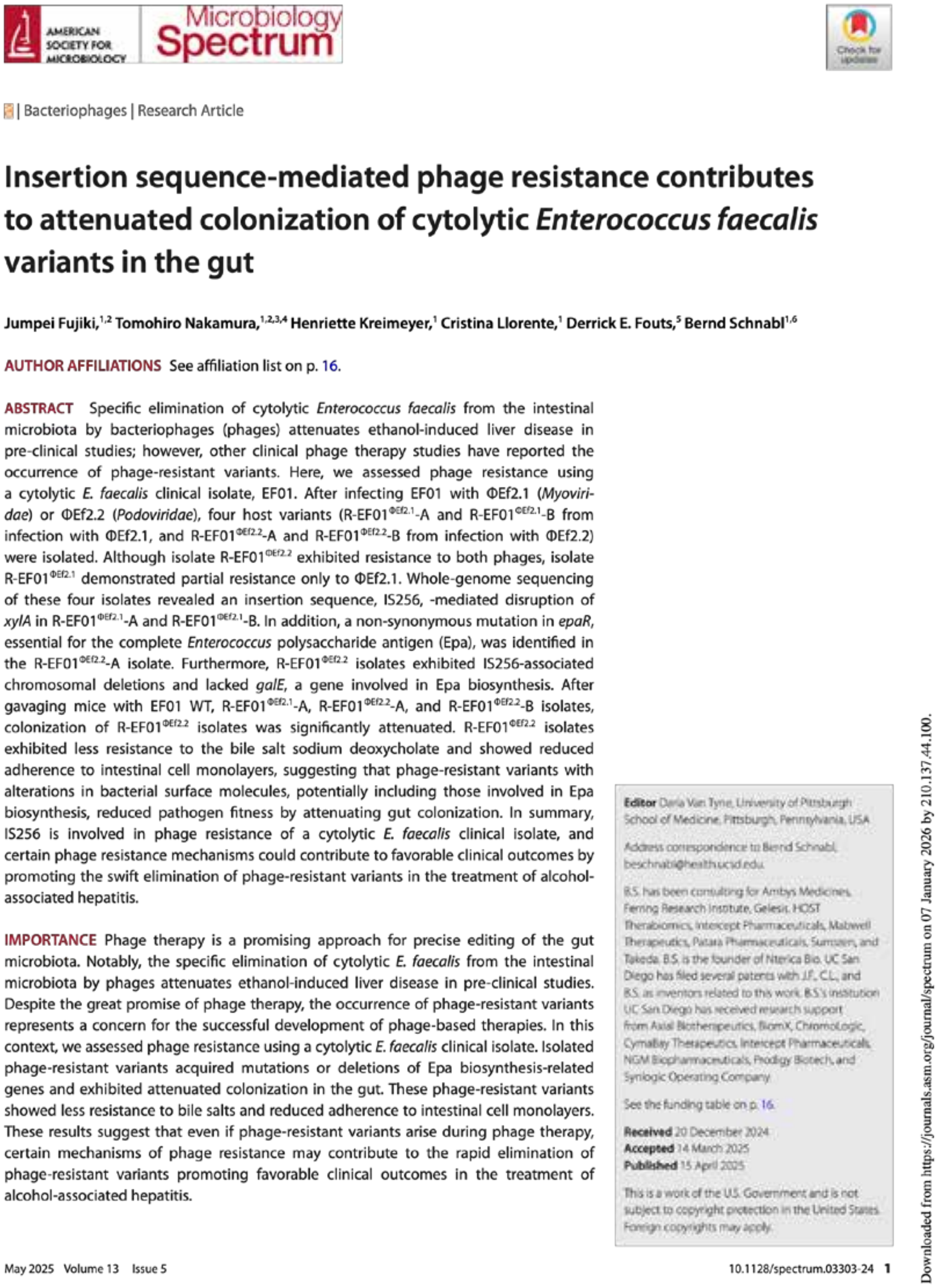
最終責任者　Hidetomo Iwano (Corresponding Author)



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| Bacteriophages | Research Article

Insertion sequence-mediated phage resistance contributes to attenuated colonization of cytolytic *Enterococcus faecalis* variants in the gut

Jumpei Fujiki,[1,2] **Tomohiro Nakamura,**[1,2,3,4] **Henriette Kreimeyer,**[1] **Cristina Llorente,**[1] **Derrick E. Fouts,**[5] **Bernd Schnabl**[1,6]

AUTHOR AFFILIATIONS See affiliation list on p. 16.

ABSTRACT Specific elimination of cytolytic *Enterococcus faecalis* from the intestinal microbiota by bacteriophages (phages) attenuates ethanol-induced liver disease in pre-clinical studies; however, other clinical phage therapy studies have reported the occurrence of phage-resistant variants. Here, we assessed phage resistance using a cytolytic *E. faecalis* clinical isolate, EF01. After infecting EF01 with ΦEf2.1 (*Myoviridae*) or ΦEf2.2 (*Podoviridae*), four host variants (R-EF01$^{\Phi Ef2.1}$-A and R-EF01$^{\Phi Ef2.1}$-B from infection with ΦEf2.1, and R-EF01$^{\Phi Ef2.2}$-A and R-EF01$^{\Phi Ef2.2}$-B from infection with ΦEf2.2) were isolated. Although isolate R-EF01$^{\Phi Ef2.2}$ exhibited resistance to both phages, isolate R-EF01$^{\Phi Ef2.1}$ demonstrated partial resistance only to ΦEf2.1. Whole-genome sequencing of these four isolates revealed an insertion sequence, IS256, -mediated disruption of *xylA* in R-EF01$^{\Phi Ef2.1}$-A and R-EF01$^{\Phi Ef2.1}$-B. In addition, a non-synonymous mutation in *epaR*, essential for the complete *Enterococcus* polysaccharide antigen (Epa), was identified in the R-EF01$^{\Phi Ef2.2}$-A isolate. Furthermore, R-EF01$^{\Phi Ef2.2}$ isolates exhibited IS256-associated chromosomal deletions and lacked *galE*, a gene involved in Epa biosynthesis. After gavaging mice with EF01 WT, R-EF01$^{\Phi Ef2.1}$-A, R-EF01$^{\Phi Ef2.2}$-A, and R-EF01$^{\Phi Ef2.2}$-B isolates, colonization of R-EF01$^{\Phi Ef2.2}$ isolates was significantly attenuated. R-EF01$^{\Phi Ef2.2}$ isolates exhibited less resistance to the bile salt sodium deoxycholate and showed reduced adherence to intestinal cell monolayers, suggesting that phage-resistant variants with alterations in bacterial surface molecules, potentially including those involved in Epa biosynthesis, reduced pathogen fitness by attenuating gut colonization. In summary, IS256 is involved in phage resistance of a cytolytic *E. faecalis* clinical isolate, and certain phage resistance mechanisms could contribute to favorable clinical outcomes by promoting the swift elimination of phage-resistant variants in the treatment of alcohol-associated hepatitis.

IMPORTANCE Phage therapy is a promising approach for precise editing of the gut microbiota. Notably, the specific elimination of cytolytic *E. faecalis* from the intestinal microbiota by phages attenuates ethanol-induced liver disease in pre-clinical studies. Despite the great promise of phage therapy, the occurrence of phage-resistant variants represents a concern for the successful development of phage-based therapies. In this context, we assessed phage resistance using a cytolytic *E. faecalis* clinical isolate. Isolated phage-resistant variants acquired mutations or deletions of Epa biosynthesis-related genes and exhibited attenuated colonization in the gut. These phage-resistant variants showed less resistance to bile salts and reduced adherence to intestinal cell monolayers. These results suggest that even if phage-resistant variants arise during phage therapy, certain mechanisms of phage resistance may contribute to the rapid elimination of phage-resistant variants promoting favorable clinical outcomes in the treatment of alcohol-associated hepatitis.

Editor Daria Van Tyne, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Address correspondence to Bernd Schnabl, beschnabl@health.ucsd.edu.

B.S. has been consulting for Ambys Medicines, Ferring Research Institute, Gelesis, HOST Therabiomics, Intercept Pharmaceuticals, Mabwell Therapeutics, Patara Pharmaceuticals, Surrozen, and Takeda. B.S. is the founder of Nterica Bio. UC San Diego has filed several patents with J.F., C.L., and B.S. as inventors related to this work. B.S.'s institution UC San Diego has received research support from Axial Biotherapeutics, BiomX, ChromoLogic, CymaBay Therapeutics, Intercept Pharmaceuticals, NGM Biopharmaceuticals, Prodigy Biotech, and Synlogic Operating Company.

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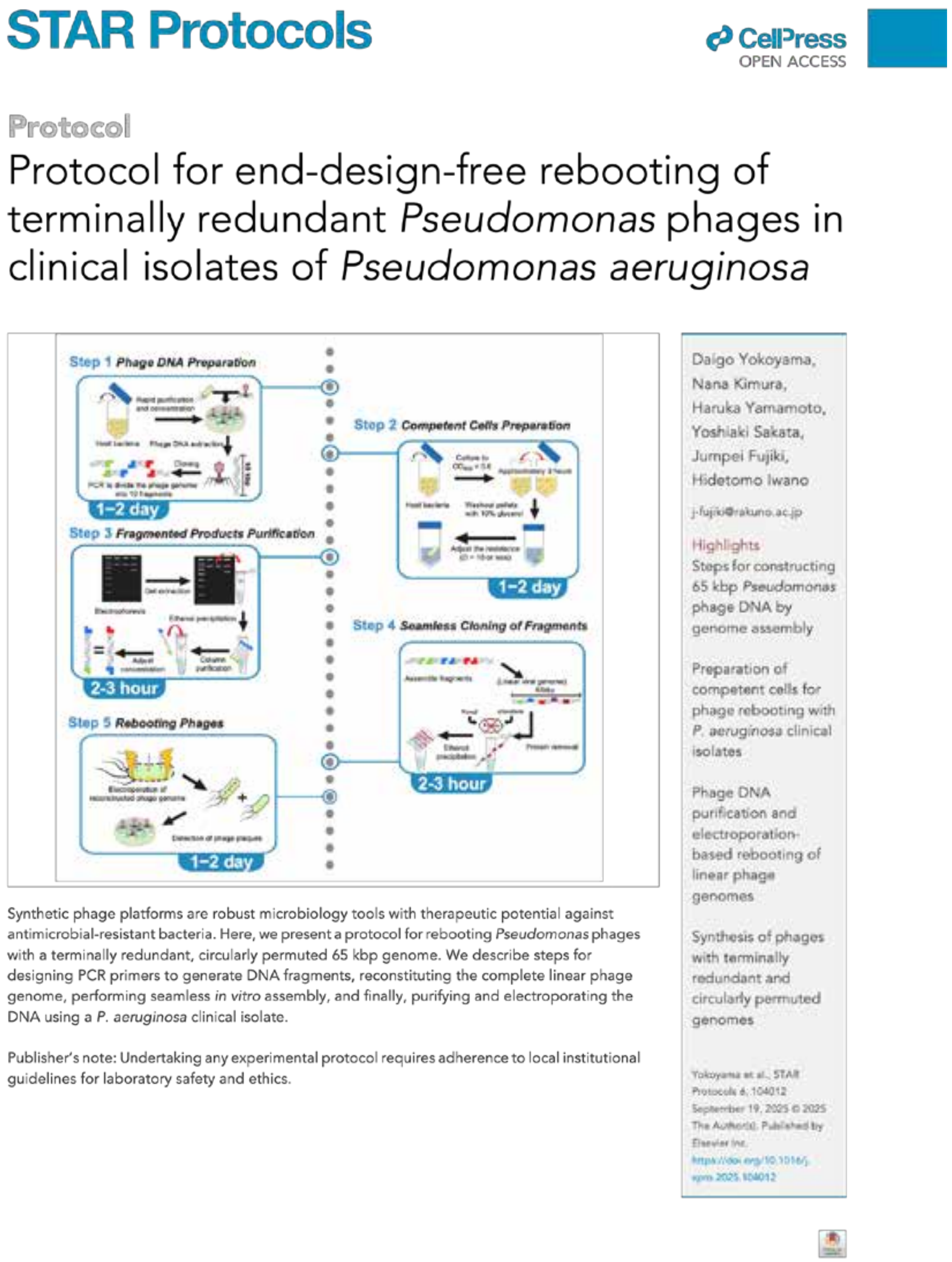
筆頭著者 Jumpei Fujiki

最終責任者 Benrd Schnabl (Corresponding Author)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Protocol

Protocol for end-design-free rebooting of terminally redundant *Pseudomonas* phages in clinical isolates of *Pseudomonas aeruginosa*

Synthetic phage platforms are robust microbiology tools with therapeutic potential against antimicrobial-resistant bacteria. Here, we present a protocol for rebooting *Pseudomonas* phages with a terminally redundant, circularly permuted 65 kbp genome. We describe steps for designing PCR primers to generate DNA fragments, reconstituting the complete linear phage genome, performing seamless *in vitro* assembly, and finally, purifying and electroporating the DNA using a *P. aeruginosa* clinical isolate.

Daigo Yokoyama,
Nana Kimura,
Haruka Yamamoto,
Yoshiaki Sakata,
Jumpei Fujiki,
Hidetomo Iwano

j-fujiki@rakuno.ac.jp

Highlights

Steps for constructing 65 kbp *Pseudomonas* phage DNA by genome assembly

Preparation of competent cells for phage rebooting with *P. aeruginosa* clinical isolates

Phage DNA purification and electroporation-based rebooting of linear phage genomes

Synthesis of phages with terminally redundant and circularly permuted genomes

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最終責任者　Jumpei Fujiki (Corresponding Author)

viruses

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Review

Biocontrol of Phage Resistance in *Pseudomonas* Infections: Insights into Directed Breaking of Spontaneous Evolutionary Selection in Phage Therapy

Jumpei Fujiki [1,*], Daigo Yokoyama [1], Haruka Yamamoto [1], Nana Kimura [1], Manaho Shimizu [1], Hinatsu Kobayashi [1], Keisuke Nakamura [1] and Hidetomo Iwano [1,2]

[1] Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; h-iwano@rakuno.ac.jp (H.I.)
[2] Phage Therapy Institute, Comprehensive Research Organization, Waseda University, Tokyo 162-8480, Japan
* Correspondence: j-fujiki@rakuno.ac.jp; Tel./Fax: +81-11-388-4806

Abstract

Phage therapy, long overshadowed by antibiotics in Western medicine, has a well-established history in some Eastern European countries and is now being revitalized as a promising strategy against antimicrobial resistance (AMR). This resurgence of phage therapy is driven by the urgent need for innovative countermeasures to AMR, which will cause an estimated 10 million deaths annually by 2050. However, the emergence of phage-resistant variants presents challenges similar to AMR, thus necessitating a deeper understanding of phage resistance mechanisms and control strategies. The highest priority must be to prevent the emergence of phage resistance. Although phage cocktails targeting multiple receptors have demonstrated a certain level of phage resistance suppression, they cannot completely suppress resistance in clinical settings. This highlights the need for strategies beyond simple resistance suppression. Notably, recent studies examining fitness trade-offs associated with phage resistance have opened new avenues in phage therapy that offer the potential of restoring antibiotic susceptibility and attenuating pathogen virulence despite phage resistance. Thus, controlling phage resistance may rely on both its suppression and strategic redirection. This review summarizes key concepts in the control of phage resistance and explores evolutionary engineering as a means of optimizing phage therapy, with a particular focus on *Pseudomonas* infections. Harnessing evolutionary dynamics by intentionally breaking the spontaneous evolutionary trajectories of target bacterial pathogens could potentially reshape bacterial adaptation by acquisition of phage resistance, unlocking potential in the application of phage therapy.

Keywords: bacteriophage; evolved phages; infection control; fitness cost; trade-offs; antimicrobial resistance

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1. Bacteriophages, Phage Therapy, and Phage-Resistant Variants

1.1. Bacteriophages

Bacteriophages (phages) are prokaryotic viruses that specifically infect and lyse bacteria. Phages are the most abundant biological entities on Earth, with an estimated total exceeding 10^{31} particles [1]. Ubiquitously distributed in a variety of environments, phages play a significant role in shaping bacterial community dynamics [2,3]. The basic structure of phages consists of a capsid that encloses genetic material and an associated tail. The tail is particularly crucial for host recognition, as it binds specifically to bacterial surface receptors

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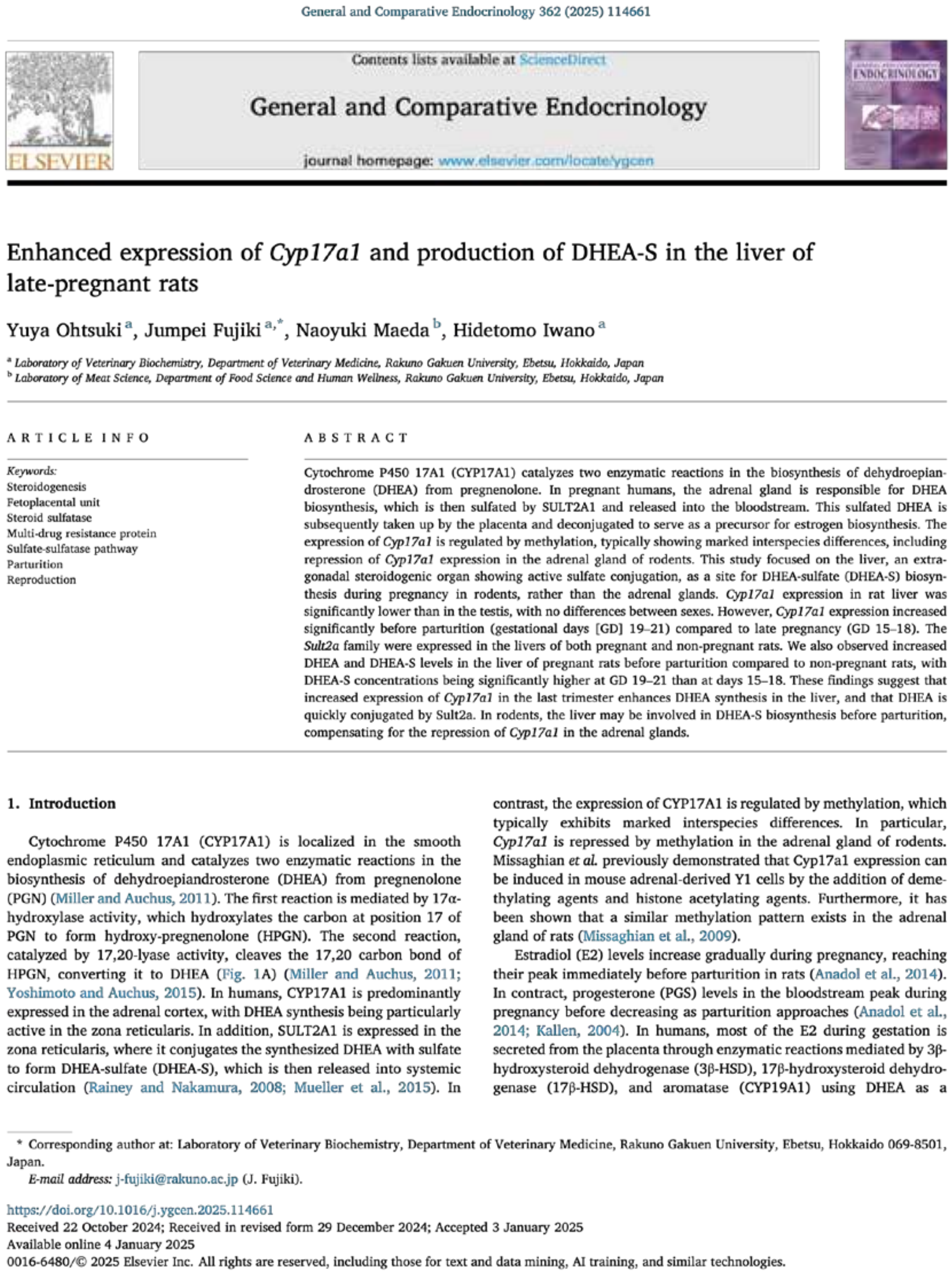

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Enhanced expression of *Cyp17a1* and production of DHEA-S in the liver of late-pregnant rats

Yuya Ohtsuki[a], Jumpei Fujiki[a,*], Naoyuki Maeda[b], Hidetomo Iwano[a]

[a] *Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*
[b] *Laboratory of Meat Science, Department of Food Science and Human Wellness, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*

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ABSTRACT

Cytochrome P450 17A1 (CYP17A1) catalyzes two enzymatic reactions in the biosynthesis of dehydroepiandrosterone (DHEA) from pregnenolone. In pregnant humans, the adrenal gland is responsible for DHEA biosynthesis, which is then sulfated by SULT2A1 and released into the bloodstream. This sulfated DHEA is subsequently taken up by the placenta and deconjugated to serve as a precursor for estrogen biosynthesis. The expression of *Cyp17a1* is regulated by methylation, typically showing marked interspecies differences, including repression of *Cyp17a1* expression in the adrenal gland of rodents. This study focused on the liver, an extra-gonadal steroidogenic organ showing active sulfate conjugation, as a site for DHEA-sulfate (DHEA-S) biosynthesis during pregnancy in rodents, rather than the adrenal glands. *Cyp17a1* expression in rat liver was significantly lower than in the testis, with no differences between sexes. However, *Cyp17a1* expression increased significantly before parturition (gestational days [GD] 19–21) compared to late pregnancy (GD 15–18). The *Sult2a* family were expressed in the livers of both pregnant and non-pregnant rats. We also observed increased DHEA and DHEA-S levels in the liver of pregnant rats before parturition compared to non-pregnant rats, with DHEA-S concentrations being significantly higher at GD 19–21 than at days 15–18. These findings suggest that increased expression of *Cyp17a1* in the last trimester enhances DHEA synthesis in the liver, and that DHEA is quickly conjugated by Sult2a. In rodents, the liver may be involved in DHEA-S biosynthesis before parturition, compensating for the repression of *Cyp17a1* in the adrenal glands.

1. Introduction

Cytochrome P450 17A1 (CYP17A1) is localized in the smooth endoplasmic reticulum and catalyzes two enzymatic reactions in the biosynthesis of dehydroepiandrosterone (DHEA) from pregnenolone (PGN) (Miller and Auchus, 2011). The first reaction is mediated by 17α-hydroxylase activity, which hydroxylates the carbon at position 17 of PGN to form hydroxy-pregnenolone (HPGN). The second reaction, catalyzed by 17,20-lyase activity, cleaves the 17,20 carbon bond of HPGN, converting it to DHEA (Fig. 1A) (Miller and Auchus, 2011; Yoshimoto and Auchus, 2015). In humans, CYP17A1 is predominantly expressed in the adrenal cortex, with DHEA synthesis being particularly active in the zona reticularis. In addition, SULT2A1 is expressed in the zona reticularis, where it conjugates the synthesized DHEA with sulfate to form DHEA-sulfate (DHEA-S), which is then released into systemic circulation (Rainey and Nakamura, 2008; Mueller et al., 2015). In contrast, the expression of CYP17A1 is regulated by methylation, which typically exhibits marked interspecies differences. In particular, *Cyp17a1* is repressed by methylation in the adrenal gland of rodents. Missaghian *et al.* previously demonstrated that Cyp17a1 expression can be induced in mouse adrenal-derived Y1 cells by the addition of demethylating agents and histone acetylating agents. Furthermore, it has been shown that a similar methylation pattern exists in the adrenal gland of rats (Missaghian et al., 2009).

Estradiol (E2) levels increase gradually during pregnancy, reaching their peak immediately before parturition in rats (Anadol et al., 2014). In contract, progesterone (PGS) levels in the bloodstream peak during pregnancy before decreasing as parturition approaches (Anadol et al., 2014; Kallen, 2004). In humans, most of the E2 during gestation is secreted from the placenta through enzymatic reactions mediated by 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), and aromatase (CYP19A1) using DHEA as a

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* Corresponding author at: Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan.
E-mail address: j-fujiki@rakuno.ac.jp (J. Fujiki).

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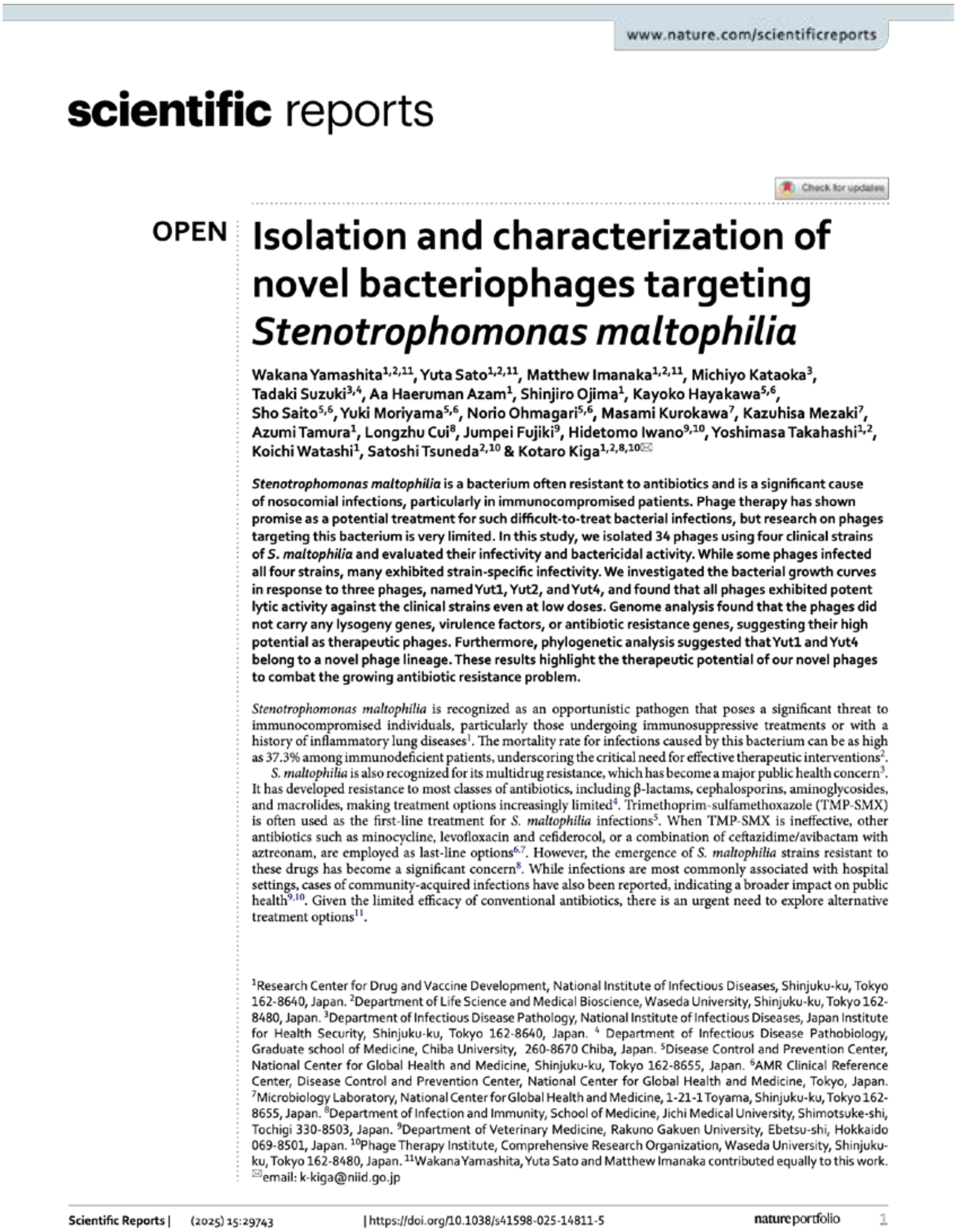

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Isolation and characterization of novel bacteriophages targeting *Stenotrophomonas maltophilia*

Wakana Yamashita[1,2,11], Yuta Sato[1,2,11], Matthew Imanaka[1,2,11], Michiyo Kataoka[3], Tadaki Suzuki[3,4], Aa Haeruman Azam[1], Shinjiro Ojima[1], Kayoko Hayakawa[5,6], Sho Saito[5,6], Yuki Moriyama[5,6], Norio Ohmagari[5,6], Masami Kurokawa[7], Kazuhisa Mezaki[7], Azumi Tamura[1], Longzhu Cui[8], Jumpei Fujiki[9], Hidetomo Iwano[9,10], Yoshimasa Takahashi[1,2], Koichi Watashi[1], Satoshi Tsuneda[2,10] & Kotaro Kiga[1,2,8,10]✉

***Stenotrophomonas maltophilia* is a bacterium often resistant to antibiotics and is a significant cause of nosocomial infections, particularly in immunocompromised patients. Phage therapy has shown promise as a potential treatment for such difficult-to-treat bacterial infections, but research on phages targeting this bacterium is very limited. In this study, we isolated 34 phages using four clinical strains of *S. maltophilia* and evaluated their infectivity and bactericidal activity. While some phages infected all four strains, many exhibited strain-specific infectivity. We investigated the bacterial growth curves in response to three phages, named Yut1, Yut2, and Yut4, and found that all phages exhibited potent lytic activity against the clinical strains even at low doses. Genome analysis found that the phages did not carry any lysogeny genes, virulence factors, or antibiotic resistance genes, suggesting their high potential as therapeutic phages. Furthermore, phylogenetic analysis suggested that Yut1 and Yut4 belong to a novel phage lineage. These results highlight the therapeutic potential of our novel phages to combat the growing antibiotic resistance problem.**

Stenotrophomonas maltophilia is recognized as an opportunistic pathogen that poses a significant threat to immunocompromised individuals, particularly those undergoing immunosuppressive treatments or with a history of inflammatory lung diseases[1]. The mortality rate for infections caused by this bacterium can be as high as 37.3% among immunodeficient patients, underscoring the critical need for effective therapeutic interventions[2].

S. maltophilia is also recognized for its multidrug resistance, which has become a major public health concern[3]. It has developed resistance to most classes of antibiotics, including β-lactams, cephalosporins, aminoglycosides, and macrolides, making treatment options increasingly limited[4]. Trimethoprim-sulfamethoxazole (TMP-SMX) is often used as the first-line treatment for *S. maltophilia* infections[5]. When TMP-SMX is ineffective, other antibiotics such as minocycline, levofloxacin and cefiderocol, or a combination of ceftazidime/avibactam with aztreonam, are employed as last-line options[6,7]. However, the emergence of *S. maltophilia* strains resistant to these drugs has become a significant concern[8]. While infections are most commonly associated with hospital settings, cases of community-acquired infections have also been reported, indicating a broader impact on public health[9,10]. Given the limited efficacy of conventional antibiotics, there is an urgent need to explore alternative treatment options[11].

[1]Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640, Japan. [2]Department of Life Science and Medical Bioscience, Waseda University, Shinjuku-ku, Tokyo 162-8480, Japan. [3]Department of Infectious Disease Pathology, National Institute of Infectious Diseases, Japan Institute for Health Security, Shinjuku-ku, Tokyo 162-8640, Japan. [4] Department of Infectious Disease Pathobiology, Graduate school of Medicine, Chiba University, 260-8670 Chiba, Japan. [5]Disease Control and Prevention Center, National Center for Global Health and Medicine, Shinjuku-ku, Tokyo 162-8655, Japan. [6]AMR Clinical Reference Center, Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan. [7]Microbiology Laboratory, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. [8]Department of Infection and Immunity, School of Medicine, Jichi Medical University, Shimotsuke-shi, Tochigi 330-8503, Japan. [9]Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu-shi, Hokkaido 069-8501, Japan. [10]Phage Therapy Institute, Comprehensive Research Organization, Waseda University, Shinjuku-ku, Tokyo 162-8480, Japan. [11]Wakana Yamashita, Yuta Sato and Matthew Imanaka contributed equally to this work. ✉email: k-kiga@niid.go.jp

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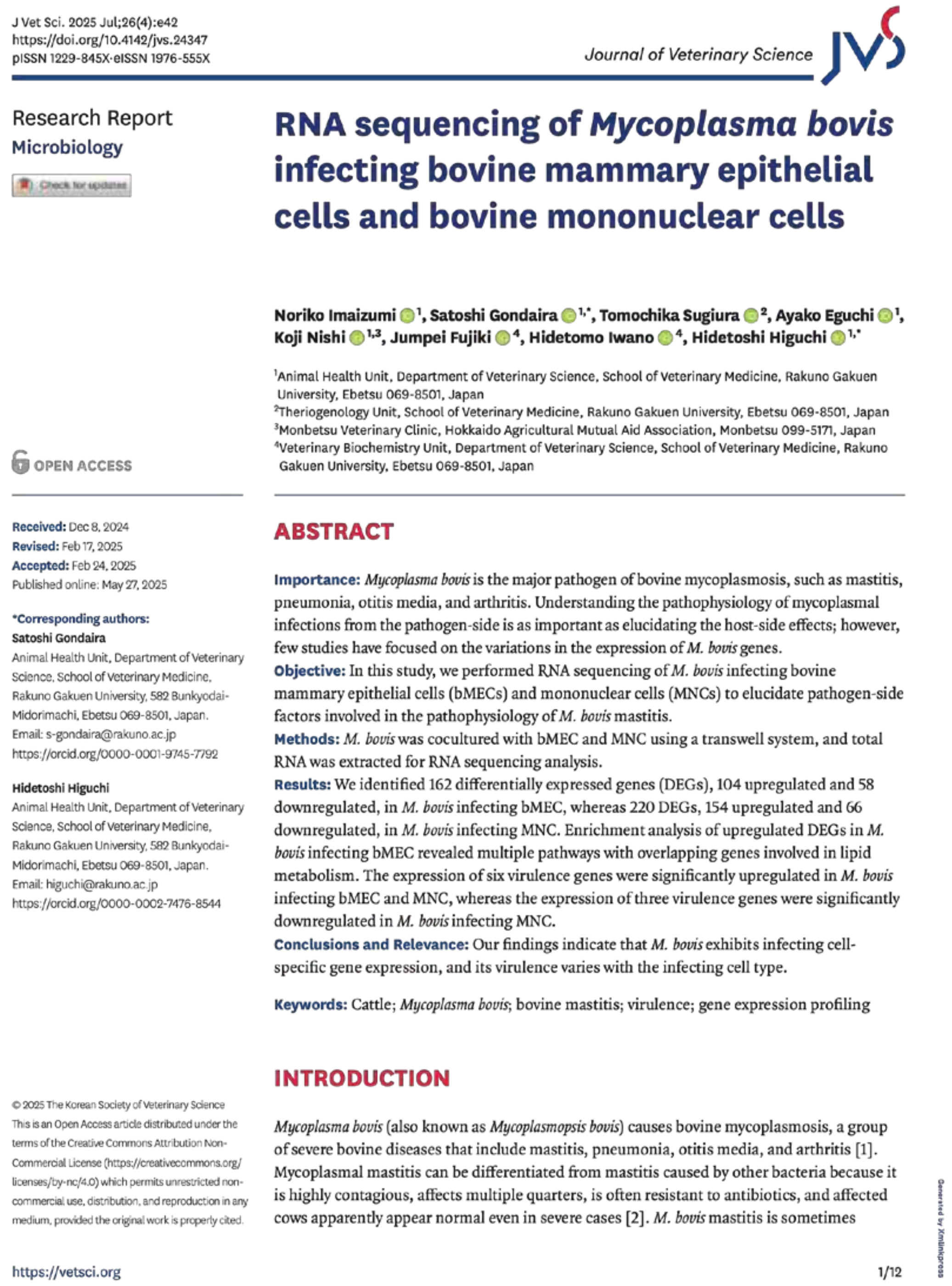

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Journal of Veterinary Science

Research Report
Microbiology

RNA sequencing of *Mycoplasma bovis* infecting bovine mammary epithelial cells and bovine mononuclear cells

Noriko Imaizumi [1], **Satoshi Gondaira** [1,*], **Tomochika Sugiura** [2], **Ayako Eguchi** [1], **Koji Nishi** [1,3], **Jumpei Fujiki** [4], **Hidetomo Iwano** [4], **Hidetoshi Higuchi** [1,*]

[1] Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[2] Theriogenology Unit, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan
[3] Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu 099-5171, Japan
[4] Veterinary Biochemistry Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan

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***Corresponding authors:**
Satoshi Gondaira
Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Japan.
Email: s-gondaira@rakuno.ac.jp
https://orcid.org/0000-0001-9745-7792

Hidetoshi Higuchi
Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu 069-8501, Japan.
Email: higuchi@rakuno.ac.jp
https://orcid.org/0000-0002-7476-8544

ABSTRACT

Importance: *Mycoplasma bovis* is the major pathogen of bovine mycoplasmosis, such as mastitis, pneumonia, otitis media, and arthritis. Understanding the pathophysiology of mycoplasmal infections from the pathogen-side is as important as elucidating the host-side effects; however, few studies have focused on the variations in the expression of *M. bovis* genes.
Objective: In this study, we performed RNA sequencing of *M. bovis* infecting bovine mammary epithelial cells (bMECs) and mononuclear cells (MNCs) to elucidate pathogen-side factors involved in the pathophysiology of *M. bovis* mastitis.
Methods: *M. bovis* was cocultured with bMEC and MNC using a transwell system, and total RNA was extracted for RNA sequencing analysis.
Results: We identified 162 differentially expressed genes (DEGs), 104 upregulated and 58 downregulated, in *M. bovis* infecting bMEC, whereas 220 DEGs, 154 upregulated and 66 downregulated, in *M. bovis* infecting MNC. Enrichment analysis of upregulated DEGs in *M. bovis* infecting bMEC revealed multiple pathways with overlapping genes involved in lipid metabolism. The expression of six virulence genes were significantly upregulated in *M. bovis* infecting bMEC and MNC, whereas the expression of three virulence genes were significantly downregulated in *M. bovis* infecting MNC.
Conclusions and Relevance: Our findings indicate that *M. bovis* exhibits infecting cell-specific gene expression, and its virulence varies with the infecting cell type.

Keywords: Cattle; *Mycoplasma bovis*; bovine mastitis; virulence; gene expression profiling

INTRODUCTION

Mycoplasma bovis (also known as *Mycoplasmopsis bovis*) causes bovine mycoplasmosis, a group of severe bovine diseases that include mastitis, pneumonia, otitis media, and arthritis [1]. Mycoplasmal mastitis can be differentiated from mastitis caused by other bacteria because it is highly contagious, affects multiple quarters, is often resistant to antibiotics, and affected cows apparently appear normal even in severe cases [2]. *M. bovis* mastitis is sometimes

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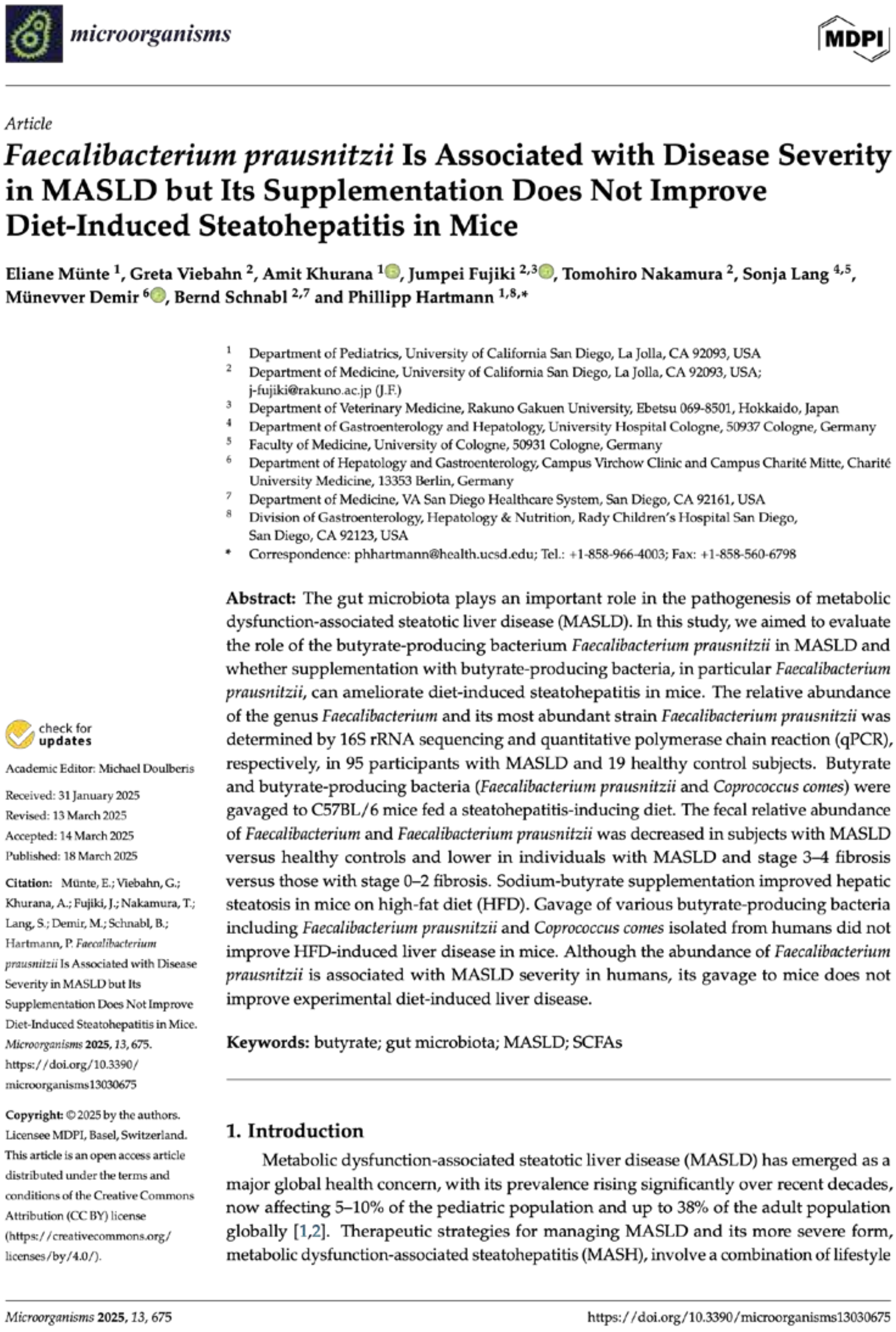
最終責任者　Satoshi Gondaira (Corresponding Author)



microorganisms

MDPI

Article

Faecalibacterium prausnitzii Is Associated with Disease Severity in MASLD but Its Supplementation Does Not Improve Diet-Induced Steatohepatitis in Mice

Eliane Münte [1], Greta Viebahn [2], Amit Khurana [1], Jumpei Fujiki [2,3], Tomohiro Nakamura [2], Sonja Lang [4,5], Münevver Demir [6], Bernd Schnabl [2,7] and Phillipp Hartmann [1,8,*]

[1] Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, USA
[2] Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA; j-fujiki@rakuno.ac.jp (J.F.)
[3] Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
[4] Department of Gastroenterology and Hepatology, University Hospital Cologne, 50937 Cologne, Germany
[5] Faculty of Medicine, University of Cologne, 50931 Cologne, Germany
[6] Department of Hepatology and Gastroenterology, Campus Virchow Clinic and Campus Charité Mitte, Charité University Medicine, 13353 Berlin, Germany
[7] Department of Medicine, VA San Diego Healthcare System, San Diego, CA 92161, USA
[8] Division of Gastroenterology, Hepatology & Nutrition, Rady Children's Hospital San Diego, San Diego, CA 92123, USA
* Correspondence: phhartmann@health.ucsd.edu; Tel.: +1-858-966-4003; Fax: +1-858-560-6798

Abstract: The gut microbiota plays an important role in the pathogenesis of metabolic dysfunction-associated steatotic liver disease (MASLD). In this study, we aimed to evaluate the role of the butyrate-producing bacterium *Faecalibacterium prausnitzii* in MASLD and whether supplementation with butyrate-producing bacteria, in particular *Faecalibacterium prausnitzii*, can ameliorate diet-induced steatohepatitis in mice. The relative abundance of the genus *Faecalibacterium* and its most abundant strain *Faecalibacterium prausnitzii* was determined by 16S rRNA sequencing and quantitative polymerase chain reaction (qPCR), respectively, in 95 participants with MASLD and 19 healthy control subjects. Butyrate and butyrate-producing bacteria (*Faecalibacterium prausnitzii* and *Coprococcus comes*) were gavaged to C57BL/6 mice fed a steatohepatitis-inducing diet. The fecal relative abundance of *Faecalibacterium* and *Faecalibacterium prausnitzii* was decreased in subjects with MASLD versus healthy controls and lower in individuals with MASLD and stage 3–4 fibrosis versus those with stage 0–2 fibrosis. Sodium-butyrate supplementation improved hepatic steatosis in mice on high-fat diet (HFD). Gavage of various butyrate-producing bacteria including *Faecalibacterium prausnitzii* and *Coprococcus comes* isolated from humans did not improve HFD-induced liver disease in mice. Although the abundance of *Faecalibacterium prausnitzii* is associated with MASLD severity in humans, its gavage to mice does not improve experimental diet-induced liver disease.

Keywords: butyrate; gut microbiota; MASLD; SCFAs

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1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as a major global health concern, with its prevalence rising significantly over recent decades, now affecting 5–10% of the pediatric population and up to 38% of the adult population globally [1,2]. Therapeutic strategies for managing MASLD and its more severe form, metabolic dysfunction-associated steatohepatitis (MASH), involve a combination of lifestyle

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食品衛生学（Food Microbiology and Food Safety）

Akira Fukuda

Lecture

講師　福田　昭

I. 筆頭または責任著者　＜First or Corresponding Author＞

1) Selection and maintenance of mobile linezolid-resistance genes and plasmids carrying them in the presence of florfenicol, an animal-specific antimicrobial.
Fukuda A, Usui M.
Access Microbiol. 7:00097.v3. 2025.
https://doi.org/10.1099/acmi.0.000997.v3
2) Complete geneme sequence of Treponema medium isolated from foot of bovine digigal dermatitis in Japan.
Fukuda A, Murakami T, Abe N, Suzuki Y, Nakajima C, Usui M.
Microbiol Res Announc. e0065625. 2025.
https://doi.org/10.1128/mra.00656-255)
3) Bacterial contamination level and characterization of antimicrobial-resistant bacteria in commercial pet foods in Japan.
Fukuda A, Yamaura K, Tokumoto K, Suzuki Y, Nakajima C, Yukawa S, Usui M.
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II. その他＜Others＞

1) Antimicrobial susceptibility and genetic diversity of Staphylococcus pseudintermedius isolated from companion animals and human clinical patients in Japan: potential zoonotic implications.Usui M, Sabala RF, Morita S, **Fukuda A**, Tsuyuki Y, Torii K, Nakamura Y, Okamura K, Komatsu T, Sasaki J, Nakajima C, Suzuki Y. *J Glob Antimicrob Resist*. 42:66-72. 2025. https://doi.org/10.1016/j.gar.2025.02.010Journal of Global Antimicrobial Resistance. 42:66-72. doi: 10.1016/j.jgar.2025.02.010
2) Surveillance of *Escherichia coli* from frozen chicken meat in Fiji: resistance characteristics and public health concernsacterial contamination level and characterization of antimicrobial-resistant bacteria in commercial pet foods in Japan
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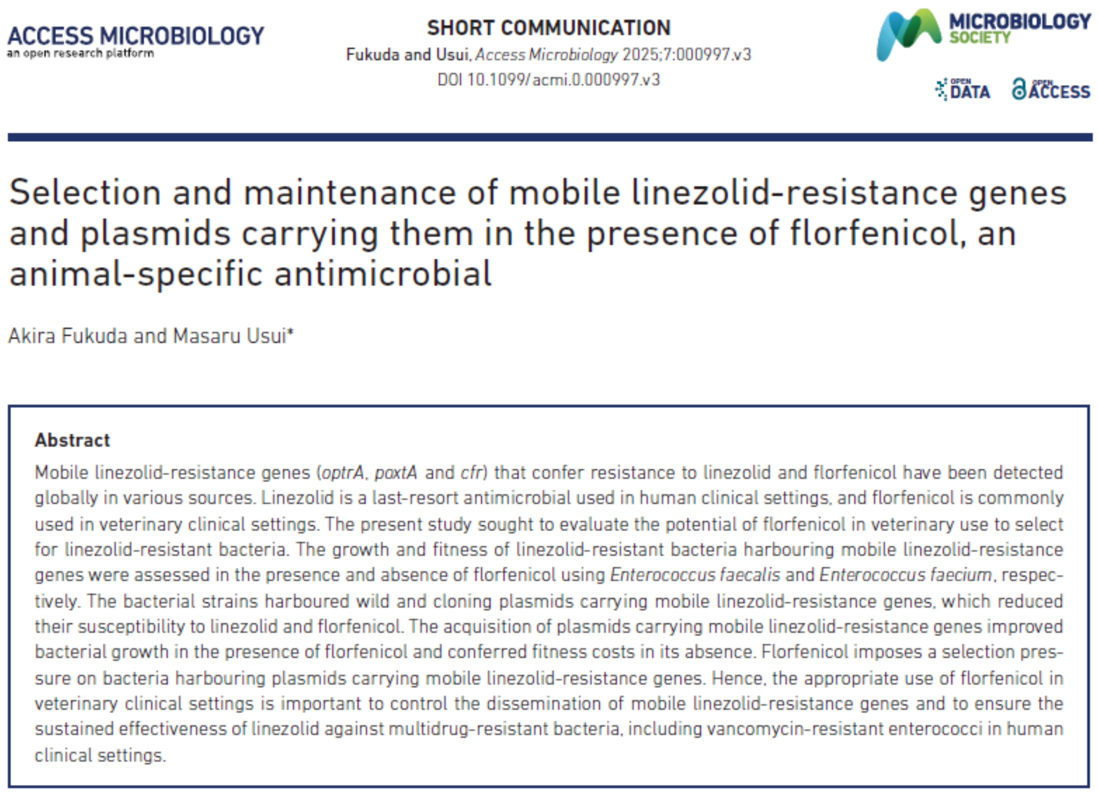

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Selection and maintenance of mobile linezolid-resistance genes and plasmids carrying them in the presence of florfenicol, an animal-specific antimicrobial

Akira Fukuda and Masaru Usui*

Abstract

Mobile linezolid-resistance genes (*optrA*, *poxtA* and *cfr*) that confer resistance to linezolid and florfenicol have been detected globally in various sources. Linezolid is a last-resort antimicrobial used in human clinical settings, and florfenicol is commonly used in veterinary clinical settings. The present study sought to evaluate the potential of florfenicol in veterinary use to select for linezolid-resistant bacteria. The growth and fitness of linezolid-resistant bacteria harbouring mobile linezolid-resistance genes were assessed in the presence and absence of florfenicol using *Enterococcus faecalis* and *Enterococcus faecium*, respectively. The bacterial strains harboured wild and cloning plasmids carrying mobile linezolid-resistance genes, which reduced their susceptibility to linezolid and florfenicol. The acquisition of plasmids carrying mobile linezolid-resistance genes improved bacterial growth in the presence of florfenicol and conferred fitness costs in its absence. Florfenicol imposes a selection pressure on bacteria harbouring plasmids carrying mobile linezolid-resistance genes. Hence, the appropriate use of florfenicol in veterinary clinical settings is important to control the dissemination of mobile linezolid-resistance genes and to ensure the sustained effectiveness of linezolid against multidrug-resistant bacteria, including vancomycin-resistant enterococci in human clinical settings.

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AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Bacteriology | Announcement

Complete genome sequence of *Treponema medium* isolated from foot of bovine digital dermatitis in Japan

Akira Fukuda,[1] **Takashi Murakami,**[2] **Noritsugu Abe,**[3] **Yasuhiko Suzuki,**[4,5,6] **Chie Nakajima,**[4,5,7] **Masaru Usui**[1]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Treponema* species are the primary causative bacteria of bovine digital dermatitis. This report describes the isolation and complete genome sequencing of *Treponema medium* strain T1 isolated from a hoof sole swab of a bovine in Japan. Genome sequences were obtained using a combination of long- and short-read sequencing technologies.

KEYWORDS *Treponema*, bovine, digital dermatitis, complete genome

Bovine digital dermatitis (BDD) is an infectious skin disease of the foot that leads to reduced dairy farm productivity. Molecular studies have identified *Treponema* species, particularly *T. medium*, *T. phagedenis*, *T. pedis*, and *T. denticola*, in BDD lesions (1, 2). Culturing and isolating *Treponema* under laboratory conditions is challenging because of their poor growth and the presence of other fast-growing bacteria in BDD lesions (3, 4). Studies on the isolation and complete genome analysis of bovine *Treponema* are very limited.

T. medium strain T1 was isolated from swabbing the plantar aspect of the interdigital cleft of a bovine exhibiting BDD symptoms (stage M2) in Hokkaido, Japan, in 2024 (5). The swab was transferred to the laboratory using an Anaerobic porter II (Terumo, Tokyo, Japan) on the day of sampling and cultured in pDDTp broth at 4°C for 3 days (3). The culture was then spotted on membrane filters (25 mm diameter and 0.45 µm pore) placed on fastidious anaerobe agar (Neogen, Lansing, MI, USA) supplemented with 5% defibrinated sheep blood, 10% fetal calf serum, and 5 mg/L each of rifampicin and enrofloxacin. After 6 h, the membrane was removed, and the agar was incubated at 37°C for 14 days (4, 6). Strain T1 was subsequently sub-cultured in mNOS medium at 37°C for 5 days, and bacterial species were identified using a phase-contrast microscope and PCR (7). The sub-culture was stored at −80°C in 15% glycerol stock (8, 9). For DNA extraction, the strain in glycerol stock was cultured using mNOS at 37°C for 5 days. All isolation procedures were performed under anaerobic conditions.

Genomic DNA was extracted for short-read sequencing using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). A DNA library was prepared using the Nextera XT Kit (Illumina, San Diego, CA, USA) and sequenced on a MiSeq platform (Illumina). The resulting 290 Mbp (~93× coverage) from 534,610 paired-end reads (300 bp) were trimmed using fastp v0.24.1 (10).

For long-read sequencing, genomic DNA was extracted using the Quick-DNA HMW MagBead Kit (Zymo Research, Irvine, CA, USA). Library preparation was performed using the Rapid Barcoding Sequencing Kit SQK-RBK114.24 (Oxford Nanopore Technologies, Oxford, UK). Sequencing was conducted using MinION with a FLO-MIN-114 R10.4.1 flow cell (Oxford Nanopore Technologies) and MinKNOW software with a 72-h run time and no alterations to voltage scripts. The obtained 214 Mbp (~68× coverage) from 53,348 reads (N_{50}; 7,996 bp) were demultiplexed and adaptor-trimmed using Porechop v0.2.4 (https://github.com/rrwick/Porechop/releases/tag/v0.2.4), followed by

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Address correspondence to Akira Fukuda, a-fukuda@rakuno.ac.jp, or Masaru Usui, musui@rakuno.ac.jp.

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Bacterial contamination level and characterization of antimicrobial-resistant bacteria in commercial pet foods in Japan

Akira Fukuda[a], Kazu Yamaura[a], Kazuyoshi Tokumoto[b], Yasuhiko Suzuki[c,d,e], Chie Nakajima[c,d,f], Shoichiro Yukawa[g], Masaru Usui[a,*]

[a] *Food Microbiology and Food Safety Unit, Division of Preventive Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*
[b] *Harmony Corporation, Tokyo, Japan*
[c] *Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Hokkaido, Japan*
[d] *International Collaboration Unit, International Institute for Zoonosis Control, Hokkaido University, Hokkaido, Japan*
[e] *Division of Research Support, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[f] *Vaccinology for Clinical Development, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[g] *Department of Comparative Animal Science, College of Life Science, Kurashiki University of Science and The Arts, Okayama, Japan*

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Antimicrobial-resistant bacteria
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ABSTRACT

With the growing pet market, various types of pet food become available. Pet foods, especially raw meat-based diets (RMBDs), are contaminated with pathogens and antimicrobial-resistant bacteria, leading to health concerns for pets and humans. The bacterial contamination levels in pet food, including the presence of antimicrobial-resistant bacteria, have not been extensively studied in Japan. To address this issue in commercial pet foods in Japan, we evaluated the standard plate counts (SPCs) of various pet food samples, and isolated and characterized *Escherichia coli* and *Enterococcus* spp. A total of 129 pet foods (48 RMBDs, 21 heat-treated foods, and 60 treats) were purchased, and SPC quantification and isolation of *E. coli* and *Enterococcus* spp. were performed. SPCs in RMBD were significantly higher than in heat-treated foods and treats. Specifically, 50.0 % of the RMBD samples had SPCs exceeding 10^6 CFU/g. *E. coli* was isolated only from 62.5 % of RMBDs. Four cephalosporin-resistant *E. coli* strains harboring *bla* genes were detected, among which, one carried *mcr* conferring resistance to colistin. *Enterococcus* spp. were isolated from 89.6 % of RMBDs and 23.3 % of treats. Linezolid-non-susceptible *E. faecalis* harboring *optrA* or *poxtA* genes were detected in four RMBDs. Pet food may be contaminated with bacteria; some RMBDs are contaminated at concentrations $>10^6$ CFU/g, and with veterinary and human clinically important antimicrobial-resistant bacteria. To prevent health risks in both humans and pets associated with pet food, ensuring the hygienic management of pet food, especially RMBDs, and promoting accurate knowledge among pet owners are important.

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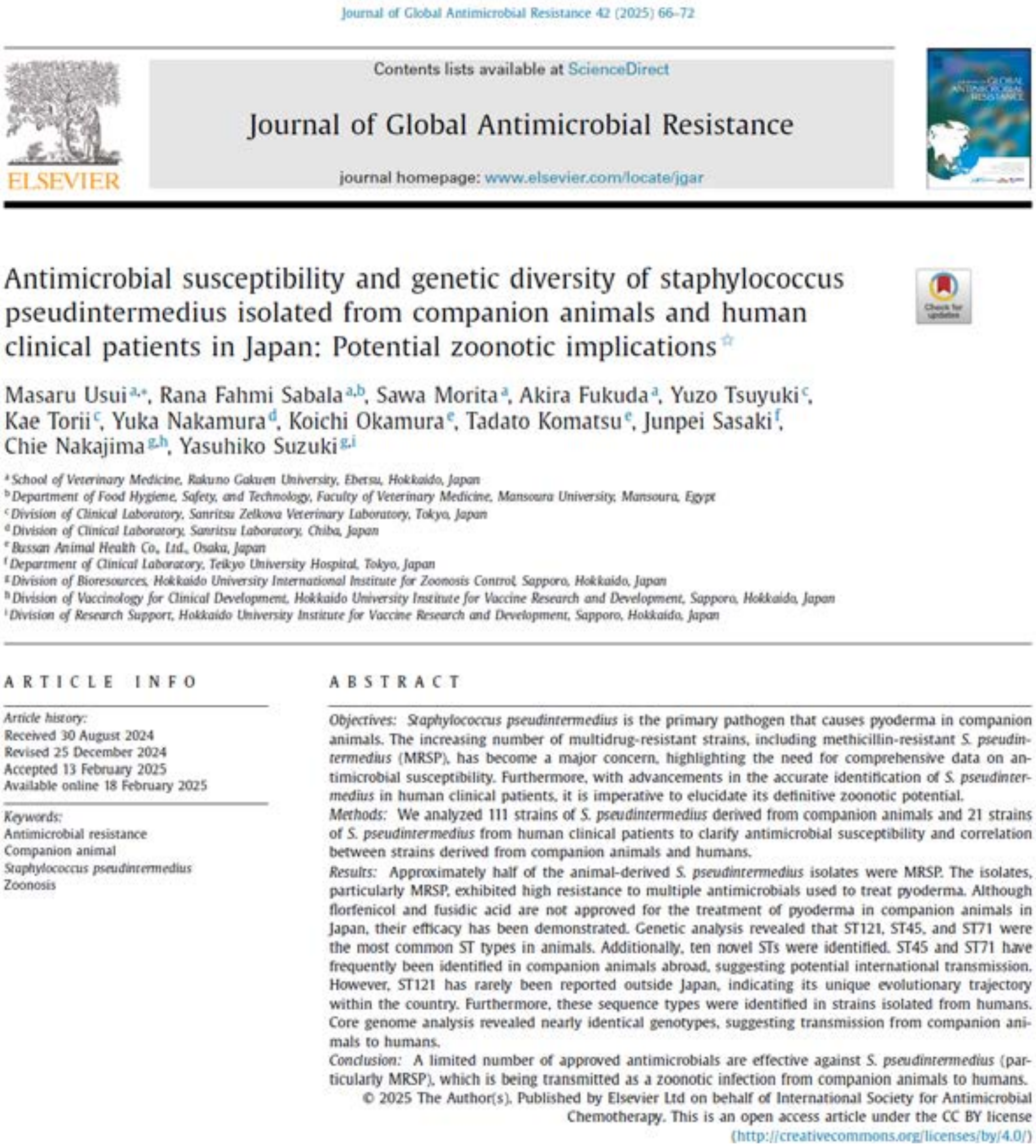

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Antimicrobial susceptibility and genetic diversity of staphylococcus pseudintermedius isolated from companion animals and human clinical patients in Japan: Potential zoonotic implications☆

Masaru Usui[a,*], Rana Fahmi Sabala[a,b], Sawa Morita[a], Akira Fukuda[a], Yuzo Tsuyuki[c], Kae Torii[c], Yuka Nakamura[d], Koichi Okamura[e], Tadato Komatsu[e], Junpei Sasaki[f], Chie Nakajima[g,h], Yasuhiko Suzuki[g,i]

[a] *School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*
[b] *Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*
[c] *Division of Clinical Laboratory, Sanritsu Zelkova Veterinary Laboratory, Tokyo, Japan*
[d] *Division of Clinical Laboratory, Sanritsu Laboratory, Chiba, Japan*
[e] *Bussan Animal Health Co., Ltd., Osaka, Japan*
[f] *Department of Clinical Laboratory, Teikyo University Hospital, Tokyo, Japan*
[g] *Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Hokkaido, Japan*
[h] *Division of Vaccinology for Clinical Development, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[i] *Division of Research Support, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*

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ABSTRACT

Objectives: *Staphylococcus pseudintermedius* is the primary pathogen that causes pyoderma in companion animals. The increasing number of multidrug-resistant strains, including methicillin-resistant *S. pseudintermedius* (MRSP), has become a major concern, highlighting the need for comprehensive data on antimicrobial susceptibility. Furthermore, with advancements in the accurate identification of *S. pseudintermedius* in human clinical patients, it is imperative to elucidate its definitive zoonotic potential.
Methods: We analyzed 111 strains of *S. pseudintermedius* derived from companion animals and 21 strains of *S. pseudintermedius* from human clinical patients to clarify antimicrobial susceptibility and correlation between strains derived from companion animals and humans.
Results: Approximately half of the animal-derived *S. pseudintermedius* isolates were MRSP. The isolates, particularly MRSP, exhibited high resistance to multiple antimicrobials used to treat pyoderma. Although florfenicol and fusidic acid are not approved for the treatment of pyoderma in companion animals in Japan, their efficacy has been demonstrated. Genetic analysis revealed that ST121, ST45, and ST71 were the most common ST types in animals. Additionally, ten novel STs were identified. ST45 and ST71 have frequently been identified in companion animals abroad, suggesting potential international transmission. However, ST121 has rarely been reported outside Japan, indicating its unique evolutionary trajectory within the country. Furthermore, these sequence types were identified in strains isolated from humans. Core genome analysis revealed nearly identical genotypes, suggesting transmission from companion animals to humans.
Conclusion: A limited number of approved antimicrobials are effective against *S. pseudintermedius* (particularly MRSP), which is being transmitted as a zoonotic infection from companion animals to humans.

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Research Article

Surveillance of *Escherichia coli* From Frozen Chicken Meat in Fiji: Resistance Characteristics and Public Health Concerns

Deepika Darshani Lata,[1] Rana Fahmi Sabala,[1,2] Akira Fukuda,[1] Chie Nakajima,[3,4] Yasuhiko Suzuki,[3,5] and Masaru Usui[1]

[1]*School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan*
[2]*Department of Food Hygiene, Safety, and Technology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt*
[3]*Division of Bioresources, Hokkaido University International Institute for Zoonosis Control, Sapporo, Hokkaido, Japan*
[4]*Division of Vaccinology for Clinical Development, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*
[5]*Division of Research Support, Hokkaido University Institute for Vaccine Research and Development, Sapporo, Hokkaido, Japan*

Correspondence should be addressed to Masaru Usui; usuima@rakuno.ac.jp

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Antimicrobial resistance (AMR) is a growing concern in human and veterinary medicine. Misuse and overuse of antimicrobials in human medicine, veterinary medicine, agriculture, and aquaculture are major drivers of AMR development, with resistant bacteria also being selected in livestock and transmitted through meat. Research on AMR in livestock and animal-derived foods is lacking in Fiji; thus, the associated risks remain unclear. Chicken is widely consumed in Fiji and is predominantly served frozen. This study is aimed at determining the prevalence and resistance profiles of *Escherichia coli* in frozen chicken meat from Fijian supermarkets. A total of 100 frozen chicken meat samples were purchased from supermarkets and retail outlets in Fiji for this study. *E. coli* was isolated from 72% of the samples. The *E. coli* isolates showed relatively high levels of resistance to ampicillin (36%), tetracycline (24%), and streptomycin (17%). Only one cefotaxime-resistant isolate was obtained, which was identified as an extended-spectrum *β*-lactamase (ESBL)–producing bacterium. This isolate harbored the ESBL-producing gene $bla_{\text{CTX-M-1}}$ and was classified as ST2522. One colistin-resistant isolate was obtained, and its resistance was attributed to a chromosomal mutation in the *pmrB* gene. The high level of intestinal bacterial contamination in frozen chicken meat suggests that improved hygiene management is necessary throughout the production and distribution chains. Furthermore, because resistance to antimicrobials is important in both human and veterinary medicine (cefotaxime- and colistin-resistant *E. coli*), careful monitoring of AMR trends in Fiji is essential. These results suggest that AMR surveillance in meat and livestock is necessary to prevent its spread in Fiji.

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最終責任者　Masaru Usui (Corresponding Author)

伴侶動物外科学（Companion Animal Surgery）

Yuji Hamamoto

Lecturer

講師　濱本　裕仁

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II. その他　＜Others＞

1） Construction of a Diagnostic Prediction Model for Feline Nasal and Nasopharyngeal Diseases in Japan Using Noninvasive Examinations.
Fujiwara-Igarashi A, Nakazawa Y, Ohshima T, Goto S, Ino M, **Hamamoto Y**, Takeuchi Y, Kanemoto H.
Vet Med Sci. 11:e70296. doi: 10.1002/vms3.70296.

Veterinary Medicine and Science

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ORIGINAL ARTICLE OPEN ACCESS

Cats

Construction of a Diagnostic Prediction Model for Feline Nasal and Nasopharyngeal Diseases in Japan Using Noninvasive Examinations

Aki Fujiwara-Igarashi[1] | Yuta Nakazawa[1] | Takafumi Ohshima[1] | Sho Goto[1] | Masatoshi Ino[1] | Yuji Hamamoto[1] | Yoshinori Takeuchi[2] | Hideyuki Kanemoto[3,4]

[1]Laboratory of Veterinary Radiology, School of Veterinary Medicine, Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan | [2]Division of Medical Statistics, Department of Social Medicine, Faculty of Medicine, Toho University, Ota-ku, Tokyo, Japan | [3]DVMs Animal Medical Center Yokohama, Yokohama, Kanagawa, Japan | [4]ER Hachioji Advanced Veterinary Medical Emergency and Critical Care Center, Hachioji, Tokyo, Japan

Correspondence: Aki Fujiwara-Igarashi (afjig@nvlu.ac.jp)

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Keywords: diagnostic prediction model | nasal and nasopharyngeal diseases | nasal and nasopharyngeal tumour | nasopharyngeal stenosis | rhinitis

ABSTRACT

Background: Although feline nasal and nasopharyngeal diseases (NNDs) often require advanced tests under general anaesthesia for definitive diagnosis, not all patients can undergo them.

Objectives: This study aimed to construct diagnostic prediction models for feline NNDs in Japan using noninvasive examinations, signalment and history.

Methods: Seventy-nine cats diagnosed with NNDs, including representative diseases in Japan—nasal and nasopharyngeal tumours (NNT), rhinitis (RS) and nasopharyngeal stenosis (NPS)—were retrospectively investigated to construct prediction models (model group, GM). Thirty-nine cats diagnosed were prospectively investigated to validate their efficacy (validation group, GV). Three predictive models were developed: Models 1 and 2 were manually constructed, with Model 1 designed to predict NNT, RS and NPS individually and Model 2 distinguishing between these diseases. Model 3 was constructed using least absolute shrinkage and selection operator logistic regression. Sensitivity, indicating the ability to identify cases of each disease, and specificity, reflecting the ability to exclude other diseases, were used to assess performance.

Results: In Model 1 of the GV, the sensitivity and specificity for NNT, RS and NPS were 1.00 and 0.73, 0.62 and 0.96 and 0.78 and 0.97, respectively. In Model 2 of the GV, the values were 0.94 and 0.86 for NNT, 0.77 and 0.92 for RS and 0.75 and 0.94 for NPS. In Model 3 of the GV, they were 0.94 and 0.05 for NNT, 0.25 and 1.00 for RS and 0.13 and 0.84 for NPS.

Conclusions: The diagnostic prediction models, particularly Models 1 and 2, could help estimate whether advanced tests are necessary.

1 | Introduction

Among feline nasal and nasopharyngeal diseases (NNDs), neoplasia has been reported to be the most common, whereas other common conditions include acute rhinitis (RS) due to viral infection, chronic rhinitis (CRS), nasopharyngeal stenosis (NPS), inflammatory polyps, foreign bodies and fungal RS (Allen et al. 1999; Fujiwara-Igarashi et al. 2024; Henderson et al. 2004; Reed and Gunn-Moore 2012). Clinical signs, such as nasal discharge, sneezing, reverse sneezing, stertor and epistaxis, are similar

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医動物学（Parasitology and Zoology）

Takahiro Ishizaki

Lecturer

講師　石﨑　隆弘

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該当なし

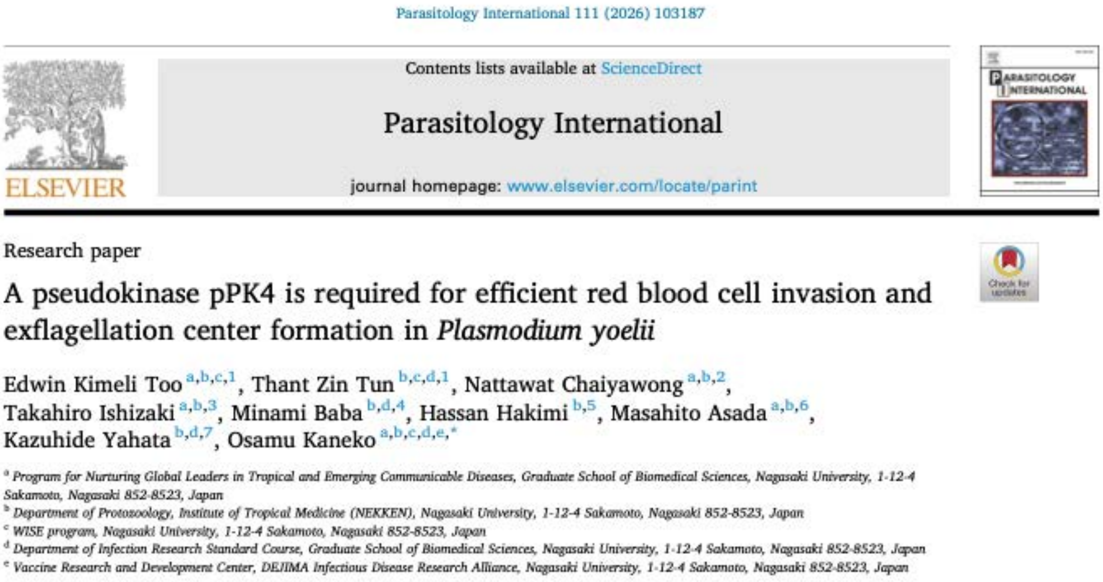
II. その他　＜Others＞

1） A pseudokinase pPK4 is required for efficient red blood cell invasion and exflagellation center formation in *Plasmodium yoelii*.
Too EK, Tun TZ, Chaiyawong N, **Ishizaki T**, Baba M, Hakimi H, Asada M, Yahata K, Kaneko O.
Parasitol Int. 103817. doi: 10.1016/j.parint.2025.103187

2） A scalable CRISPR-Cas9 gene editing system facilitates CRISPR screens in the malaria parasite *Plasmodium berghei*.
Jonsdottir TK, Paoletta MS, **Ishizaki T**, Hernandez S, Ivanova M, Herrera Curbelo A, Saiki PA, Selinger M, Das D, Henriksson J, Bushell ESC.
Nucleic Acids Res. 53:gkaf005. 2025. doi: 10.1093/nar/gkaf005.

3） Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (*Choloepus didactylus*).

4） Murakami M, Iwasa A, Okamoto M, **Ishizaki T**, Suzuki K, Hirata H.
J Vet Med Sci. 87:972-975. 2025. doi: 10.1292/jvms.25-0063.



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Research paper

A pseudokinase pPK4 is required for efficient red blood cell invasion and exflagellation center formation in *Plasmodium yoelii*

Edwin Kimeli Too [a,b,c,1], Thant Zin Tun [b,c,d,1], Nattawat Chaiyawong [a,b,2],
Takahiro Ishizaki [a,b,3], Minami Baba [b,d,4], Hassan Hakimi [b,5], Masahito Asada [a,b,6],
Kazuhide Yahata [b,d,7], Osamu Kaneko [a,b,c,d,e,*]

[a] *Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[b] *Department of Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[c] *WISE program, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[d] *Department of Infection Research Standard Course, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[e] *Vaccine Research and Development Center, DEJIMA Infectious Disease Research Alliance, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*

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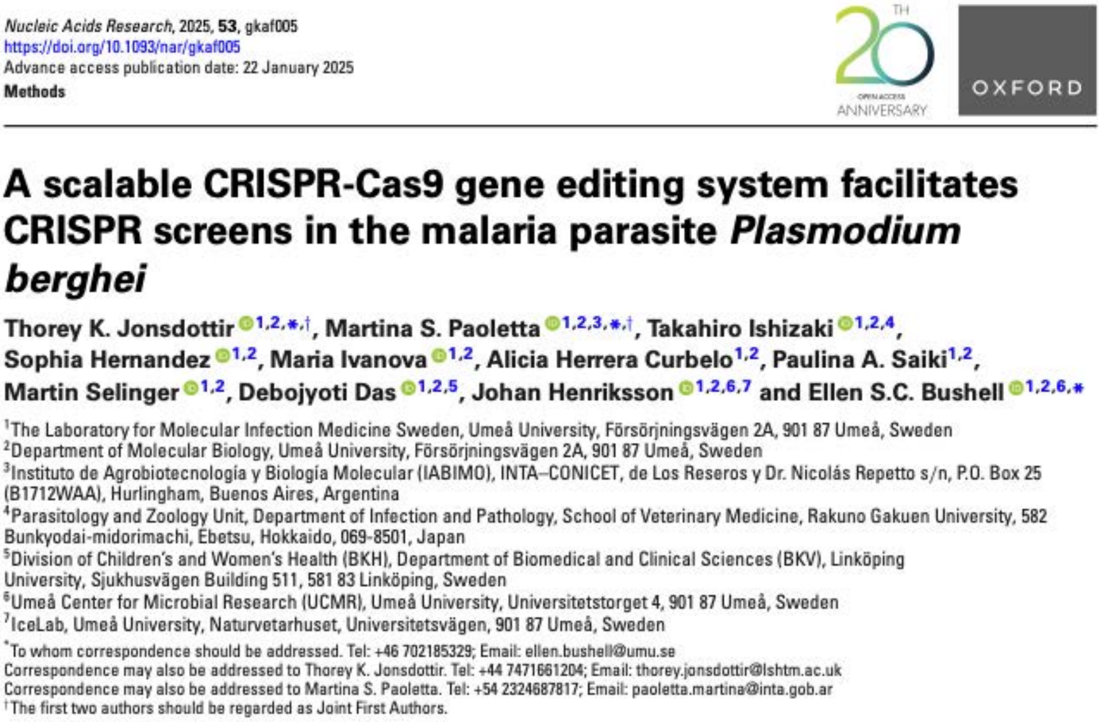
最終責任者　Osamu Kaneko (Corresponding Author)



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Methods

A scalable CRISPR-Cas9 gene editing system facilitates CRISPR screens in the malaria parasite *Plasmodium berghei*

Thorey K. Jonsdottir[1,2,*,†], **Martina S. Paoletta**[1,2,3,*,†], **Takahiro Ishizaki**[1,2,4], **Sophia Hernandez**[1,2], **Maria Ivanova**[1,2], **Alicia Herrera Curbelo**[1,2], **Paulina A. Saiki**[1,2], **Martin Selinger**[1,2], **Debojyoti Das**[1,2,5], **Johan Henriksson**[1,2,6,7] and **Ellen S.C. Bushell**[1,2,6,*]

[1]The Laboratory for Molecular Infection Medicine Sweden, Umeå University, Försörjningsvägen 2A, 901 87 Umeå, Sweden
[2]Department of Molecular Biology, Umeå University, Försörjningsvägen 2A, 901 87 Umeå, Sweden
[3]Instituto de Agrobiotecnologia y Biología Molecular (IABIMO), INTA–CONICET, de Los Reseros y Dr. Nicolás Repetto s/n, P.O. Box 25 (B1712WAA), Hurlingham, Buenos Aires, Argentina
[4]Parasitology and Zoology Unit, Department of Infection and Pathology, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-midorimachi, Ebetsu, Hokkaido, 069-8501, Japan
[5]Division of Children's and Women's Health (BKH), Department of Biomedical and Clinical Sciences (BKV), Linköping University, Sjukhusvägen Building 511, 581 83 Linköping, Sweden
[6]Umeå Center for Microbial Research (UCMR), Umeå University, Universitetstorget 4, 901 87 Umeå, Sweden
[7]IceLab, Umeå University, Naturvetarhuset, Universitetsvägen, 901 87 Umeå, Sweden

[*]To whom correspondence should be addressed. Tel: +46 702185329; Email: ellen.bushell@umu.se
Correspondence may also be addressed to Thorey K. Jonsdottir. Tel: +44 7471661204; Email: thorey.jonsdottir@lshtm.ac.uk
Correspondence may also be addressed to Martina S. Paoletta. Tel: +54 2324687817; Email: paoletta.martina@inta.gob.ar

[†]The first two authors should be regarded as Joint First Authors.

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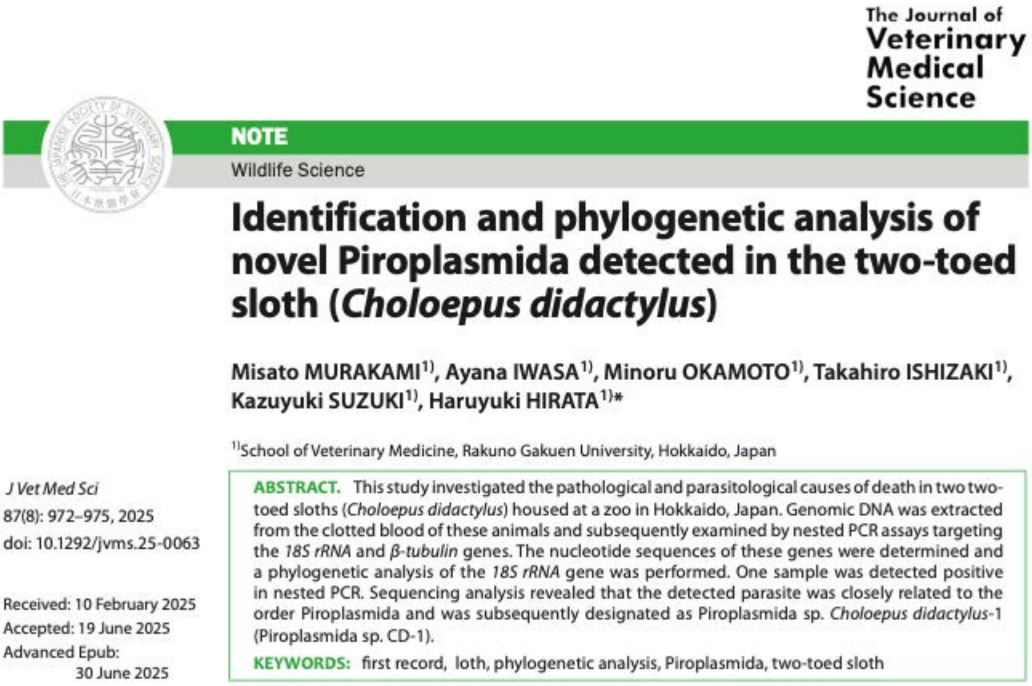
最終責任者　Ellen S C Bushell (Corresponding Author)



The Journal of
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Science

NOTE
Wildlife Science

Identification and phylogenetic analysis of novel Piroplasmida detected in the two-toed sloth (*Choloepus didactylus*)

Misato MURAKAMI[1], Ayana IWASA[1], Minoru OKAMOTO[1], Takahiro ISHIZAKI[1], Kazuyuki SUZUKI[1], Haruyuki HIRATA[1]*

[1]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. This study investigated the pathological and parasitological causes of death in two two-toed sloths (*Choloepus didactylus*) housed at a zoo in Hokkaido, Japan. Genomic DNA was extracted from the clotted blood of these animals and subsequently examined by nested PCR assays targeting the *18S rRNA* and *β-tubulin* genes. The nucleotide sequences of these genes were determined and a phylogenetic analysis of the *18S rRNA* gene was performed. One sample was detected positive in nested PCR. Sequencing analysis revealed that the detected parasite was closely related to the order Piroplasmida and was subsequently designated as Piroplasmida sp. *Choloepus didactylus*-1 (Piroplasmida sp. CD-1).

KEYWORDS: first record, loth, phylogenetic analysis, Piroplasmida, two-toed sloth

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最終責任者　Haruyuki Hirata (Corresponding Author)

Kenjirou Miyoshi

Lecturer

講師　三好健二郎

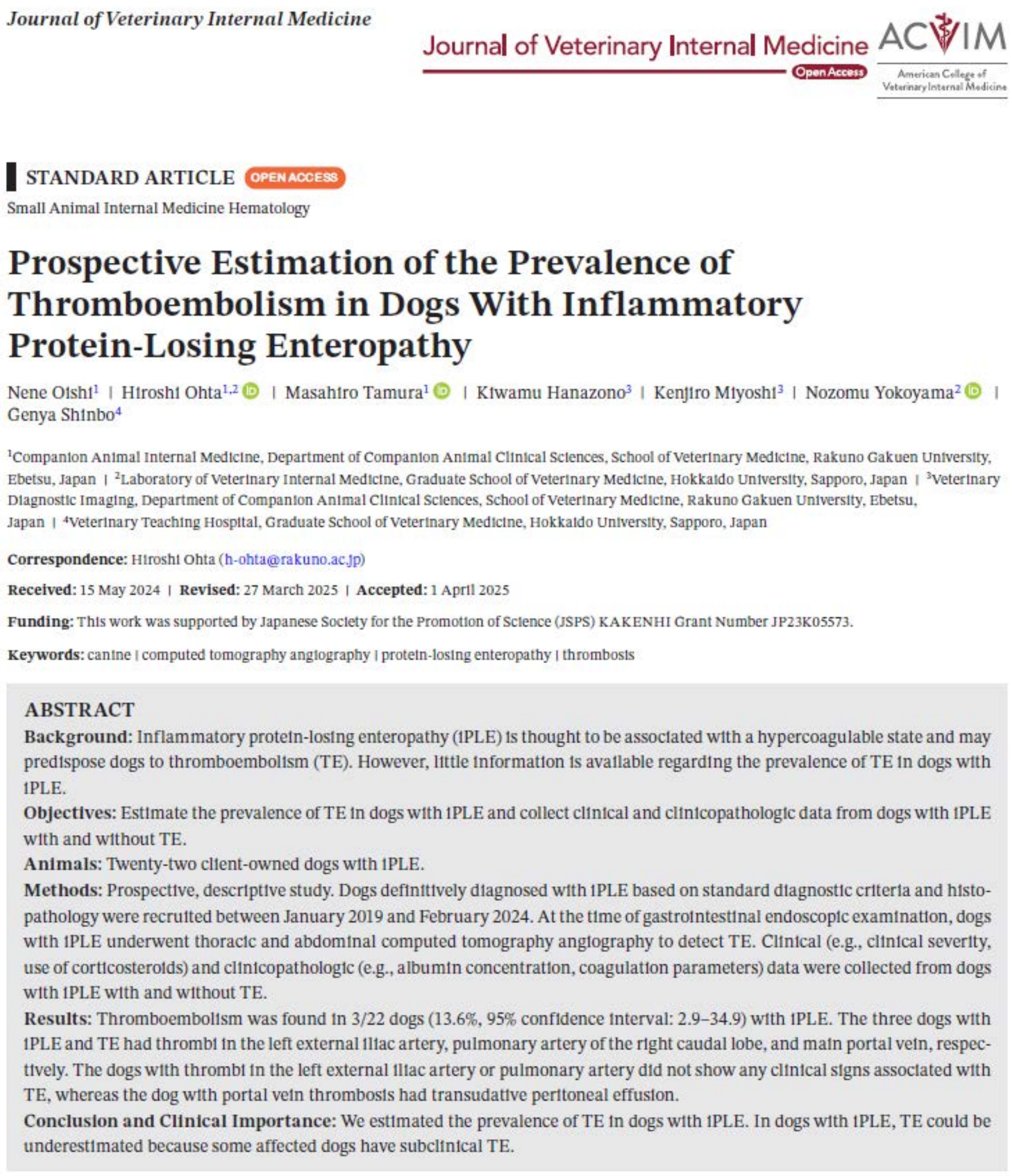
I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他＜Others＞

1）Prospective Estimation of the Prevalence of Thromboembolism in Dogs With Inflammatory Protein-Losing Enteropathy
Oishi N, Ohta H**,** Tamura M, Hanazono K, **Miyoshi K**, Yokoyama N, Shinbo G.
J Vet Intern Med. 39:e70098. 2025. doi: 10.1111/jvim.70098.

2）Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study.
Goda Y, Yamamoto S, **Miyoshi K**, Hanazono K, Morimura M, Sakai T, Tamura M, Deguchi T, Endo Y, Kadosawa T, Fujita M, Itami T, Torisu S.
Open Vet. J. 15: 5739-5747. 2025. doi: 10.5455/OVJ.2025.v15.i11.29



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Small Animal Internal Medicine Hematology

Prospective Estimation of the Prevalence of Thromboembolism in Dogs With Inflammatory Protein-Losing Enteropathy

Nene Oishi[1] | Hiroshi Ohta[1,2] | Masahiro Tamura[1] | Kiwamu Hanazono[3] | Kenjiro Miyoshi[3] | Nozomu Yokoyama[2] |
Genya Shinbo[4]

[1]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Laboratory of Veterinary Internal Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan | [3]Veterinary Diagnostic Imaging, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [4]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Correspondence: Hiroshi Ohta (h-ohta@rakuno.ac.jp)

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Keywords: canine | computed tomography angiography | protein-losing enteropathy | thrombosis

ABSTRACT

Background: Inflammatory protein-losing enteropathy (iPLE) is thought to be associated with a hypercoagulable state and may predispose dogs to thromboembolism (TE). However, little information is available regarding the prevalence of TE in dogs with iPLE.

Objectives: Estimate the prevalence of TE in dogs with iPLE and collect clinical and clinicopathologic data from dogs with iPLE with and without TE.

Animals: Twenty-two client-owned dogs with iPLE.

Methods: Prospective, descriptive study. Dogs definitively diagnosed with iPLE based on standard diagnostic criteria and histopathology were recruited between January 2019 and February 2024. At the time of gastrointestinal endoscopic examination, dogs with iPLE underwent thoracic and abdominal computed tomography angiography to detect TE. Clinical (e.g., clinical severity, use of corticosteroids) and clinicopathologic (e.g., albumin concentration, coagulation parameters) data were collected from dogs with iPLE with and without TE.

Results: Thromboembolism was found in 3/22 dogs (13.6%, 95% confidence interval: 2.9–34.9) with iPLE. The three dogs with iPLE and TE had thrombi in the left external iliac artery, pulmonary artery of the right caudal lobe, and main portal vein, respectively. The dogs with thrombi in the left external iliac artery or pulmonary artery did not show any clinical signs associated with TE, whereas the dog with portal vein thrombosis had transudative peritoneal effusion.

Conclusion and Clinical Importance: We estimated the prevalence of TE in dogs with iPLE. In dogs with iPLE, TE could be underestimated because some affected dogs have subclinical TE.

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Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study

Yoshimichi Goda[1#], Shushi Yamamoto[1#], Kenjiro Miyoshi[1], Kiwamu Hanazono[1], Mai Morimura[1], Toshikazu Sakai[1], Masahiro Tamura[1], Tatsuya Deguchi[1], Yoshifumi Endo[1,2], Tsuyoshi Kadosawa[1,3], Mayu Fujita[1], Takaharu Itami[1] and Shidow Torisu[1]*

[1]*Department of Companion Animal Clinical Sciences, School of Veterinary Medicine,*
Rakuno Gakuen University, Ebetsu, Japan
[2]*Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, Japan*
[3]*Japan Small Animal Medical Center, Tokorozawa-Shi, Japan*
[#]*Both authors are equally contributed to this work*

ABSTRACT

Background: Recently, the usefulness of triple-phase contrast-enhanced computed tomography (CT) scans for liver tumors in canines has been reported. However, detailed information on the CT findings of cholangiocarcinoma (CCA) and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) remains limited.

Aim: This study aimed to retrospectively evaluate triple-phase contrast-enhanced CT findings in canine CCA and cHCC-CCA using objective parameters, such as CT values and morphological characteristics.

Methods: This study included eight dogs that underwent triple-phase contrast-enhanced CT scans and surgical removal and were pathologically diagnosed. Three CCA cases and five cHCC-CCA cases were analyzed. Parameters included CT values and mass sizes, CT values and lymph node sizes, and rim enhancement. "Rim enhancement" was defined as the contrast enhancement effect at the liver parenchyma–mass boundary.

Results: CT values for CCA were as follows: pre-contrast: 43.9 ± 3.6 Hounsfield units (HUs); arterial phase: 76.9 ± 22.5 HU; portal phase: 98.8 ± 37.7 HU; equilibrium phase: 90.9 ± 27.1 HU. For cHCC-CCA, the mean CT values were as follows: pre-contrast: 50.7 ± 6.1 HU; arterial phase: 80.2 ± 19.2 HU; portal phase: 95.0 ± 21.3 HU; equilibrium phase: 86.1 ± 13.4 HU. The peak contrast enhancement for both CCA and cHCC-CCA was in the portal phase. Rim enhancement appeared in the arterial phase in all CCA cases, whereas it appeared in the portal phase in 80% of cHCC-CCA cases, indicating a significant difference.

Conclusion: Rim enhancement may help distinguish between CCA and cHCC-CCA. However, this study has a limited number of cases, and future large-scale, multicenter studies are warranted.

Keywords: Canine, Cholangiocarcinoma, Combined hepatocellular-cholangiocarcinoma, Computed tomography, Dog.

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最終責任者　Shidow Torisu. (Corresponding Author)

生産動物外科学（Farm Animal Surgery）

Takashi Murakami

Lecturer

講師　村上　高志

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該当なし

II. その他　＜Others＞

1) Complete geneme sequence of Treponema medium isolated from foot of bovine digigal dermatitis in Japan.
   Fukuda A, **Murakami T**, Abe N, Suzuki Y, Nakajima C, Usui M.
   Microbiol Res Announc. e0065625. 2025.
   https://doi.org/10.1128/mra.00656-255)
2) Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow.
   Ohsaki K, Sano Y, **Murakami T**, Ishiguro Y, Ohtake A, Sato A, Nakada,K, Sugiura T.
   Front Vet Sci, 12:170489215. 2025.
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Complete genome sequence of *Treponema medium* isolated from foot of bovine digital dermatitis in Japan

Akira Fukuda,[1] **Takashi Murakami,**[2] **Noritsugu Abe,**[3] **Yasuhiko Suzuki,**[4,5,6] **Chie Nakajima,**[4,5,7] **Masaru Usui**[1]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT *Treponema* species are the primary causative bacteria of bovine digital dermatitis. This report describes the isolation and complete genome sequencing of *Treponema medium* strain T1 isolated from a hoof sole swab of a bovine in Japan. Genome sequences were obtained using a combination of long- and short-read sequencing technologies.

KEYWORDS *Treponema*, bovine, digital dermatitis, complete genome

Bovine digital dermatitis (BDD) is an infectious skin disease of the foot that leads to reduced dairy farm productivity. Molecular studies have identified *Treponema* species, particularly *T. medium*, *T. phagedenis*, *T. pedis*, and *T. denticola*, in BDD lesions (1, 2). Culturing and isolating *Treponema* under laboratory conditions is challenging because of their poor growth and the presence of other fast-growing bacteria in BDD lesions (3, 4). Studies on the isolation and complete genome analysis of bovine *Treponema* are very limited.

T. medium strain T1 was isolated from swabbing the plantar aspect of the interdigital cleft of a bovine exhibiting BDD symptoms (stage M2) in Hokkaido, Japan, in 2024 (5). The swab was transferred to the laboratory using an Anaerobic porter II (Terumo, Tokyo, Japan) on the day of sampling and cultured in pDDTp broth at 4°C for 3 days (3). The culture was then spotted on membrane filters (25 mm diameter and 0.45 µm pore) placed on fastidious anaerobe agar (Neogen, Lansing, MI, USA) supplemented with 5% defibrinated sheep blood, 10% fetal calf serum, and 5 mg/L each of rifampicin and enrofloxacin. After 6 h, the membrane was removed, and the agar was incubated at 37°C for 14 days (4, 6). Strain T1 was subsequently sub-cultured in mNOS medium at 37°C for 5 days, and bacterial species were identified using a phase-contrast microscope and PCR (7). The sub-culture was stored at −80°C in 15% glycerol stock (8, 9). For DNA extraction, the strain in glycerol stock was cultured using mNOS at 37°C for 5 days. All isolation procedures were performed under anaerobic conditions.

Genomic DNA was extracted for short-read sequencing using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). A DNA library was prepared using the Nextera XT Kit (Illumina, San Diego, CA, USA) and sequenced on a MiSeq platform (Illumina). The resulting 290 Mbp (~93× coverage) from 534,610 paired-end reads (300 bp) were trimmed using fastp v0.24.1 (10).

For long-read sequencing, genomic DNA was extracted using the Quick-DNA HMW MagBead Kit (Zymo Research, Irvine, CA, USA). Library preparation was performed using the Rapid Barcoding Sequencing Kit SQK-RBK114.24 (Oxford Nanopore Technologies, Oxford, UK). Sequencing was conducted using MinION with a FLO-MIN-114 R10.4.1 flow cell (Oxford Nanopore Technologies) and MinKNOW software with a 72-h run time and no alterations to voltage scripts. The obtained 214 Mbp (~68× coverage) from 53,348 reads (N_{50}; 7,996 bp) were demultiplexed and adaptor-trimmed using Porechop v0.2.4 (https://github.com/rrwick/Porechop/releases/tag/v0.2.4), followed by

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Address correspondence to Akira Fukuda, a-fukuda@rakuno.ac.jp, or Masaru Usui, musui@rakuno.ac.jp.

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Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow

Koto Ohsaki[1], Yuto Sano[2], Takashi Murakami[3], Yuki Ishiguro[2], Ayaka Ohtake[3], Ayano Sato[3], Ken Nakada[1] and Tomochika Sugiura[1]*

[1]Laboratory of Theriogenology, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [2]Laboratory of Veterinary Pathology, Pathobiology, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [3]Laboratory of Farm Animal Surgery, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan

This case report describes a rare instance of a bovine fetus with concurrent umbilical cord torsion and entanglement, which resulted in fetal growth restriction (FGR) and prolonged gestation. A 36-month-old primiparous Holstein cow, 285 days pregnant, was examined after failing to show signs of parturition. Fetal heartbeat was confirmed via abdominal ultrasonography until 295 days post-artificial insemination (AI) but was not detected thereafter, leading to a presumptive diagnosis of fetal death at 313 days post-AI. Following induction, the dead fetus was delivered at 316 days post-AI. Despite a gestational age of approximately 10.5 months, the fetus exhibited severe growth restriction that is equivalent to that of a normal 7-month-old fetus. The umbilical cord measured 40 cm—abnormally long for a 7-month-old fetus—and was tightly wrapped around the right hind limb with more than 360 degrees of torsion along its long axis. Maternal serum analysis revealed persistently high progesterone and markedly low estradiol concentrations before parturition, suggesting that the physiological process of parturition had not been initiated. It is speculated that the FGR resulted from chronic blood flow obstruction likely associated with the umbilical cord abnormalities, which were hypothesized to have occurred during the second trimester (approximately 4 months). The consequent absence of normal fetal signals to initiate parturition and the lack of periparturient endocrine changes contributed to prolonged gestation and underdeveloped mammary glands in the dam. This report represents the first detailed description of intrauterine umbilical cord abnormalities in cattle, demonstrating their potential to cause fetal developmental delay, prolonged gestation, and impaired mammary gland development.

KEYWORDS

bovine fetus, umbilical cord torsion, umbilical cord entanglement, fetal growth restriction, maternal serum analysis, parturition

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Carolina Smok,
Universidad de La Frontera, Chile
Jagmohan Rajput,
Nanaji Deshmukh Veterinary Science
University, India

*CORRESPONDENCE
Tomochika Sugiura
✉ t-sugiura@rakuno.ac.jp

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最終責任者　Tomochika Sugiura (Corresponding Author)

伴侶動物外科学（Companion Animal Surgery）

Eunryel Nam

Lecturer

講師　南　垠列

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Correction of pivot shift phenomenon following tibial plateau leveling osteotomy using lateral fabellotibial suture in a toy-breed dog with cranial cruciate ligament rupture. **Nam E**, Amano M, Mochizuki M, Honnami M *Open Vet J.* 5:3369–3377. 2025 doi: 10.5455/OVJ.2025.v15.i7.50.

II. その他　＜Others＞

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Correction of pivot shift phenomenon following tibial plateau leveling osteotomy using lateral fabellotibial suture in a toy-breed dog with cranial cruciate ligament rupture

Eunryel Nam[1,2*], Madoka Amano[1], Manabu Mochizuki[1,3] and Muneki Honnami[1]

[1]*Veterinary Medical Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*
[2]*Laboratory of Companion Animal Surgery, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*
[3]*Laboratory of Veterinary Emergency Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*

ABSTRACT

Background: Cranial cruciate ligament rupture (CCLR) is a leading cause of lameness in dogs, often requiring surgical stabilization. Tibial plateau leveling osteotomy (TPLO) is a widely used procedure; however, postoperative instability, including the pivot shift phenomenon, can occur. This case highlights the need for additional stabilization techniques when TPLO alone is insufficient.

Case Description: A 5-kg toy poodle presented with right hind limb CCLR. The tibial plateau angle (TPA) was 25°, and no injury was observed in the medial meniscus. TPLO was performed; however, postoperative instability due to the pivot shift phenomenon was observed. To restore joint stability, a lateral fabellotibial suture (LFTS) was placed. Eight weeks later, lameness developed in the left hind limb, and orthopedic examination confirmed CCLR with a TPA of 25° and an intact medial meniscus. LFTS was applied to the left stifle joint due to recurrent instability following TPLO. Postoperatively, stifle joint stability was regained in both hind limbs.

Conclusion: While TPLO is an effective treatment for CCLR, some cases may require additional stabilization. This case suggests that LFTS can be a valuable adjunct for managing postoperative instability in small-breed dogs, improving surgical outcomes when the pivot shift phenomenon occurs.

Keywords: Cruciate ligament rupture, Lateral fabellotibial suture, Pivot shift phenomenon, Tibial plateau leveling osteotomy, Toy-breed dog.

Introduction

Cranial cruciate ligament rupture (CCLR) is a frequently observed orthopedic condition in dogs. While CCLR is more prevalent in large-breed dogs, its incidence has been increasing among toy-breed dogs (Brioschi and Arthurs, 2021). Damage to the cranial cruciate ligament can lead to chronic complications such as meniscus detachment, tearing, and arthritis owing to cranial tibial subluxation and instability of the stifle joint. Osteotomy procedures, such as cranial tibial wedge osteotomy, tibial plateau leveling osteotomy (TPLO), and tibial tuberosity advancement, can restore the stability of the stifle joint (Comerford *et al.*, 2013). Lameness caused by CCLR in toy-breed dogs can be managed conservatively through the administration, such as the administration of non-steroidal anti-inflammatory drugs, weight loss, and cage rest, as their body weight (<15 kg) is lower than that of medium and large breeds (Kwananocha *et al.*, 2024). Notably, TPLO has been shown to shorten the period of postoperative lameness even in toy-breed dogs (Knight and Danielski, 2018; Tikekar *et al.*, 2022; Schuenemann and Kaczmarek, 2023).

A combination of TPLO and lateral fabellotibial suture (LFTS) has been widely used to limit the postoperative instability of the stifle joint in large-breed dogs with CCLR (Schaible *et al.*, 2017). These integrated surgical procedures temporarily promote adequate periarticular fibrosis, decrease cranial tibial subluxation, and strengthen the hamstring muscles, thereby maintaining long-term stability and facilitating functional recovery of the stifle joint postoperatively (Harasen, 2008).

This report presents a case wherein a greater postoperative stability of the stifle joint was achieved

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***Corresponding Author:** Eunryel Nam. Laboratory of Companion Animal Surgery, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan. Email: *eunryelnam@gmail.com*

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最終責任者　Eunryel Nam (Corresponding Author)

獣医病理学（**Veterinary Pathology**）

Yuto Sano

Lecturer

講師　佐野　悠人

I. 筆頭または責任著者　＜First or Corresponding Author＞

1) Bilateral congenital cystic eye with intraocular tissue differentiation in a horse.
Sano Y, Miura C, Kinoshita Y, Kakimoto M, Tsuda T, Matsuda K.
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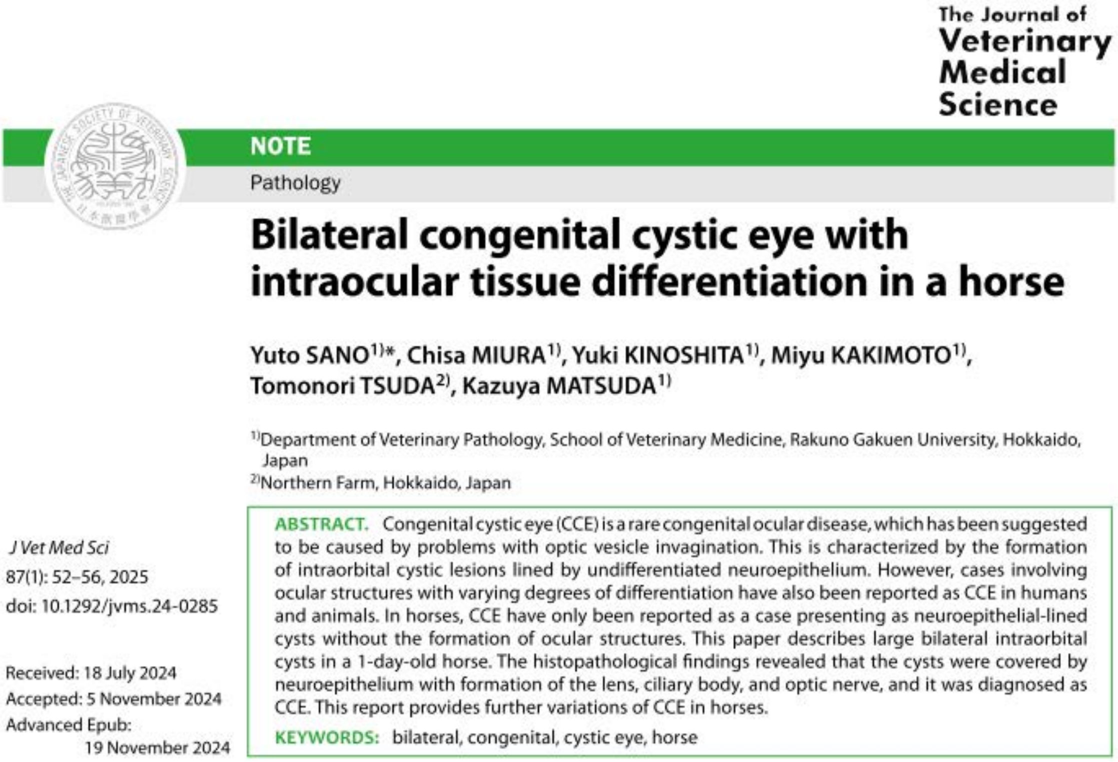

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NOTE

Pathology

Bilateral congenital cystic eye with intraocular tissue differentiation in a horse

Yuto SANO[1]*, Chisa MIURA[1], Yuki KINOSHITA[1], Miyu KAKIMOTO[1], Tomonori TSUDA[2], Kazuya MATSUDA[1]

[1]Department of Veterinary Pathology, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2]Northern Farm, Hokkaido, Japan

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ABSTRACT. Congenital cystic eye (CCE) is a rare congenital ocular disease, which has been suggested to be caused by problems with optic vesicle invagination. This is characterized by the formation of intraorbital cystic lesions lined by undifferentiated neuroepithelium. However, cases involving ocular structures with varying degrees of differentiation have also been reported as CCE in humans and animals. In horses, CCE have only been reported as a case presenting as neuroepithelial-lined cysts without the formation of ocular structures. This paper describes large bilateral intraorbital cysts in a 1-day-old horse. The histopathological findings revealed that the cysts were covered by neuroepithelium with formation of the lens, ciliary body, and optic nerve, and it was diagnosed as CCE. This report provides further variations of CCE in horses.

KEYWORDS: bilateral, congenital, cystic eye, horse

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最終責任者　Yuto Sano (Corresponding Author)

生産動物外科学（Farm Animal Surgery）

Ayano Sato

Senior Lecturer

講師　佐藤　綾乃

I. 筆頭または責任著者　＜First or Corresponding Author＞

II. その他　＜Others＞

1) Biofilm characterisation of *Mycoplasma bovis* co-cultured with Trueperella pyogenes.
   Nishi K, Gondaira S, Hirano Y, Ohashi M, **Sato A,** Matsuda K, Iwasaki T, Kanda T, Uemura R, Higuchi H.
   Vet Res. 56:22, 2025. doi: 10.1186/s13567-025-01468-1.

2) Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow.
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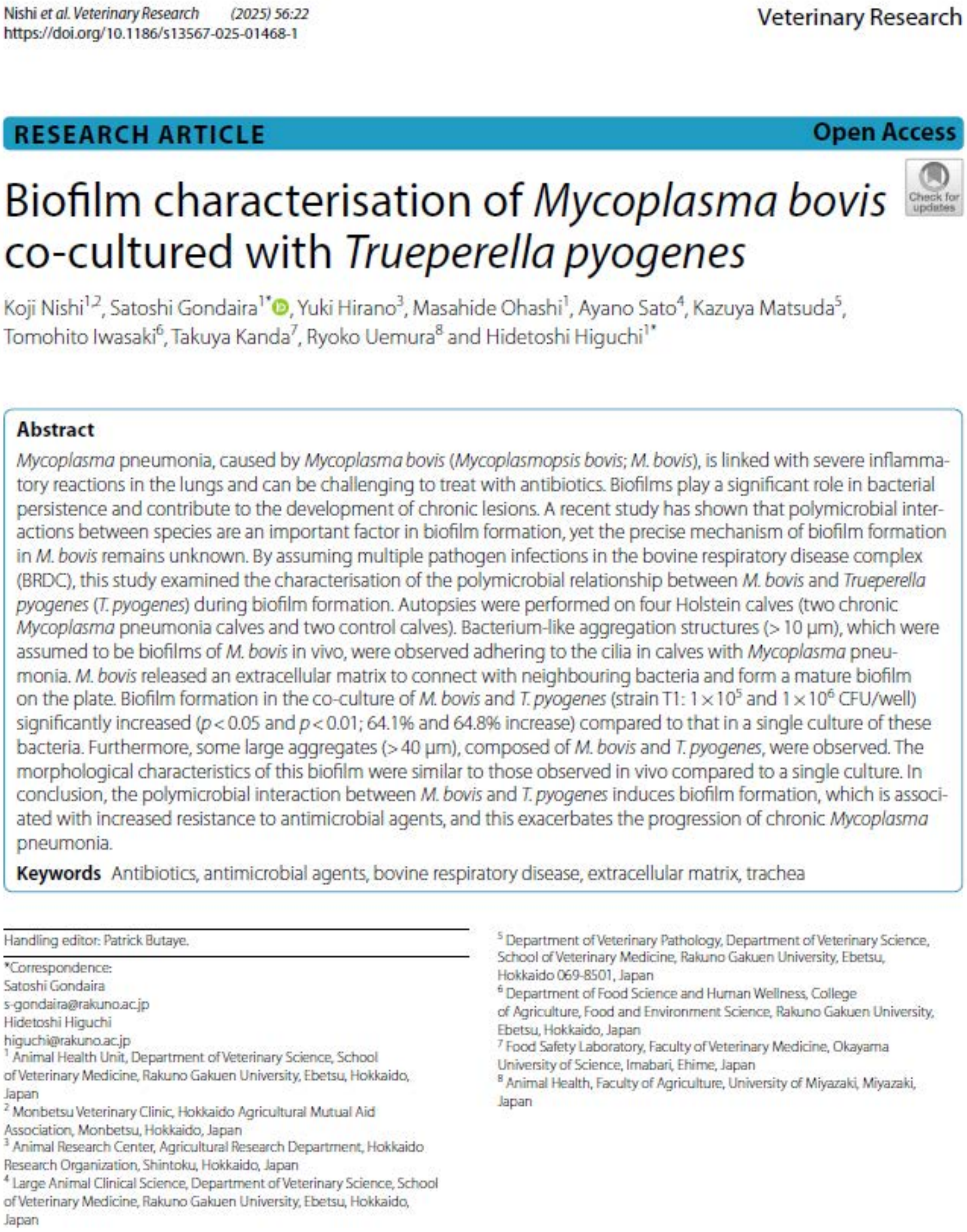

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Veterinary Research

RESEARCH ARTICLE Open Access

Biofilm characterisation of *Mycoplasma bovis* co-cultured with *Trueperella pyogenes*

Koji Nishi[1,2], Satoshi Gondaira[1*], Yuki Hirano[3], Masahide Ohashi[1], Ayano Sato[4], Kazuya Matsuda[5], Tomohito Iwasaki[6], Takuya Kanda[7], Ryoko Uemura[8] and Hidetoshi Higuchi[1*]

Abstract

Mycoplasma pneumonia, caused by *Mycoplasma bovis* (*Mycoplasmopsis bovis*; *M. bovis*), is linked with severe inflammatory reactions in the lungs and can be challenging to treat with antibiotics. Biofilms play a significant role in bacterial persistence and contribute to the development of chronic lesions. A recent study has shown that polymicrobial interactions between species are an important factor in biofilm formation, yet the precise mechanism of biofilm formation in *M. bovis* remains unknown. By assuming multiple pathogen infections in the bovine respiratory disease complex (BRDC), this study examined the characterisation of the polymicrobial relationship between *M. bovis* and *Trueperella pyogenes* (*T. pyogenes*) during biofilm formation. Autopsies were performed on four Holstein calves (two chronic *Mycoplasma* pneumonia calves and two control calves). Bacterium-like aggregation structures (> 10 µm), which were assumed to be biofilms of *M. bovis* in vivo, were observed adhering to the cilia in calves with *Mycoplasma* pneumonia. *M. bovis* released an extracellular matrix to connect with neighbouring bacteria and form a mature biofilm on the plate. Biofilm formation in the co-culture of *M. bovis* and *T. pyogenes* (strain T1: 1×10^5 and 1×10^6 CFU/well) significantly increased ($p < 0.05$ and $p < 0.01$; 64.1% and 64.8% increase) compared to that in a single culture of these bacteria. Furthermore, some large aggregates (> 40 µm), composed of *M. bovis* and *T. pyogenes*, were observed. The morphological characteristics of this biofilm were similar to those observed in vivo compared to a single culture. In conclusion, the polymicrobial interaction between *M. bovis* and *T. pyogenes* induces biofilm formation, which is associated with increased resistance to antimicrobial agents, and this exacerbates the progression of chronic *Mycoplasma* pneumonia.

Keywords Antibiotics, antimicrobial agents, bovine respiratory disease, extracellular matrix, trachea

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*Correspondence:
Satoshi Gondaira
s-gondaira@rakuno.ac.jp
Hidetoshi Higuchi
higuchi@rakuno.ac.jp
[1] Animal Health Unit, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[2] Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu, Hokkaido, Japan
[3] Animal Research Center, Agricultural Research Department, Hokkaido Research Organization, Shintoku, Hokkaido, Japan
[4] Large Animal Clinical Science, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[5] Department of Veterinary Pathology, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[6] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[7] Food Safety Laboratory, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan
[8] Animal Health, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

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Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow

Koto Ohsaki[1], Yuto Sano[2], Takashi Murakami[3], Yuki Ishiguro[2], Ayaka Ohtake[3], Ayano Sato[3], Ken Nakada[1] and Tomochika Sugiura[1]*

[1]Laboratory of Theriogenology, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [2]Laboratory of Veterinary Pathology, Pathobiology, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [3]Laboratory of Farm Animal Surgery, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan

This case report describes a rare instance of a bovine fetus with concurrent umbilical cord torsion and entanglement, which resulted in fetal growth restriction (FGR) and prolonged gestation. A 36-month-old primiparous Holstein cow, 285 days pregnant, was examined after failing to show signs of parturition. Fetal heartbeat was confirmed via abdominal ultrasonography until 295 days post-artificial insemination (AI) but was not detected thereafter, leading to a presumptive diagnosis of fetal death at 313 days post-AI. Following induction, the dead fetus was delivered at 316 days post-AI. Despite a gestational age of approximately 10.5 months, the fetus exhibited severe growth restriction that is equivalent to that of a normal 7-month-old fetus. The umbilical cord measured 40 cm—abnormally long for a 7-month-old fetus—and was tightly wrapped around the right hind limb with more than 360 degrees of torsion along its long axis. Maternal serum analysis revealed persistently high progesterone and markedly low estradiol concentrations before parturition, suggesting that the physiological process of parturition had not been initiated. It is speculated that the FGR resulted from chronic blood flow obstruction likely associated with the umbilical cord abnormalities, which were hypothesized to have occurred during the second trimester (approximately 4 months). The consequent absence of normal fetal signals to initiate parturition and the lack of periparturient endocrine changes contributed to prolonged gestation and underdeveloped mammary glands in the dam. This report represents the first detailed description of intrauterine umbilical cord abnormalities in cattle, demonstrating their potential to cause fetal developmental delay, prolonged gestation, and impaired mammary gland development.

KEYWORDS

bovine fetus, umbilical cord torsion, umbilical cord entanglement, fetal growth restriction, maternal serum analysis, parturition

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Amal M. Aboelmaaty,
National Research Centre, Egypt

REVIEWED BY
Carolina Smok,
Universidad de La Frontera, Chile
Jagmohan Rajput,
Nanaji Deshmukh Veterinary Science University, India

*CORRESPONDENCE
Tomochika Sugiura
✉ t-sugiura@rakuno.ac.jp

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最終責任者　Tomochika Sugiura (Corresponding Author)

獣医解剖学（Veterinary Anatomy）

Naoki Takahashi

Lecture

講師　髙橋　直紀

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1）Segmented ring-mesh model of glycosaminoglycan chains based on the 3D analysis of normal individual and musculocontractural Ehlers–Danlos syndrome skin using scanning transmission electron microscopy
Takahashi N, Hirose T, Kametani K, Iwasaki T, Imamura Y, Kosho T, Watanabe T.
Microscopy. 74: 358-366. 2025. doi:10.1093/jmicro/dfaf012

II. その他　＜Others＞

1）Injury and Fibrosis at the Myoaponeurotic Junction of Pectoralis Major and Supracoracoideus Muscles in Broiler Chickens
Kawasaki T, Iwasaki T, Watanabe T, Yamada M, Maeda N, Hasegawa Y, **Takahashi N**, Kobayashi R.
J Poult Sci. 62: 2025014. 2025 doi: 10.2141/jpsa.2025014

2）Suppression of USP2 in mouse skeletal muscle: a model of oxidative stress in muscle tissue
Fujimoto M, Iwasaki T, Hosotani Saito M, **Takahashi N**, Hashimoto M, Takahashi E, Kitamura H.
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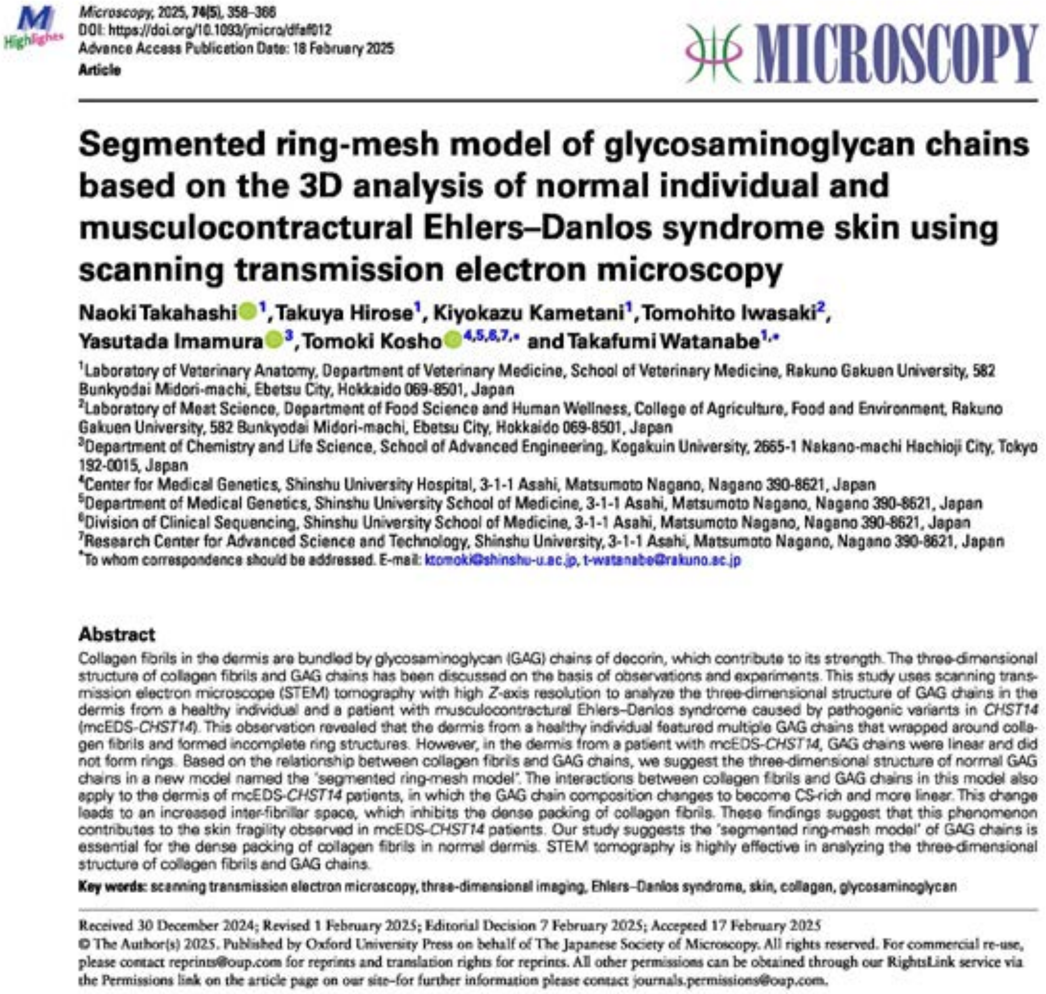

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Article

Segmented ring-mesh model of glycosaminoglycan chains based on the 3D analysis of normal individual and musculocontractural Ehlers–Danlos syndrome skin using scanning transmission electron microscopy

Naoki Takahashi[1], Takuya Hirose[1], Kiyokazu Kametani[1], Tomohito Iwasaki[2], Yasutada Imamura[3], Tomoki Kosho[4,5,6,7,*] and Takafumi Watanabe[1,*]

[1]Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midori-machi, Ebetsu City, Hokkaido 069-8501, Japan
[2]Laboratory of Meat Science, Department of Food Science and Human Wellness, College of Agriculture, Food and Environment, Rakuno Gakuen University, 582 Bunkyodai Midori-machi, Ebetsu City, Hokkaido 069-8501, Japan
[3]Department of Chemistry and Life Science, School of Advanced Engineering, Kogakuin University, 2665-1 Nakano-machi Hachioji City, Tokyo 192-0015, Japan
[4]Center for Medical Genetics, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[5]Department of Medical Genetics, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[6]Division of Clinical Sequencing, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[7]Research Center for Advanced Science and Technology, Shinshu University, 3-1-1 Asahi, Matsumoto Nagano, Nagano 390-8621, Japan
[*]To whom correspondence should be addressed. E-mail: ktomoki@shinshu-u.ac.jp, t-watanabe@rakuno.ac.jp

Abstract

Collagen fibrils in the dermis are bundled by glycosaminoglycan (GAG) chains of decorin, which contribute to its strength. The three-dimensional structure of collagen fibrils and GAG chains has been discussed on the basis of observations and experiments. This study uses scanning transmission electron microscope (STEM) tomography with high *Z*-axis resolution to analyze the three-dimensional structure of GAG chains in the dermis from a healthy individual and a patient with musculocontractural Ehlers–Danlos syndrome caused by pathogenic variants in *CHST14* (mcEDS-*CHST14*). This observation revealed that the dermis from a healthy individual featured multiple GAG chains that wrapped around collagen fibrils and formed incomplete ring structures. However, in the dermis from a patient with mcEDS-*CHST14*, GAG chains were linear and did not form rings. Based on the relationship between collagen fibrils and GAG chains, we suggest the three-dimensional structure of normal GAG chains in a new model named the 'segmented ring-mesh model'. The interactions between collagen fibrils and GAG chains in this model also apply to the dermis of mcEDS-*CHST14* patients, in which the GAG chain composition changes to become CS-rich and more linear. This change leads to an increased inter-fibrillar space, which inhibits the dense packing of collagen fibrils. These findings suggest that this phenomenon contributes to the skin fragility observed in mcEDS-*CHST14* patients. Our study suggests the 'segmented ring-mesh model' of GAG chains is essential for the dense packing of collagen fibrils in normal dermis. STEM tomography is highly effective in analyzing the three-dimensional structure of collagen fibrils and GAG chains.

Key words: scanning transmission electron microscopy, three-dimensional imaging, Ehlers–Danlos syndrome, skin, collagen, glycosaminoglycan

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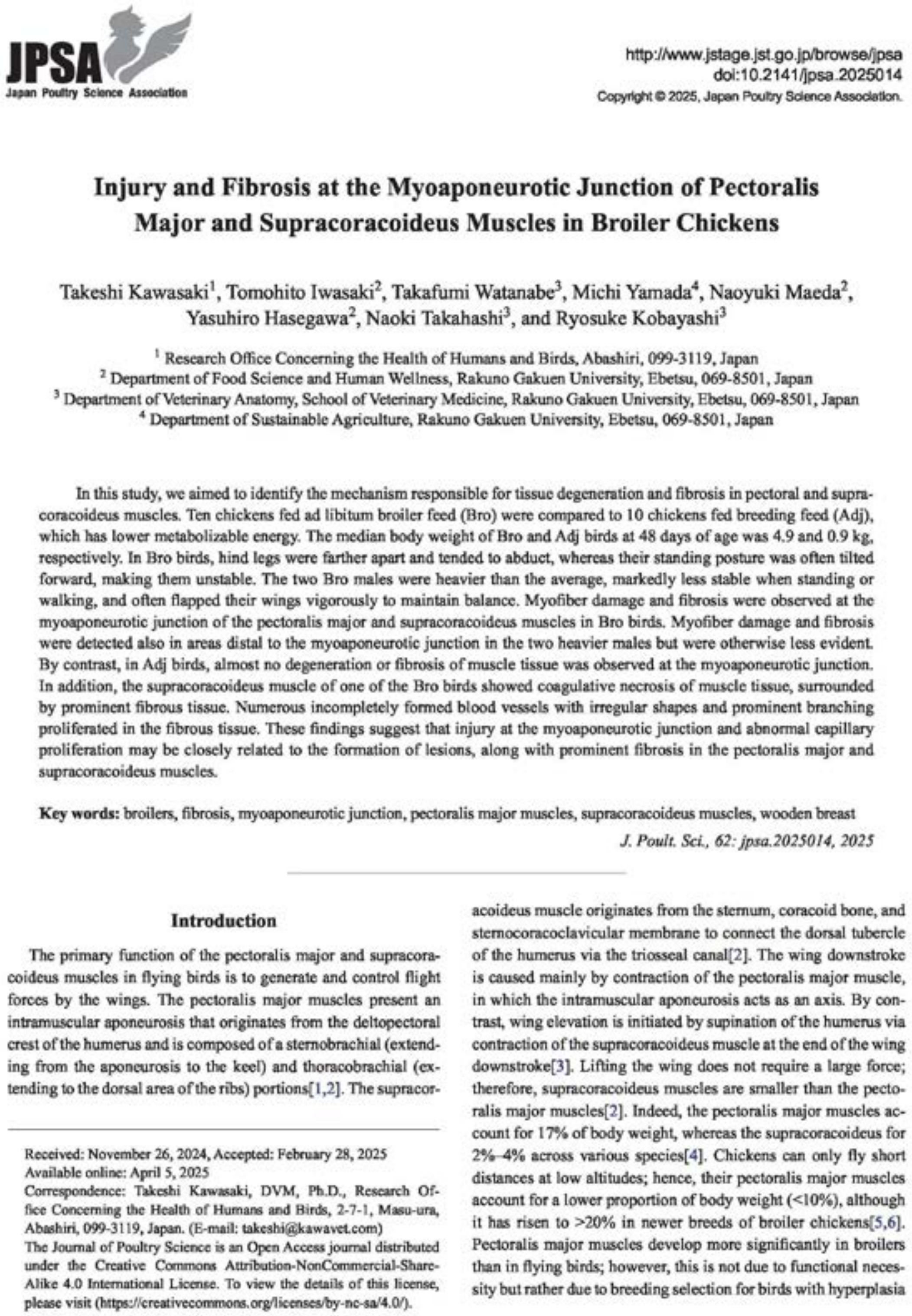

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Injury and Fibrosis at the Myoaponeurotic Junction of Pectoralis Major and Supracoracoideus Muscles in Broiler Chickens

Takeshi Kawasaki[1], Tomohito Iwasaki[2], Takafumi Watanabe[3], Michi Yamada[4], Naoyuki Maeda[2],
Yasuhiro Hasegawa[2], Naoki Takahashi[3], and Ryosuke Kobayashi[3]

[1] Research Office Concerning the Health of Humans and Birds, Abashiri, 099-3119, Japan
[2] Department of Food Science and Human Wellness, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[3] Department of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[4] Department of Sustainable Agriculture, Rakuno Gakuen University, Ebetsu, 069-8501, Japan

In this study, we aimed to identify the mechanism responsible for tissue degeneration and fibrosis in pectoral and supracoracoideus muscles. Ten chickens fed ad libitum broiler feed (Bro) were compared to 10 chickens fed breeding feed (Adj), which has lower metabolizable energy. The median body weight of Bro and Adj birds at 48 days of age was 4.9 and 0.9 kg, respectively. In Bro birds, hind legs were farther apart and tended to abduct, whereas their standing posture was often tilted forward, making them unstable. The two Bro males were heavier than the average, markedly less stable when standing or walking, and often flapped their wings vigorously to maintain balance. Myofiber damage and fibrosis were observed at the myoaponeurotic junction of the pectoralis major and supracoracoideus muscles in Bro birds. Myofiber damage and fibrosis were detected also in areas distal to the myoaponeurotic junction in the two heavier males but were otherwise less evident. By contrast, in Adj birds, almost no degeneration or fibrosis of muscle tissue was observed at the myoaponeurotic junction. In addition, the supracoracoideus muscle of one of the Bro birds showed coagulative necrosis of muscle tissue, surrounded by prominent fibrous tissue. Numerous incompletely formed blood vessels with irregular shapes and prominent branching proliferated in the fibrous tissue. These findings suggest that injury at the myoaponeurotic junction and abnormal capillary proliferation may be closely related to the formation of lesions, along with prominent fibrosis in the pectoralis major and supracoracoideus muscles.

Key words: broilers, fibrosis, myoaponeurotic junction, pectoralis major muscles, supracoracoideus muscles, wooden breast

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Introduction

The primary function of the pectoralis major and supracoracoideus muscles in flying birds is to generate and control flight forces by the wings. The pectoralis major muscles present an intramuscular aponeurosis that originates from the deltopectoral crest of the humerus and is composed of a sternobrachial (extending from the aponeurosis to the keel) and thoracobrachial (extending to the dorsal area of the ribs) portions[1,2]. The supracoracoideus muscle originates from the sternum, coracoid bone, and sternocoracoclavicular membrane to connect the dorsal tubercle of the humerus via the triosseal canal[2]. The wing downstroke is caused mainly by contraction of the pectoralis major muscle, in which the intramuscular aponeurosis acts as an axis. By contrast, wing elevation is initiated by supination of the humerus via contraction of the supracoracoideus muscle at the end of the wing downstroke[3]. Lifting the wing does not require a large force; therefore, supracoracoideus muscles are smaller than the pectoralis major muscles[2]. Indeed, the pectoralis major muscles account for 17% of body weight, whereas the supracoracoideus for 2%–4% across various species[4]. Chickens can only fly short distances at low altitudes; hence, their pectoralis major muscles account for a lower proportion of body weight (<10%), although it has risen to >20% in newer breeds of broiler chickens[5,6]. Pectoralis major muscles develop more significantly in broilers than in flying birds; however, this is not due to functional necessity but rather due to breeding selection for birds with hyperplasia

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Correspondence: Takeshi Kawasaki, DVM, Ph.D., Research Office Concerning the Health of Humans and Birds, 2-7-1, Masu-ura, Abashiri, 099-3119, Japan. (E-mail: takeshi@kawavet.com)

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Original

Suppression of USP2 in mouse skeletal muscle: a model of oxidative stress in muscle tissue

Masaki FUJIMOTO1), Tomohito IWASAKI2), Marina Hosotani SAITO3), Naoki TAKAHASHI4),
Mayuko HASHIMOTO5), Eiki TAKAHASHI6) and Hiroshi KITAMURA7)

1)Laboratory of Disease Models, School of Veterinary Medicines, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan
2)Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan
3)Department of Otolaryngology, University of Texas Medical Branch, 400 Harborside Dr Unit 123, Galveston, Texas 77555-0521, USA
4)Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan
5)Laboratory of Immunology, Faculty of Pharmacy, Osaka Ohtani University, Kita 3-11-1, Nishikiori, Tondabayashi, Osaka 584-8540, Japan
6)Department of Biomedicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan
7)Department of Laboratory Animal Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan

Abstract: Emerging evidence indicates that oxidative stress in skeletal muscle is a prerequisite for sarcopenia in diabetic patients. In this study, we show that ubiquitin-specific protease (USP) 2 mitigates the accumulation of reactive oxygen species (ROS) in mature muscle cells. Treatment with ML364, a canonical USP2 inhibitor, robustly increased mitochondrial ROS in mouse C2C12 myotubes and caused an accompanying increase in the glutathione disulfide (GSSG)/glutathione (GSH) ratio. ML364 also caused mitochondrial damage in C2C12 myotubes, resulting in a reduction in intracellular adenosine triphosphate levels. Correspondingly, under diabetic condition, the muscle-specific *Usp2*-knockout (ms*Usp2*KO) C57BL/6N mice exhibited a significantly higher lipid peroxide level and GSSG/GSH ratio in skeletal muscle than the control mice. The ms*Usp2*KO mice also exhibited augmented insulin resistance and glucose intolerance, but showed no obvious deterioration in muscle weight or histology relative to the control mice. However, damaged mitochondria in the soleus muscle were more frequently observed in ms*Usp2*KO mice than in the control mice. Together, these data suggest that USP2 mitigates ROS accumulation and subsequent mitochondrial damage in muscle cells in mice.

Key words: diabetes, oxidative stress, skeletal muscle, ubiquitin-specific protease (USP) 2

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Corresponding author: H. Kitamura. email: hiroshi.kitamura.d8@tohoku.ac.jp
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最終責任者　Hiroshi Kitamura (Corresponding Author)

伴侶動物内科学（Companion Animal Internal Medicine）

Masahiro Tamura

Lecturer

講師　田村　昌大

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1) Evaluating the influence of hepatic triglyceride and lipoprotein lipases on lipase assays used to diagnose pancreatitis in dogs and cats.
Harada H, Tamura M, Ishii K, Osuga T, **Ohta H**.*Vet Clin Pathol.* 54:132-141. 2025.　doi: 10.1111/vcp.70002.

Ⅱ. その他　＜Others＞

1）Prospective estimation of the prevalence of thromboembolism in dogs with inflammatory protein-losing enteropathy.
Oishi N, Ohta H, **Tamura M,** Hanazono K, Miyoshi K, Yokoyama N, Shinbo G.
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Am J Vet Res. 2025;86:ajvr.25.04.0138. doi: 10.2460/ajvr.25.04.0138.
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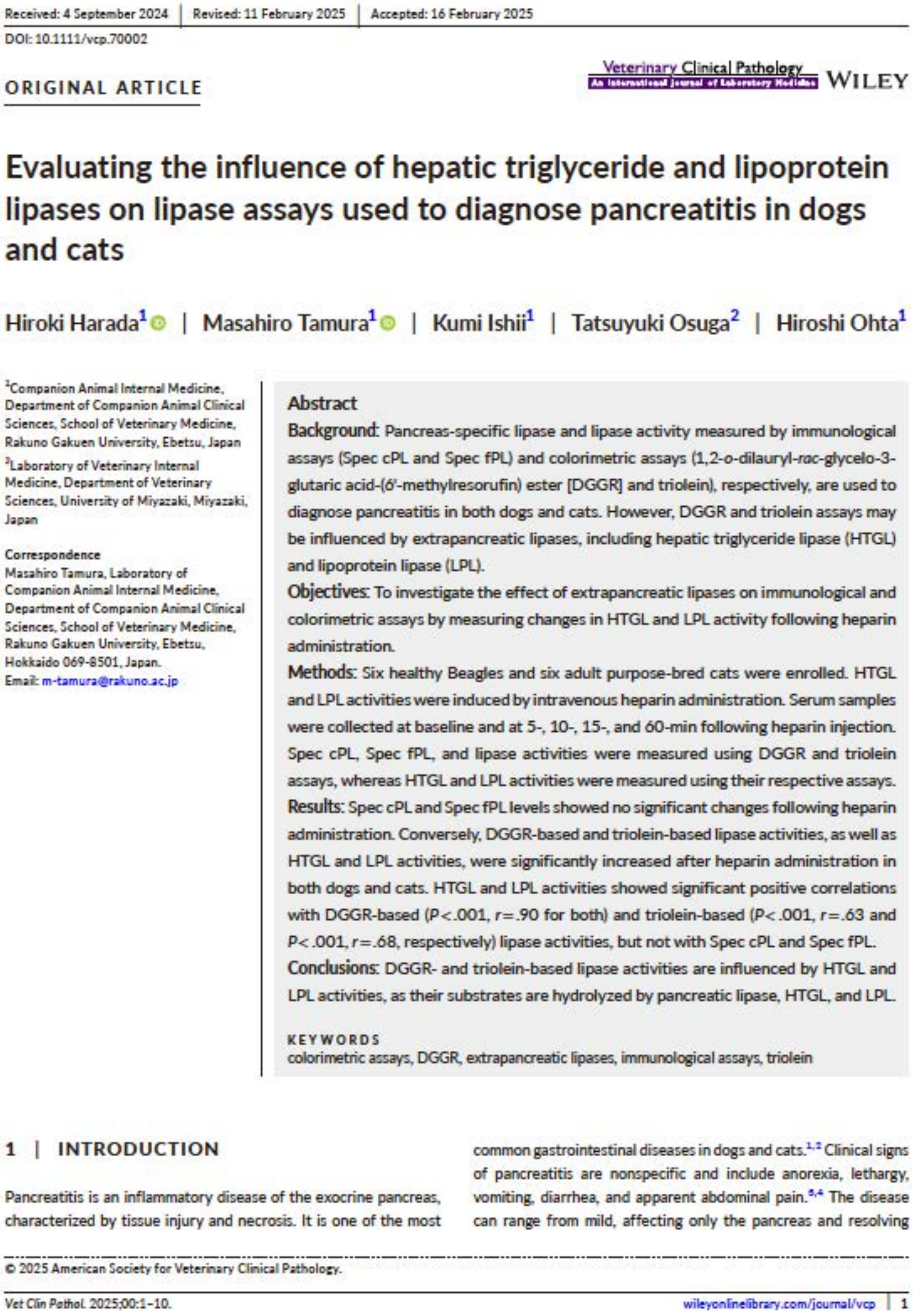

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Evaluating the influence of hepatic triglyceride and lipoprotein lipases on lipase assays used to diagnose pancreatitis in dogs and cats

Hiroki Harada[1] | Masahiro Tamura[1] | Kumi Ishii[1] | Tatsuyuki Osuga[2] | Hiroshi Ohta[1]

[1]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

[2]Laboratory of Veterinary Internal Medicine, Department of Veterinary Sciences, University of Miyazaki, Miyazaki, Japan

Correspondence
Masahiro Tamura, Laboratory of Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan.
Email: m-tamura@rakuno.ac.jp

Abstract

Background: Pancreas-specific lipase and lipase activity measured by immunological assays (Spec cPL and Spec fPL) and colorimetric assays (1,2-*o*-dilauryl-*rac*-glycelo-3-glutaric acid-(6'-methylresorufin) ester [DGGR] and triolein), respectively, are used to diagnose pancreatitis in both dogs and cats. However, DGGR and triolein assays may be influenced by extrapancreatic lipases, including hepatic triglyceride lipase (HTGL) and lipoprotein lipase (LPL).

Objectives: To investigate the effect of extrapancreatic lipases on immunological and colorimetric assays by measuring changes in HTGL and LPL activity following heparin administration.

Methods: Six healthy Beagles and six adult purpose-bred cats were enrolled. HTGL and LPL activities were induced by intravenous heparin administration. Serum samples were collected at baseline and at 5-, 10-, 15-, and 60-min following heparin injection. Spec cPL, Spec fPL, and lipase activities were measured using DGGR and triolein assays, whereas HTGL and LPL activities were measured using their respective assays.

Results: Spec cPL and Spec fPL levels showed no significant changes following heparin administration. Conversely, DGGR-based and triolein-based lipase activities, as well as HTGL and LPL activities, were significantly increased after heparin administration in both dogs and cats. HTGL and LPL activities showed significant positive correlations with DGGR-based ($P < .001$, $r = .90$ for both) and triolein-based ($P < .001$, $r = .63$ and $P < .001$, $r = .68$, respectively) lipase activities, but not with Spec cPL and Spec fPL.

Conclusions: DGGR- and triolein-based lipase activities are influenced by HTGL and LPL activities, as their substrates are hydrolyzed by pancreatic lipase, HTGL, and LPL.

KEYWORDS
colorimetric assays, DGGR, extrapancreatic lipases, immunological assays, triolein

1 | INTRODUCTION

Pancreatitis is an inflammatory disease of the exocrine pancreas, characterized by tissue injury and necrosis. It is one of the most common gastrointestinal diseases in dogs and cats.[1,2] Clinical signs of pancreatitis are nonspecific and include anorexia, lethargy, vomiting, diarrhea, and apparent abdominal pain.[3,4] The disease can range from mild, affecting only the pancreas and resolving

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Small Animal Internal Medicine Hematology

Prospective Estimation of the Prevalence of Thromboembolism in Dogs With Inflammatory Protein-Losing Enteropathy

Nene Oishi[1] | Hiroshi Ohta[1,2] | Masahiro Tamura[1] | Kiwamu Hanazono[3] | Kenjiro Miyoshi[3] | Nozomu Yokoyama[2] | Genya Shinbo[4]

[1]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [2]Laboratory of Veterinary Internal Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan | [3]Veterinary Diagnostic Imaging, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [4]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Correspondence: Hiroshi Ohta (h-ohta@rakuno.ac.jp)

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Keywords: canine | computed tomography angiography | protein-losing enteropathy | thrombosis

ABSTRACT

Background: Inflammatory protein-losing enteropathy (iPLE) is thought to be associated with a hypercoagulable state and may predispose dogs to thromboembolism (TE). However, little information is available regarding the prevalence of TE in dogs with iPLE.

Objectives: Estimate the prevalence of TE in dogs with iPLE and collect clinical and clinicopathologic data from dogs with iPLE with and without TE.

Animals: Twenty-two client-owned dogs with iPLE.

Methods: Prospective, descriptive study. Dogs definitively diagnosed with iPLE based on standard diagnostic criteria and histopathology were recruited between January 2019 and February 2024. At the time of gastrointestinal endoscopic examination, dogs with iPLE underwent thoracic and abdominal computed tomography angiography to detect TE. Clinical (e.g., clinical severity, use of corticosteroids) and clinicopathologic (e.g., albumin concentration, coagulation parameters) data were collected from dogs with iPLE with and without TE.

Results: Thromboembolism was found in 3/22 dogs (13.6%, 95% confidence interval: 2.9–34.9) with iPLE. The three dogs with iPLE and TE had thrombi in the left external iliac artery, pulmonary artery of the right caudal lobe, and main portal vein, respectively. The dogs with thrombi in the left external iliac artery or pulmonary artery did not show any clinical signs associated with TE, whereas the dog with portal vein thrombosis had transudative peritoneal effusion.

Conclusion and Clinical Importance: We estimated the prevalence of TE in dogs with iPLE. In dogs with iPLE, TE could be underestimated because some affected dogs have subclinical TE.

Abbreviations: ALB, albumin; AT, antithrombin; CCECAI, canine chronic enteropathy clinical activity index; CIBDAI, canine inflammatory bowel disease activity index; CIE, chronic inflammatory enteropathy; CT, computed tomography; IBD, inflammatory bowel disease; iPLE, inflammatory protein-losing enteropathy; PLE, protein-losing enteropathy; PTE, pulmonary thromboembolism; RI, Reference interval; TE, thromboembolism; WSAVA, World Small Animal Veterinary Association.

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Effects of cardiac volume loading on left atrial stiffness estimated by 2-dimensional echocardiography in healthy dogs

Tatsuyuki Osuga, DVM, PhD[1]; Masahiro Tamura, DVM, PhD[1]; Noboru Sasaki, DVM, PhD[2];
Mitsuyoshi Takiguchi, DVM, PhD[2]*

[1]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan
[2]Laboratory of Veterinary Internal Medicine, Department of Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

*Corresponding author: Dr. Takiguchi (mtaki@vetmed.hokudai.ac.jp)

Objective
To elucidate the effect of cardiac volume loading, which should physiologically increase left atrial (LA) stiffness (LASt), on echocardiographically estimated LASt in healthy dogs.

Methods
6 healthy Beagle dogs were anesthetized. In each dog, a Swan-Ganz catheter was placed to measure the mean pulmonary capillary wedge pressure. Cardiac volume loading was performed by IV infusion with hydroxyethylated starch solution. Echocardiography was transthoracically conducted at the baseline and at mean pulmonary capillary wedge pressures of 10 to 15, 15 to 20, 20 to 25, and 25 to 30 mm Hg. The echocardiographic indicators of LA pressure, including the peak velocity of early diastolic transmitral flow (E), ratio of E to the isovolumic relaxation time (E/IVRT), ratio of E to the peak velocity of early diastolic mitral annular motion (E/E′), and LA reservoir strain, were determined to estimate LASt.

Results
During cardiac volume loading, E, E/IVRT, and LA reservoir strain were increased, and E/E′ was decreased, when compared with the baseline. Consequently, LASts estimated by using E, E/IVRT, and E/E′ were decreased or remained unchanged during cardiac volume loading.

Conclusions
Echocardiographically estimated LASt may not be increased by cardiac volume loading in healthy dogs.

Clinical Relevance
Healthy dogs may not be an appropriate model to elucidate the effect of cardiac volume loading on echocardiographically estimated LASt. This is problematic, especially for dogs with myxomatous mitral valve disease that are older and may have cardiac dysfunction.

Keywords: left atrial stiffness, canine, echocardiography, strain imaging, volume overload

The left atrium (LA) modulates left ventricular filling to maintain left ventricular output through its 3 phasic functions. The LA phasic function is comprised of a reservoir function (expansion associated with blood inflow from the pulmonary veins during ventricular systole), a conduit function (passage of blood from the pulmonary veins to the left ventricle during ventricular diastole), and a booster-pump function (active contraction during late ventricular diastole).[1] In dogs with myxomatous mitral valve disease, the most common heart disease of dogs, we and other researchers have shown that deterioration of the LA phasic function evaluated by echocardiography is associated with increased disease severity[2–5] and shortened survival time.[5–7] Common methods for echocardiographic evaluation of the LA phasic function in dogs include the calculation of fractional changes of the LA size (eg, area, volume) and determination of LA strains and strain rates with the use of tissue Doppler imaging or 2-D speckle tracking echocardiography (2D-STE).[2–8]

Preload dependency of the fractional changes of the LA size and LA strains and strain rates can be problematic when evaluating the LA phasic function. Preload dependency could be most markedly of concern for dogs with myxomatous mitral valve disease because deterioration of the LA phasic

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最終責任者　Mitsuyoshi Takiguchi (Corresponding Author)

Masaki Fujimoto

Assistant Professor

助教　藤本　政毅

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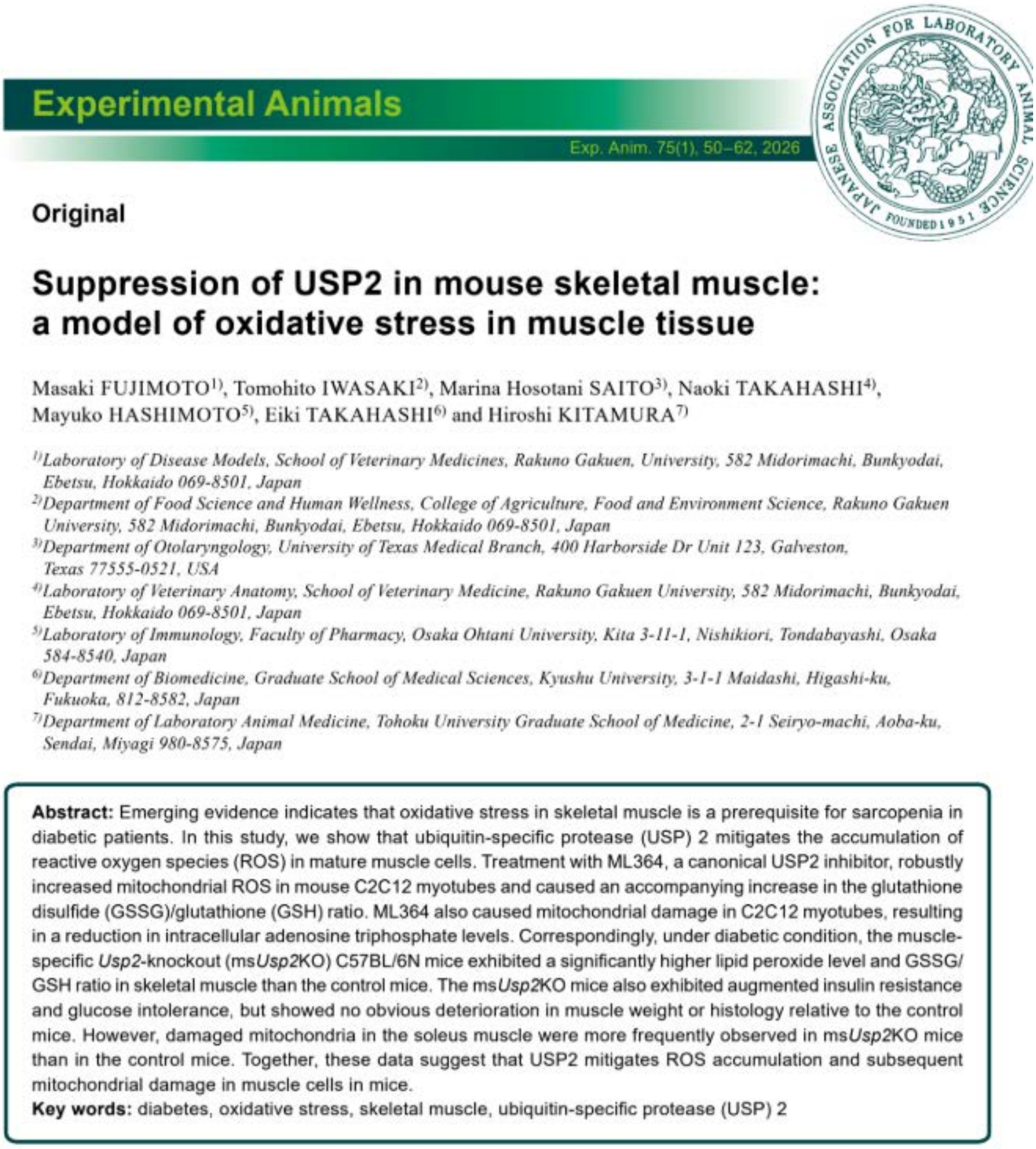
1）Suppression of USP2 in mouse skeletal muscle: a model of oxidative stress in muscle tissue.
Fujimoto M, Iwasaki T, Hosotani Saito M, Hashimoto M, Takahashi E, Kitamura H
Exp Anim. 75: 50-62. 2025. https://doi.org/10.1538/expanim.25-0032

II. その他　＜Others＞

1）Muscular USP2 is dispensable for MASLD-associated disorders in the liver and skeletal muscle in mice
Iida A, **Fujimoto M**, Kitamura H
Biomed Res. 46:203-214. 2025. https://doi.org/10.2220/biomedres.46.203



Experimental Animals

Exp. Anim. 75(1), 50–62, 2026

Original

Suppression of USP2 in mouse skeletal muscle: a model of oxidative stress in muscle tissue

Masaki FUJIMOTO[1], Tomohito IWASAKI[2], Marina Hosotani SAITO[3], Naoki TAKAHASHI[4], Mayuko HASHIMOTO[5], Eiki TAKAHASHI[6] and Hiroshi KITAMURA[7]

[1]*Laboratory of Disease Models, School of Veterinary Medicines, Rakuno Gakuen, University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*

[2]*Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*

[3]*Department of Otolaryngology, University of Texas Medical Branch, 400 Harborside Dr Unit 123, Galveston, Texas 77555-0521, USA*

[4]*Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*

[5]*Laboratory of Immunology, Faculty of Pharmacy, Osaka Ohtani University, Kita 3-11-1, Nishikiori, Tondabayashi, Osaka 584-8540, Japan*

[6]*Department of Biomedicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan*

[7]*Department of Laboratory Animal Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan*

Abstract: Emerging evidence indicates that oxidative stress in skeletal muscle is a prerequisite for sarcopenia in diabetic patients. In this study, we show that ubiquitin-specific protease (USP) 2 mitigates the accumulation of reactive oxygen species (ROS) in mature muscle cells. Treatment with ML364, a canonical USP2 inhibitor, robustly increased mitochondrial ROS in mouse C2C12 myotubes and caused an accompanying increase in the glutathione disulfide (GSSG)/glutathione (GSH) ratio. ML364 also caused mitochondrial damage in C2C12 myotubes, resulting in a reduction in intracellular adenosine triphosphate levels. Correspondingly, under diabetic condition, the muscle-specific *Usp2*-knockout (ms*Usp2*KO) C57BL/6N mice exhibited a significantly higher lipid peroxide level and GSSG/GSH ratio in skeletal muscle than the control mice. The ms*Usp2*KO mice also exhibited augmented insulin resistance and glucose intolerance, but showed no obvious deterioration in muscle weight or histology relative to the control mice. However, damaged mitochondria in the soleus muscle were more frequently observed in ms*Usp2*KO mice than in the control mice. Together, these data suggest that USP2 mitigates ROS accumulation and subsequent mitochondrial damage in muscle cells in mice.

Key words: diabetes, oxidative stress, skeletal muscle, ubiquitin-specific protease (USP) 2

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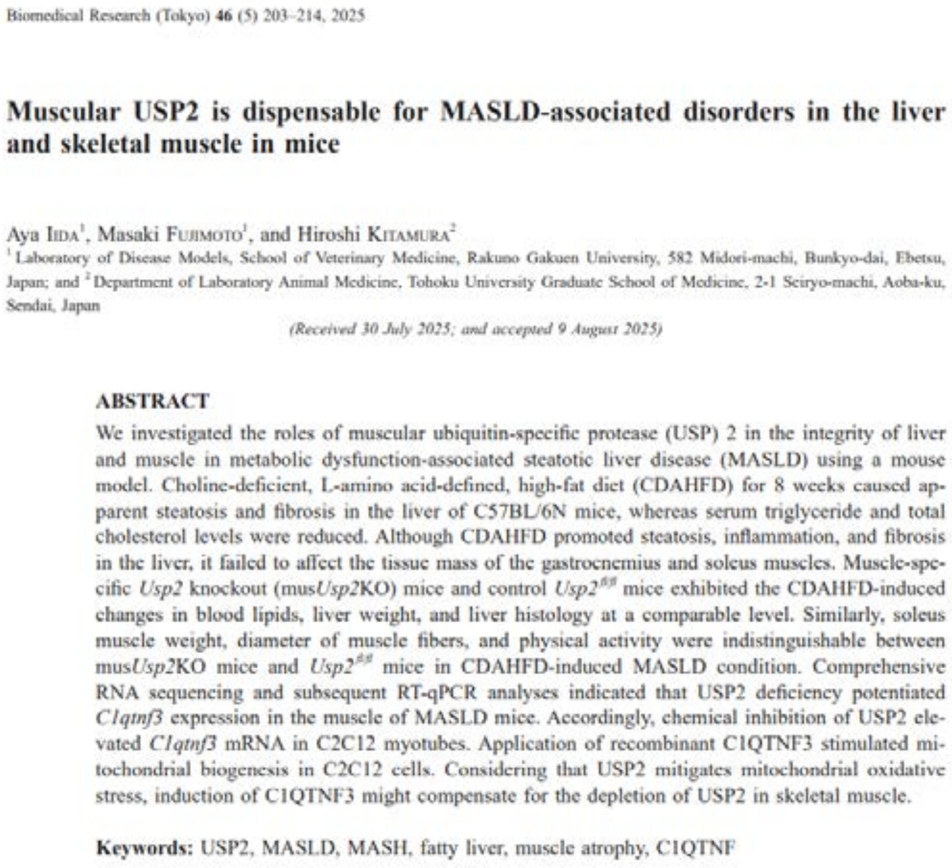

Biomedical Research (Tokyo) **46** (5) 203–214, 2025

Muscular USP2 is dispensable for MASLD-associated disorders in the liver and skeletal muscle in mice

Aya IIDA[1], Masaki FUJIMOTO[1], and Hiroshi KITAMURA[2]
[1] Laboratory of Disease Models, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midori-machi, Bunkyo-dai, Ebetsu, Japan; and [2] Department of Laboratory Animal Medicine, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Japan

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ABSTRACT

We investigated the roles of muscular ubiquitin-specific protease (USP) 2 in the integrity of liver and muscle in metabolic dysfunction-associated steatotic liver disease (MASLD) using a mouse model. Choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD) for 8 weeks caused apparent steatosis and fibrosis in the liver of C57BL/6N mice, whereas serum triglyceride and total cholesterol levels were reduced. Although CDAHFD promoted steatosis, inflammation, and fibrosis in the liver, it failed to affect the tissue mass of the gastrocnemius and soleus muscles. Muscle-specific *Usp2* knockout (mus*Usp2*KO) mice and control $Usp2^{fl/fl}$ mice exhibited the CDAHFD-induced changes in blood lipids, liver weight, and liver histology at a comparable level. Similarly, soleus muscle weight, diameter of muscle fibers, and physical activity were indistinguishable between mus*Usp2*KO mice and $Usp2^{fl/fl}$ mice in CDAHFD-induced MASLD condition. Comprehensive RNA sequencing and subsequent RT-qPCR analyses indicated that USP2 deficiency potentiated *C1qtnf3* expression in the muscle of MASLD mice. Accordingly, chemical inhibition of USP2 elevated *C1qtnf3* mRNA in C2C12 myotubes. Application of recombinant C1QTNF3 stimulated mitochondrial biogenesis in C2C12 cells. Considering that USP2 mitigates mitochondrial oxidative stress, induction of C1QTNF3 might compensate for the depletion of USP2 in skeletal muscle.

Keywords: USP2, MASLD, MASH, fatty liver, muscle atrophy, C1QTNF

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伴侶動物内科学（Companion Animal Internal Medicine）

Mayu Fujita

Assistant Professor

助教　藤田　麻由

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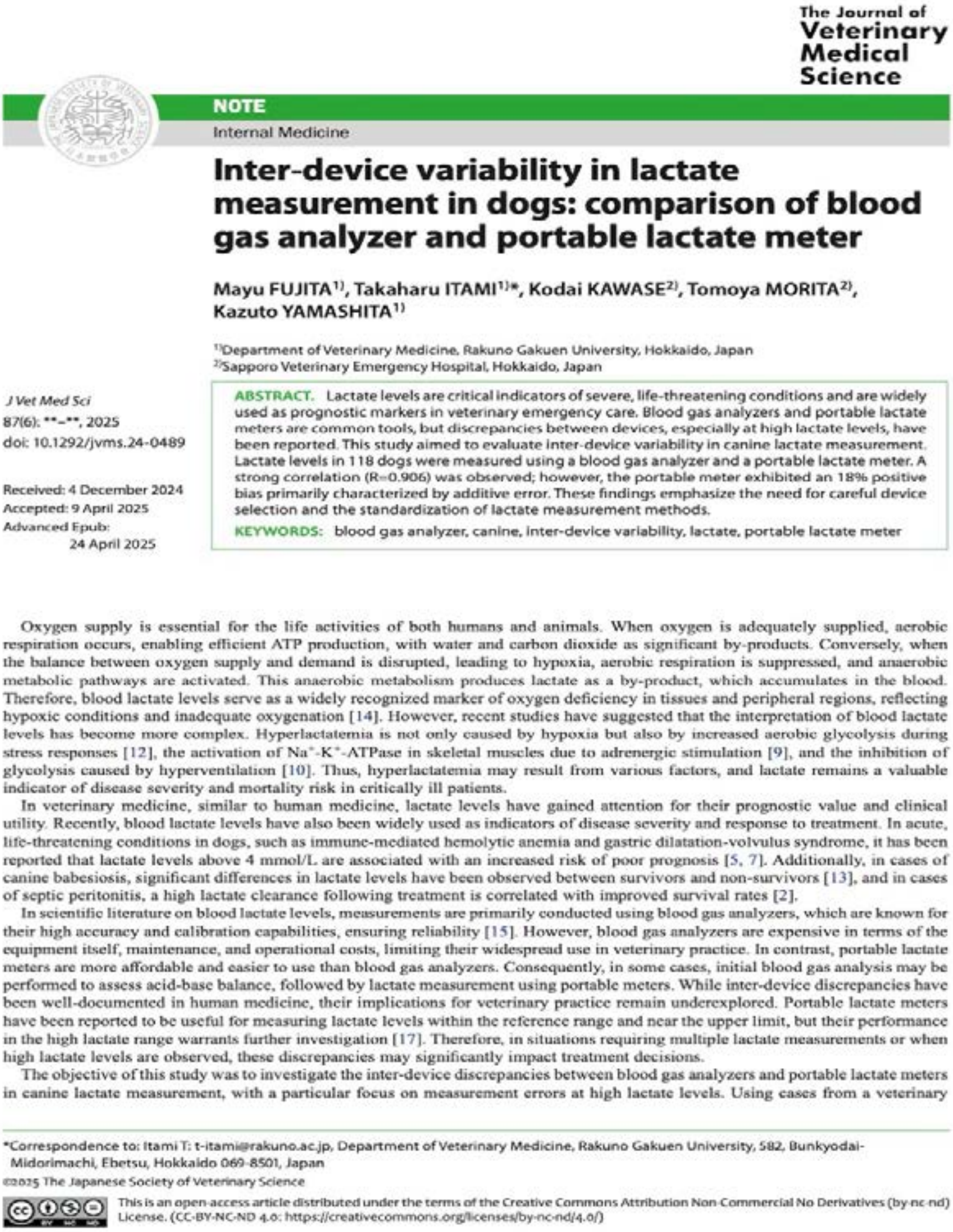
1) Inter-device variability in lactate measurement in dogs: comparison of blood gas analyzer and portable lactate meter.
Fujita M, Itami T, Kawase K, Morita T, Yamashita K.
J Vet Med Sci. 87: 697–701. 2025. doi: 10.1292/jvms.24-0489.

II. その他＜Others＞

該当なし



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Inter-device variability in lactate measurement in dogs: comparison of blood gas analyzer and portable lactate meter

Mayu FUJITA[1)], Takaharu ITAMI[1)]*, Kodai KAWASE[2)], Tomoya MORITA[2)], Kazuto YAMASHITA[1)]

[1)]Department of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]Sapporo Veterinary Emergency Hospital, Hokkaido, Japan

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ABSTRACT. Lactate levels are critical indicators of severe, life-threatening conditions and are widely used as prognostic markers in veterinary emergency care. Blood gas analyzers and portable lactate meters are common tools, but discrepancies between devices, especially at high lactate levels, have been reported. This study aimed to evaluate inter-device variability in canine lactate measurement. Lactate levels in 118 dogs were measured using a blood gas analyzer and a portable lactate meter. A strong correlation (R=0.906) was observed; however, the portable meter exhibited an 18% positive bias primarily characterized by additive error. These findings emphasize the need for careful device selection and the standardization of lactate measurement methods.

KEYWORDS: blood gas analyzer, canine, inter-device variability, lactate, portable lactate meter

Oxygen supply is essential for the life activities of both humans and animals. When oxygen is adequately supplied, aerobic respiration occurs, enabling efficient ATP production, with water and carbon dioxide as significant by-products. Conversely, when the balance between oxygen supply and demand is disrupted, leading to hypoxia, aerobic respiration is suppressed, and anaerobic metabolic pathways are activated. This anaerobic metabolism produces lactate as a by-product, which accumulates in the blood. Therefore, blood lactate levels serve as a widely recognized marker of oxygen deficiency in tissues and peripheral regions, reflecting hypoxic conditions and inadequate oxygenation [14]. However, recent studies have suggested that the interpretation of blood lactate levels has become more complex. Hyperlactatemia is not only caused by hypoxia but also by increased aerobic glycolysis during stress responses [12], the activation of Na^+-K^+-ATPase in skeletal muscles due to adrenergic stimulation [9], and the inhibition of glycolysis caused by hyperventilation [10]. Thus, hyperlactatemia may result from various factors, and lactate remains a valuable indicator of disease severity and mortality risk in critically ill patients.

In veterinary medicine, similar to human medicine, lactate levels have gained attention for their prognostic value and clinical utility. Recently, blood lactate levels have also been widely used as indicators of disease severity and response to treatment. In acute, life-threatening conditions in dogs, such as immune-mediated hemolytic anemia and gastric dilatation-volvulus syndrome, it has been reported that lactate levels above 4 mmol/L are associated with an increased risk of poor prognosis [5, 7]. Additionally, in cases of canine babesiosis, significant differences in lactate levels have been observed between survivors and non-survivors [13], and in cases of septic peritonitis, a high lactate clearance following treatment is correlated with improved survival rates [2].

In scientific literature on blood lactate levels, measurements are primarily conducted using blood gas analyzers, which are known for their high accuracy and calibration capabilities, ensuring reliability [15]. However, blood gas analyzers are expensive in terms of the equipment itself, maintenance, and operational costs, limiting their widespread use in veterinary practice. In contrast, portable lactate meters are more affordable and easier to use than blood gas analyzers. Consequently, in some cases, initial blood gas analysis may be performed to assess acid-base balance, followed by lactate measurement using portable meters. While inter-device discrepancies have been well-documented in human medicine, their implications for veterinary practice remain underexplored. Portable lactate meters have been reported to be useful for measuring lactate levels within the reference range and near the upper limit, but their performance in the high lactate range warrants further investigation [17]. Therefore, in situations requiring multiple lactate measurements or when high lactate levels are observed, these discrepancies may significantly impact treatment decisions.

The objective of this study was to investigate the inter-device discrepancies between blood gas analyzers and portable lactate meters in canine lactate measurement, with a particular focus on measurement errors at high lactate levels. Using cases from a veterinary

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*Correspondence to: Itami T: t-itami@rakuno.ac.jp, Department of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

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最終責任者 Takaharu Itami (Corresponding Author)

画像診断学（Veterinary Diagnostic Imaging）

Yoshimichi Goda

Assistant Professor

助教　五田　嘉倫

I. 筆頭または責任著者　＜First or Corresponding Author＞

1) Shannon entropy as a stable diffusion tensor parameter for evaluating normal spinal cord regions in dogs.
   Goda Y, Mizutani Y, Hirayama M, Asanuma T, Satoh H.
   Res Vet Sci. 200:106036. doi: 10.1016/j.rvsc.2025.106036.
2) Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: pilot study.
   Goda Y, Yamamoto S, Miyoshi K, Hanazono K, Morimura M, Sakai T, Tamura M, Deguchi T, Endo Y, Kadosawa T, Fujita M, Itami T, Torisu S.
   Open Vet J. 15:5739-5747. doi: 10.5455/OVJ.2025.v15.i11.29.

II. その他　＜Others＞

1) Computed tomography-based thymoma-to-cranial intrathoracic volume ratio predicts the feasibility of video-assisted thoracoscopic surgery in a small dog: A case report.
   Mizutani S, Kageyama S, **Goda Y**, Okamura Y, Mitsui I, Akashi N, Ohnishi A, Kanda T, Asanuma T.
   Open Vet J. 15:4744-4749. doi: 10.5455/OVJ.2025.v15.i9.79.
2) Dynamic Computed Tomography for detecting pulmonary cysts in canine Paragonimiasis: a case report.
   Mizutani S, Mizutani Y, **Goda Y**, Satoh H, Asanuma T, Yoshida A, Torisu S.
   J Vet Med Sci. 87: 986-989. doi: 10.1292/jvms.25-0169.

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Can Vet J. 66:267-273.

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Shannon entropy as a stable diffusion tensor parameter for evaluating normal spinal cord regions in dogs

Yoshimichi Goda [a,1], Yuko Mizutani [b], Mizuki Hirayama [c], Taketoshi Asanuma [d], Hiroyuki Satoh [a,c,*]

[a] Graduate School of Medicine and Veterinary Medicine, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan
[b] Veterinary Teaching Hospital, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan
[c] Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan
[d] Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan

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ABSTRACT

Diffusion tensor imaging (DTI) has been used in veterinary medicine for cerebral infarctions and degenerative diseases in recent years. Defining normal values for the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) to detect lesions is challenging because these values depend on anatomical differences in the spinal cord, magnetic resonance imaging (MRI) machine model, magnetic field strength, and measurement protocol. Shannon entropy is the average amount of information and is a measure of the variability or complexity, of distributed numbers. The purpose of this study is to evaluate whether Shannon entropy can serve as a useful new indicator for interpreting the structural integrity of the canine spinal cord. We used six beagle dogs with no spinal cord disease, as confirmed by neurological examination and magnetic resonance imaging. We calculated the respective values for the cervical and thoracolumbar spinal cord under the 3 T magnetic field. The ADC and FA values (mean ± standard error) of the cervical spinal cord were $1.11 \pm 0.05 \times 10^{-3}$ mm^2/s and 0.64 ± 0.02. The thoracolumbar spinal cord had values of $1.16 \pm 0.04 \times 10^{-3}$ mm^2/s and 0.58 ± 0.02. The ADC and FA values of the spinal cord reflected anatomical differences, and no fixed normal values were observed for the entire spinal cord. Shannon entropy was 5.67 ± 0.45. No significant differences were observed in the values at any intervertebral level. These results suggest that Shannon entropy is more useful than the ADC and FA values. Shannon entropy may be able to differentiate between normal tissue and lesions, independent of various MRI conditions.

1. Introduction

Conventional magnetic resonance imaging (MRI) is difficult to accurately diagnose and detect lesions in acute stroke and degenerative disease [Aoki et al., 2010; Bellomo et al., 2025; Burdette et al., 1999; Coates et al., 2007; Hobert et al., 2013; Naito et al., 2022]. Diffusion tensor imaging (DTI), an imaging technique that visualizes the diffusion anisotropy of water molecules in tissues, is useful for diagnosis [Bellomo et al., 2025; Boudreau et al., 2022; Sumei et al., 2006; Okamoto et al., 2000]. The diffusion of water molecules is a Brownian motion, which is the random motion of fine particles in a solvent [Hashemi et al., 2004]. The apparent diffusion coefficient (ADC) value, an index of the diffusion degree, and the fractional anisotropy (FA) value, an index of the strength of diffusion anisotropy, are known parameters of DTI. In recent years, ADC and FA values have been reported in veterinary medicine for various neurological diseases [Lewis et al., 2021; Pease and Miller, 2011; Shinn et al., 2022] and normal parameter measurements have also been reported [Arai et al., 2023; Hobert et al., 2013]. However, no

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Abbreviations: ADC, Apparent diffusion coefficient; CSM, Cervical spondylotic myelopathy; DTI, Diffusion tensor imaging; EPI, Echo-planar imaging; FA, Fractional anisotropy; FOV, Field of view; MRI, Magnetic resonance imaging; ROI, Regions of interest; SE, Spin echo; TE, Echo time; TR, Repetition time.

[*] Corresponding author at: Graduate School of Medicine and Veterinary Medicine, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan.

E-mail address: hsatoh@miyazaki-u.ac.jp (H. Satoh).

[1] Present affiliation: Laboratory of Veterinary Diagnostic Imaging, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan.

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Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study

Yoshimichi Goda[1#], Shushi Yamamoto[1#], Kenjiro Miyoshi[1], Kiwamu Hanazono[1], Mai Morimura[1], Toshikazu Sakai[1], Masahiro Tamura[1], Tatsuya Deguchi[1], Yoshifumi Endo[1,2], Tsuyoshi Kadosawa[1,3], Mayu Fujita[1], Takaharu Itami[1] and Shidow Torisu[1*]

[1]*Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*
[2]*Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, Japan*
[3]*Japan Small Animal Medical Center, Tokorozawa-Shi, Japan*
[#]*Both authors are equally contributed to this work*

ABSTRACT

Background: Recently, the usefulness of triple-phase contrast-enhanced computed tomography (CT) scans for liver tumors in canines has been reported. However, detailed information on the CT findings of cholangiocarcinoma (CCA) and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) remains limited.

Aim: This study aimed to retrospectively evaluate triple-phase contrast-enhanced CT findings in canine CCA and cHCC-CCA using objective parameters, such as CT values and morphological characteristics.

Methods: This study included eight dogs that underwent triple-phase contrast-enhanced CT scans and surgical removal and were pathologically diagnosed. Three CCA cases and five cHCC-CCA cases were analyzed. Parameters included CT values and mass sizes, CT values and lymph node sizes, and rim enhancement. "Rim enhancement" was defined as the contrast enhancement effect at the liver parenchyma–mass boundary.

Results: CT values for CCA were as follows: pre-contrast: 43.9 ± 3.6 Hounsfield units (HUs); arterial phase: 76.9 ± 22.5 HU; portal phase: 98.8 ± 37.7 HU; equilibrium phase: 90.9 ± 27.1 HU. For cHCC-CCA, the mean CT values were as follows: pre-contrast: 50.7 ± 6.1 HU; arterial phase: 80.2 ± 19.2 HU; portal phase: 95.0 ± 21.3 HU; equilibrium phase: 86.1 ± 13.4 HU. The peak contrast enhancement for both CCA and cHCC-CCA was in the portal phase. Rim enhancement appeared in the arterial phase in all CCA cases, whereas it appeared in the portal phase in 80% of cHCC-CCA cases, indicating a significant difference.

Conclusion: Rim enhancement may help distinguish between CCA and cHCC-CCA. However, this study has a limited number of cases, and future large-scale, multicenter studies are warranted.

Keywords: Canine, Cholangiocarcinoma, Combined hepatocellular-cholangiocarcinoma, Computed tomography, Dog.

Introduction

Primary hepatic tumors in canines are rare, accounting for 0.6%–1.3% of all canine tumors (Patnaik *et al.*, 1981). The most common malignant liver tumor is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma (CCA) (Liptak *et al.*, 2019). Unlike in humans, the cause of HCC in dogs remains unknown, and the risk of occurrence based on breed or sex is unclear. However, there are reports indicating a higher incidence in male dogs. Infection with trematodes has been reported as a risk factor for the occurrence of CCA, but cases have also been reported outside the infection area, and the relationship remains unclear (Liptak *et al.*, 2019). Combined hepatocellular-cholangiocarcinoma (cHCC-CCA), which shows the histological features of both HCC and CCA, has also been reported (Patnaik *et al.*, 1980; Trigo *et al.*, 1982; Terai *et al.*, 2022). It has been reported that among malignant canine hepatic tumors, the incidence of HCC is 77%, CCA is 9%–41%, and HCC-cCCA is 4.6%, respectively (Liptak *et al.*, 2019; Terai *et al.*, 2022). The diagnosis of HCC and CCA involves blood tests, X-ray, and ultrasonography. As advanced examinations, triple-phase contrast-enhanced computed tomography (CT) and magnetic

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***Corresponding Author:** Shidow Torisu. Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan. Email: *s-torisu@rakuno.ac.jp*

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最終責任者 Sidow Torisu (Corresponding Author)

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Computed tomography-based thymoma-to-cranial intrathoracic volume ratio predicts the feasibility of video-assisted thoracoscopic surgery in a small dog: A case report

Shinya Mizutani[1]*, Saki Kageyama[2], Yoshimichi Goda[3], Yasuhiko Okamura[1], Ikki Mitsui[4], Natsuki Akashi[1], Akihiro Ohnishi[1], Teppei Kanda[1,2] and Taketoshi Asanuma[1]

[1]*Department of Small Animal Surgery, Field of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Japan*
[2]*Graduate School of Veterinary Science, Okayama University of Science, Imabari, Japan*
[3]*Laboratory of Veterinary Diagnostic Imaging, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*
[4]*No Boundaries Animal Pathology, Fuchu, Japan*

ABSTRACT

Background: Canine thymoma (CT) is the most common neoplastic disease of the cranial intrathoracic cavity. Reports of video-assisted thoracoscopic surgery-thymectomy (VATS-T) in dogs are increasing, but the surgical indication criteria remain unclear. This report highlights the value of a new criterion called the thymoma volume to cranial intrathoracic volume ratio (T/CI ratio) for evaluating the feasibility of VATS-T.

Case Description: A neutered 9-year-old Papillon weighing 3.5 kg had a mass of approximately 20 mm in the cranial intrathoracic cavity. The dog was in good general condition and had no clinical symptoms. Computed tomography revealed a solitary mass with clear boundaries and weak contrast enhancement. The T/CI ratio obtained from CT examination was 2.4%. The dog underwent thoracoscopic tumor resection. Pathological examination revealed that the tumor was a thymoma. The dog was discharged without any postoperative complications. The T/CI ratios obtained from CT data of dogs of the same breed and weight, which ranged from 1.1% to 4.1%, were calculated based on previous reports that measured the volume of thymomas.

Conclusion: The T/CI ratio may serve as a new objective criterion for evaluating the relationship between cranial intrathoracic volume and thymoma volume for VATS-T, regardless of dog body size. Although this case demonstrates a promising correlation between the CT-based T/CI ratio and the feasibility of VATS-T, the findings are limited to a single case. Further studies involving a larger number of patients are necessary to validate this observation.

Keywords: Computed tomography, Dog, Thoracoscopy, Video-assisted thoracoscopic surgery, Thymoma.

Introduction

Canine thymoma is a rare neoplastic disease and is considered a common tumor in the cranial intrathoracic cavity (Mayhew and Friedberg, 2008; Zitz *et al.*, 2008). Surgical resection is the primary treatment, and the available approaches are intercostal thoracotomy, median sternotomy, and Video-assisted thoracoscopic surgery-thymectomy (VATS-T) (Mayhew and Friedberg, 2008; Zitz *et al.*, 2008). Median sternotomy provides a wide surgical field but is highly invasive. In contrast, the narrow surgical fields of intercostal thoracotomy and thoracoscopic approaches make them less suitable for large or infiltrative thymomas (Atwater *et al.*, 1994; MacIver *et al.*, 2017). Cases of thymoma associated with tumor-associated syndromes (especially myasthenia gravis) in humans are managed with minimally invasive approaches whenever possible, as this improves prognosis (Wang *et al.*, 2019). VATS-T is recommended when feasible (Wang *et al.*, 2019). VATS-T has been increasingly reported in the field of veterinary medicine in recent years (Mayhew & Friedberg, 2008; MacIver *et al.*, 2017; Alwen *et al.*, 2015; Carroll *et al.*, 2024). However, its indications have not been established. We report a case of a thymoma in a small dog weighing 3.5 kg that was removed using VATS-T.

Case Details

A neutered 9-year-old male Papillon weighing 3.5 kg was referred to the Okayama University of Science Veterinary Medical Teaching Hospital for examination

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*__Corresponding Author:__ Shinya Mizutani. Department of Small Animal Surgery, Field of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Japan. Email: *s-mizutani@ous.ac.jp*

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NOTE
Parasitology

Dynamic Computed Tomography for detecting pulmonary cysts in canine Paragonimiasis: a case report

Shinya MIZUTANI[1)]*, Yuko MIZUTANI[2)], Yoshimichi GODA[3)], Hiroyuki SATOH[4)], Taketoshi ASANUMA[1)], Ayako YOSHIDA[5)], Shidow TORISU[6)]

[1)] Department of Small Animal Surgery, Field of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Ehime, Japan
[2)] University of Miyazaki Veterinary Teaching Hospital, Miyazaki, Japan
[3)] Laboratory of Veterinary Diagnostic Imaging, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[4)] Laboratory of Veterinary Clinical Radiology, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
[5)] Centre for Animal Disease Control (CADIC), University of Miyazaki, Miyazaki, Japan; Laboratory of Veterinary Parasitic Diseases, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan
[6)] Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

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ABSTRACT. An 11-year-old male French bulldog presented with incidental cystic lesions by the pulmonary CT. CT revealed two cysts in the right caudal lobe of the lung that were suspicious for emphysematous lesions. These cysts were divided into an air layer and a soft tissue-like layer. Dynamic computed tomography (DCT) of cysts revealed undulating motion in the soft tissue-like layer. The dog's rearing environment included brackish water, and it was evident that the dog had been eating brackish water crabs from the river. The dog was diagnosed with paragonimiasis. This DCT imaging revealed movement of live worms inside the cysts. This study is the first report that DCT may be useful for differentiating paragonimiasis from other pulmonary lesions.

KEYWORDS: dog, dynamic computed tomography, endemic disease, paragonimiasis, parasitic disease

Paragonimiasis is an important zoonosis caused by *Paragonimus* spp. in humans, dogs, and cats and is endemic, particularly in Asian countries [2, 18]. Paragonimiasis in dogs is primarily caused by the ingestion of a second intermediate host. However, cases involving the consumption of raw meat from wild boars and deer, which serve as paratenic hosts, have also been reported [2, 4, 6, 19]. *Paragonimus* spp. parasitize the lungs of definitive hosts (e.g., humans, dogs and cats), forming worm cysts. Symptoms of paragonimiasis include chronic respiratory symptoms such as coughing, respiratory urgency, pneumothorax, and bloody sputum [19].

Dynamic computed tomography (DCT) is an imaging technique that allows dynamic evaluation through a series of images of a single tomogram [8, 10–13]. DCT has been used to dynamically evaluate hemodynamics in tumors, information on blood flow in organs and the presence of inflammation, following contrast agent administration in humans and dogs [8, 10–13]. It has also been used to evaluate dynamic stenosis in the human lung and nasopharyngeal collapse in dogs [12, 13]. In this study, we report the use of DCT for the dynamic evaluation of *Paragonimus* spp. within the worm cysts of a dog with paragonimiasis due to natural infection.

An 11-year-old male French bulldog weighing 11.1 kg presented to our veterinary teaching hospital with epistaxis from the left nostril. At the time of presentation, the dog was in good condition, had a good appetite and had no obvious respiratory symptoms other than epistaxis from the left nostril. The dog's house was near brackish water area, and he often ate brackish water crabs from the river. Blood tests revealed no major abnormalities, and eosinophil counts were normal in our veterinary teaching hospital examination. Chest X-ray (right lateral, left lateral, ventral-dorsal) revealed no significant abnormalities, and abdominal X-ray revealed a high-density body, similar to a metallic body in the stomach. These X-ray evaluations were performed by a veterinarian trained in diagnostic imaging in our veterinary teaching hospital.

CT was then performed to examine the epistaxis in this dog. CT was performed under general anesthesia in the prone position under

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*Correspondence to: Mizutani S: s-mizutani@ous.ac.jp, Field of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan

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最終責任者　Sinya Mizutani (Corresponding Author)

CASE REPORT
RAPPORT DE CAS

Shinya Mizutani, Yuko Mizutani,
Yoshimichi Goda, Taketoshi Asanuma,
Shidow Torisu

Usefulness of transnasal volume-reduction treatment for nasal tumors by ultrasonic emulsification suction in 2 dogs

ABSTRACT

Intranasal tumors in dogs are rare neoplastic diseases with obvious clinical signs, such as epistaxis, nasal congestion, and facial deformity. Radiation therapy is the treatment of choice but is not always accessible due to geographical location, logistics, or financial constraints. Other treatments may not be used because of various restrictions. We performed transnasal intranasal tumor volume reduction in 2 dogs with intranasal tumors; 1 dog had an adenocarcinoma and the other had a fibrosarcoma. In both cases, improvement in the quality of life (QOL), including reduced clinical signs and increased activity, was observed ~1 wk after treatment. No obvious complications were associated with this treatment. Although tumor recurrence was noted in both cases, this treatment could be applied multiple times. In conclusion, transnasal nasal tumor volume reduction is a palliative method that contributes to improvement of clinical signs and QOL.

Key clinical message:
We report palliative treatment of intranasal tumors in dogs. The treatment described in this report is available to most veterinarians and helps improve the QOL of dogs with nasal tumors, including improving respiratory status.

RÉSUMÉ

Utilité du traitement de réduction transnasale du volume pour les tumeurs nasales par émulsification et succion chez 2 chiens

Les tumeurs intranasales chez le chien sont des maladies néoplasiques rares qui présentent des symptômes cliniques évidents, tels que l'épistaxis, la congestion nasale et la déformation de la face. La radiothérapie est le traitement de choix, mais elle n'est pas toujours accessible en raison de la situation géographique ou de contraintes logistiques et financières. Cependant, ces traitements peuvent ne pas être réalisés en raison de diverses restrictions. Nous avons procédé à une réduction du volume de la tumeur intranasale par voie transnasale chez deux chiens atteints de tumeurs intranasales. Les tumeurs intranasales de la cavité nasale étaient des adénocarcinomes et des fibrosarcomes. Dans les deux cas, une amélioration de la qualité de vie, telle que l'amélioration des symptômes cliniques et de l'activité, a été observée environ une semaine après le traitement. Aucune complication évidente n'a été associée à ce traitement. Une récidive tumorale a été constatée dans les deux cas. Ce traitement a pu être réalisé plusieurs fois. Cette réduction transnasale du volume de la tumeur nasale est une méthode palliative. Elle est considérée comme un traitement qui contribue à l'amélioration des symptômes cliniques et de la qualité de vie.

Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan (Mizutani S, Asanuma); University of Miyazaki Veterinary Teaching Hospital, 1-1 Gakuen Kibana-dai Nishi, Miyazaki, Miyazaki 889-2192, Japan (Mizutani Y); Okayama University of Science Veterinary Medical Teaching Hospital, 1-3 Ikoinooka, Imabari, Ehime 794-8555, Japan (Goda); Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan (Torisu).

Address all correspondence to Shinya Mizutani; email: s-mizutani@ous.ac.jp.

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最終責任者 Sinya Mizutani (Corresponding Author)

獣医解剖学（Veterinary Anatomy）

Ryosuke Kobayashi

Assistant professor

助教　小林　良祐

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Understanding epigenetic regulation in the endometrium – lessons from mouse models with implantation defects.
Kobayashi R, Hatada I.
Epigenomics. 17:541-554. 2025. doi: 10.1080/17501911.2025.2491298.

II. その他　＜Others＞

1）Injury and Fibrosis at the Myoaponeurotic Junction of Pectoralis Major and Supracoracoideus Muscles in Broiler Chickens.
Kawasaki T, Iwasaki T, Watanabe T, Yamada M, Maeda N, Hasegawa Y, Takahashi N, **Kobayashi R.**
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2）Induction of MASH-like pathogenesis in the Nwd1$^{-/-}$ mouse liver.
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EPIGENOMICS
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REVIEW

Understanding epigenetic regulation in the endometrium – lessons from mouse models with implantation defects

Ryosuke Kobayashi [a] and Izuho Hatada [b,c]

[a]Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan; [b]Laboratory of Genome Science, Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Gunma, Japan; [c]Viral Vector Core, Gunma University Initiative for Advanced Research (GIAR), Gunma, Japan

ABSTRACT

Endometrial function, crucial for successful embryo implantation, is significantly influenced by epigenetic regulation. This review investigates the crucial roles of DNA methylation, histone modifications, chromatin remodeling, and RNA methylation in endometrial receptivity and implantation, based on a survey of recent literature on knockout mouse models with implantation defects. These models illuminate how epigenetic disruptions contribute to implantation failure, a significant human reproductive health concern. DNA methylation and histone modifications modulate endometrial receptivity by affecting gene silencing and chromatin structure, respectively. Chromatin remodeling factors also play a critical role in endometrial dynamics, influencing gene expression. Furthermore, RNA methylation emerges as critical in implantation through transcriptional and translational control. While human studies provide limited epigenetic snapshots, mouse models with suppressed epigenetic regulators reveal direct causal links between epigenetic alterations and implantation failure. Understanding these epigenetic interactions offers potential for novel therapies addressing reproductive disorders.

PLAIN LANGUAGE SUMMARY

For a pregnancy to start, an embryo must implant in the uterus. The uterus lining (endometrium) must be prepared for embryo in advance, like soil for a seed. "Epigenetic switches" control how the endometrium develops by turning genes "on" or "off." Studying mice with implantation problems helps us understand these epigenetic switches. This review, summarizes recent research on these mice, highlighting the importance of epigenetic switches for successful implantation. These knowledges could lead to new fertility treatments.

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KEYWORDS
Embryo implantation; endometrium; infertility; mouse models; DNA methylation; histone modifications; chromatin remodeling; RNA methylation

1. Introduction

> *If men and women, after they have reached a certain age, do not have children after cohabitation, the fault sometimes rests with both, and sometimes in only one of them. And first, it is requisite to examine the uterus of the female … Aristotle*

The ancient Greek philosopher Aristotle had discussed infertility in his seminal work *Historia Animalium*, highlighting uterine disorders as a potential cause of infertility [1]. This early recognition of the uterus's central role in reproduction underscores humanity's enduring quest to understand and improve fertility. While over two millennia have passed since Aristotle's writings, the complexities of human reproduction, particularly the molecular and cellular mechanisms governing endometrial function during embryo implantation, continue to be an active area of research.

The endometrium, the inner layer of the uterus that Aristotle emphasized as needing examination, is a highly dynamic tissue in the human body, characterized by cyclical regeneration and shedding. This tissue remodeling allows the endometrium to provide an optimal environment for embryo implantation, subsequent placental formation, and embryo nutrition, thereby contributing to the establishment of pregnancy. This process involves the precise regulation of various cell types within the endometrium, including epithelial, stromal, and immune cells. The spatiotemporal interaction of these cells enables the endometrium to acquire receptivity to the embryo during early pregnancy [2,3]. However, this carefully regulated process can be disrupted by a variety of factors, leading to implantation failure, which is a major cause of infertility [4]. Moreover, implantation failures present a significant challenge in assisted reproductive technologies such as *in vitro* fertilization and embryo transfer. For patients suffering from recurrent implantation failure (RIF), understanding the underlying causes and developing effective treatments are essential.

The endometrium undergoes significant transcriptional changes during the peri-implantation period to become receptive to the embryo [3,5]. Dysregulation of these processes is implicated in implantation failure and infertility [6], making the investigation of the underlying epigenetic mechanisms a natural progression for implantation research. Ovarian steroid hormones,

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CONTACT Ryosuke Kobayashi r-kobayashi@rakuno.ac.jp Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan; Izuho Hatada hatada@gunma-u.ac.jp Laboratory of Genome Science, Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Gunma 371-8512, Japan

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Injury and Fibrosis at the Myoaponeurotic Junction of Pectoralis Major and Supracoracoideus Muscles in Broiler Chickens

Takeshi Kawasaki[1], Tomohito Iwasaki[2], Takafumi Watanabe[3], Michi Yamada[4], Naoyuki Maeda[2],
Yasuhiro Hasegawa[2], Naoki Takahashi[3], and Ryosuke Kobayashi[3]

[1] Research Office Concerning the Health of Humans and Birds, Abashiri, 099-3119, Japan
[2] Department of Food Science and Human Wellness, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[3] Department of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[4] Department of Sustainable Agriculture, Rakuno Gakuen University, Ebetsu, 069-8501, Japan

In this study, we aimed to identify the mechanism responsible for tissue degeneration and fibrosis in pectoral and supracoracoideus muscles. Ten chickens fed ad libitum broiler feed (Bro) were compared to 10 chickens fed breeding feed (Adj), which has lower metabolizable energy. The median body weight of Bro and Adj birds at 48 days of age was 4.9 and 0.9 kg, respectively. In Bro birds, hind legs were farther apart and tended to abduct, whereas their standing posture was often tilted forward, making them unstable. The two Bro males were heavier than the average, markedly less stable when standing or walking, and often flapped their wings vigorously to maintain balance. Myofiber damage and fibrosis were observed at the myoaponeurotic junction of the pectoralis major and supracoracoideus muscles in Bro birds. Myofiber damage and fibrosis were detected also in areas distal to the myoaponeurotic junction in the two heavier males but were otherwise less evident. By contrast, in Adj birds, almost no degeneration or fibrosis of muscle tissue was observed at the myoaponeurotic junction. In addition, the supracoracoideus muscle of one of the Bro birds showed coagulative necrosis of muscle tissue, surrounded by prominent fibrous tissue. Numerous incompletely formed blood vessels with irregular shapes and prominent branching proliferated in the fibrous tissue. These findings suggest that injury at the myoaponeurotic junction and abnormal capillary proliferation may be closely related to the formation of lesions, along with prominent fibrosis in the pectoralis major and supracoracoideus muscles.

Key words: broilers, fibrosis, myoaponeurotic junction, pectoralis major muscles, supracoracoideus muscles, wooden breast

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Introduction

The primary function of the pectoralis major and supracoracoideus muscles in flying birds is to generate and control flight forces by the wings. The pectoralis major muscles present an intramuscular aponeurosis that originates from the deltopectoral crest of the humerus and is composed of a sternobrachial (extending from the aponeurosis to the keel) and thoracobrachial (extending to the dorsal area of the ribs) portions[1,2]. The supracoracoideus muscle originates from the sternum, coracoid bone, and sternocoracoclavicular membrane to connect the dorsal tubercle of the humerus via the triosseal canal[2]. The wing downstroke is caused mainly by contraction of the pectoralis major muscle, in which the intramuscular aponeurosis acts as an axis. By contrast, wing elevation is initiated by supination of the humerus via contraction of the supracoracoideus muscle at the end of the wing downstroke[3]. Lifting the wing does not require a large force; therefore, supracoracoideus muscles are smaller than the pectoralis major muscles[2]. Indeed, the pectoralis major muscles account for 17% of body weight, whereas the supracoracoideus for 2%–4% across various species[4]. Chickens can only fly short distances at low altitudes; hence, their pectoralis major muscles account for a lower proportion of body weight (<10%), although it has risen to >20% in newer breeds of broiler chickens[5,6]. Pectoralis major muscles develop more significantly in broilers than in flying birds; however, this is not due to functional necessity but rather due to breeding selection for birds with hyperplasia

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Correspondence: Takeshi Kawasaki, DVM, Ph.D., Research Office Concerning the Health of Humans and Birds, 2-7-1, Masu-ura, Abashiri, 099-3119, Japan. (E-mail: takeshi@kawavet.com)

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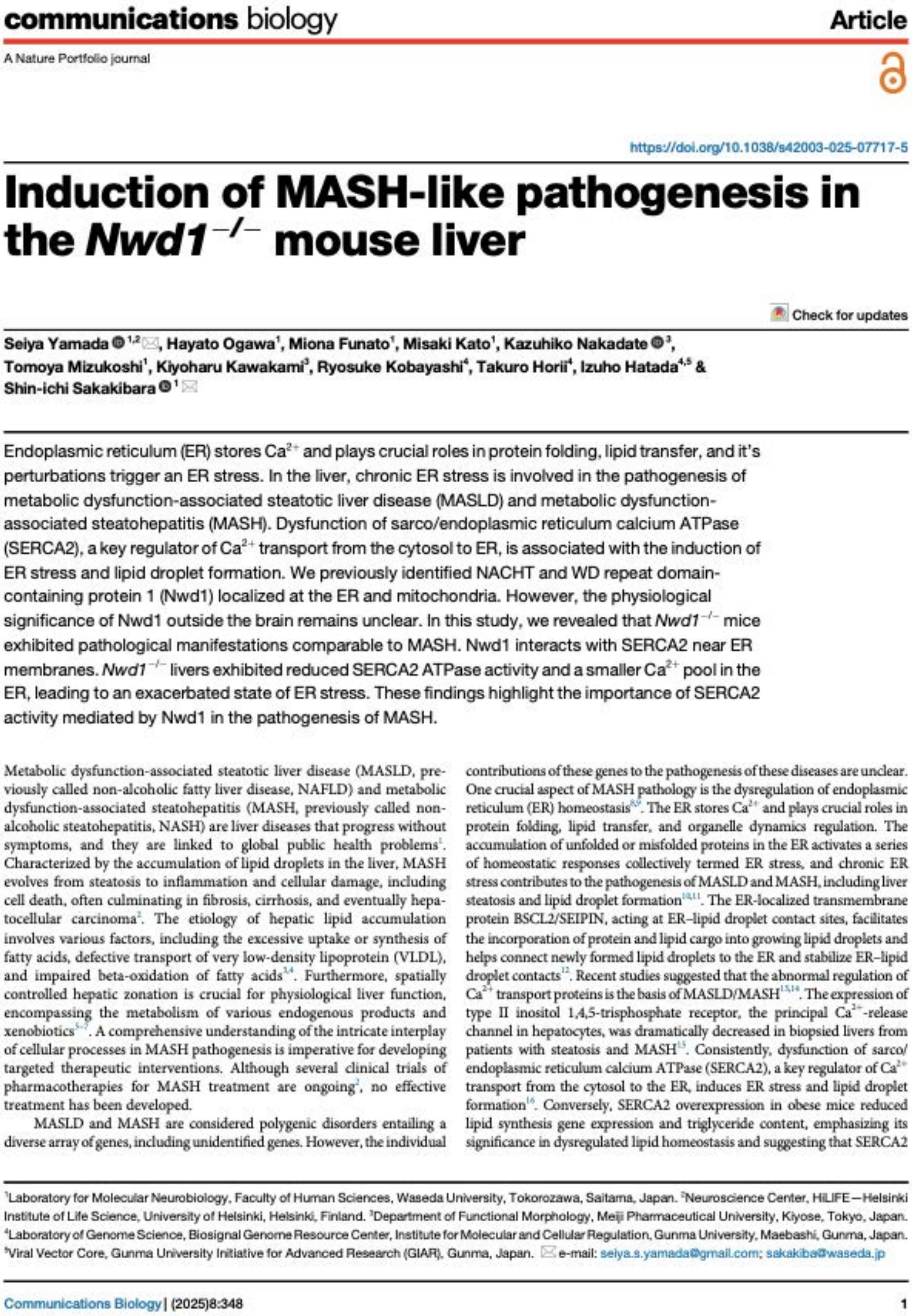

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Article

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Induction of MASH-like pathogenesis in the *Nwd1*$^{-/-}$ mouse liver

Seiya Yamada [1,2] ✉, **Hayato Ogawa** [1], **Miona Funato** [1], **Misaki Kato** [1], **Kazuhiko Nakadate** [3], **Tomoya Mizukoshi** [1], **Kiyoharu Kawakami** [3], **Ryosuke Kobayashi** [4], **Takuro Horii** [4], **Izuho Hatada** [4,5] & **Shin-ichi Sakakibara** [1] ✉

Endoplasmic reticulum (ER) stores Ca^{2+} and plays crucial roles in protein folding, lipid transfer, and it's perturbations trigger an ER stress. In the liver, chronic ER stress is involved in the pathogenesis of metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH). Dysfunction of sarco/endoplasmic reticulum calcium ATPase (SERCA2), a key regulator of Ca^{2+} transport from the cytosol to ER, is associated with the induction of ER stress and lipid droplet formation. We previously identified NACHT and WD repeat domain-containing protein 1 (Nwd1) localized at the ER and mitochondria. However, the physiological significance of Nwd1 outside the brain remains unclear. In this study, we revealed that *Nwd1*$^{-/-}$ mice exhibited pathological manifestations comparable to MASH. Nwd1 interacts with SERCA2 near ER membranes. *Nwd1*$^{-/-}$ livers exhibited reduced SERCA2 ATPase activity and a smaller Ca^{2+} pool in the ER, leading to an exacerbated state of ER stress. These findings highlight the importance of SERCA2 activity mediated by Nwd1 in the pathogenesis of MASH.

Metabolic dysfunction-associated steatotic liver disease (MASLD, previously called non-alcoholic fatty liver disease, NAFLD) and metabolic dysfunction-associated steatohepatitis (MASH, previously called non-alcoholic steatohepatitis, NASH) are liver diseases that progress without symptoms, and they are linked to global public health problems[1]. Characterized by the accumulation of lipid droplets in the liver, MASH evolves from steatosis to inflammation and cellular damage, including cell death, often culminating in fibrosis, cirrhosis, and eventually hepatocellular carcinoma[2]. The etiology of hepatic lipid accumulation involves various factors, including the excessive uptake or synthesis of fatty acids, defective transport of very low-density lipoprotein (VLDL), and impaired beta-oxidation of fatty acids[3,4]. Furthermore, spatially controlled hepatic zonation is crucial for physiological liver function, encompassing the metabolism of various endogenous products and xenobiotics[5–7]. A comprehensive understanding of the intricate interplay of cellular processes in MASH pathogenesis is imperative for developing targeted therapeutic interventions. Although several clinical trials of pharmacotherapies for MASH treatment are ongoing[2], no effective treatment has been developed.

MASLD and MASH are considered polygenic disorders entailing a diverse array of genes, including unidentified genes. However, the individual contributions of these genes to the pathogenesis of these diseases are unclear. One crucial aspect of MASH pathology is the dysregulation of endoplasmic reticulum (ER) homeostasis[8,9]. The ER stores Ca^{2+} and plays crucial roles in protein folding, lipid transfer, and organelle dynamics regulation. The accumulation of unfolded or misfolded proteins in the ER activates a series of homeostatic responses collectively termed ER stress, and chronic ER stress contributes to the pathogenesis of MASLD and MASH, including liver steatosis and lipid droplet formation[10,11]. The ER-localized transmembrane protein BSCL2/SEIPIN, acting at ER–lipid droplet contact sites, facilitates the incorporation of protein and lipid cargo into growing lipid droplets and helps connect newly formed lipid droplets to the ER and stabilize ER–lipid droplet contacts[12]. Recent studies suggested that the abnormal regulation of Ca^{2+} transport proteins is the basis of MASLD/MASH[13,14]. The expression of type II inositol 1,4,5-trisphosphate receptor, the principal Ca^{2+}-release channel in hepatocytes, was dramatically decreased in biopsied livers from patients with steatosis and MASH[15]. Consistently, dysfunction of sarco/endoplasmic reticulum calcium ATPase (SERCA2), a key regulator of Ca^{2+} transport from the cytosol to the ER, induces ER stress and lipid droplet formation[16]. Conversely, SERCA2 overexpression in obese mice reduced lipid synthesis gene expression and triglyceride content, emphasizing its significance in dysregulated lipid homeostasis and suggesting that SERCA2

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[1] Laboratory for Molecular Neurobiology, Faculty of Human Sciences, Waseda University, Tokorozawa, Saitama, Japan. [2] Neuroscience Center, HiLIFE—Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland. [3] Department of Functional Morphology, Meiji Pharmaceutical University, Kiyose, Tokyo, Japan. [4] Laboratory of Genome Science, Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Gunma, Japan. [5] Viral Vector Core, Gunma University Initiative for Advanced Research (GIAR), Gunma, Japan. ✉ e-mail: seiya.s.yamada@gmail.com; sakakiba@waseda.jp

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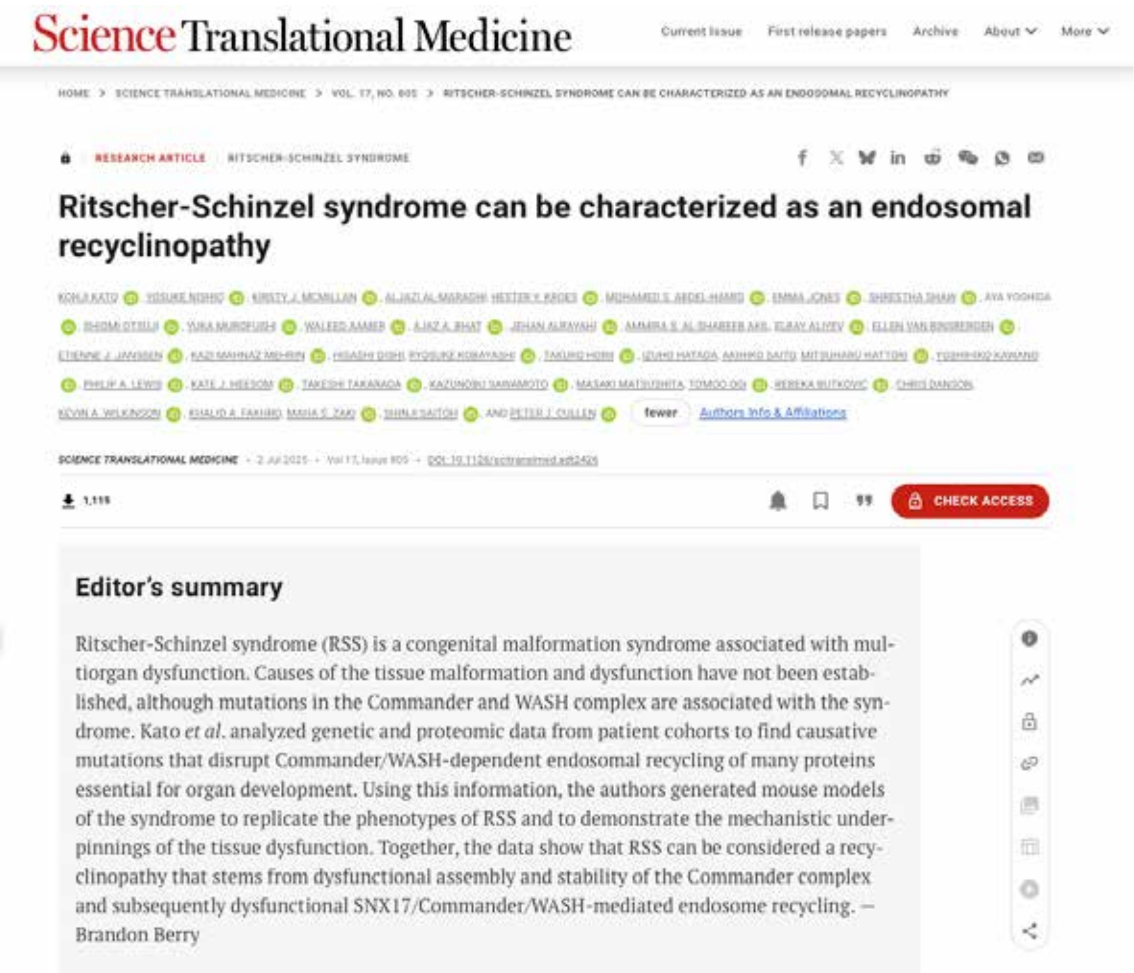
最終責任者　Shin-Ichi Sakakibara (Corresponding Author)



Ritscher-Schinzel syndrome can be characterized as an endosomal recyclinopathy

KOHJI KATO, YOSUKE NISHIO, KIRSTY J. MCMILLAN, ALJAZI AL-MARAGHI, HESTER Y. KROES, MOHAMED S. ABDEL-HAMID, EMMA JONES, SHRESTHA SHAW, AYA YOSHIDA, SHIOMI OTSUJI, YUKA MUROFUSHI, WALEED AAMER, AJAZ A. BHAT, JEHAN ALRAYAHI, AMMIRA S. AL-SHABEEB AKIL, ELBAY ALIYEV, ELLEN VAN BINSBERGEN, ETIENNE J. JANSSEN, KAZI MAHNAZ MEHRIN, HISASHI OISHI, RYOSUKE KOBAYASHI, TAKURO HORII, IZUHO HATADA, AKIHIKO SAITO, MITSUHARU HATTORI, YOSHIHIKO KAWANO, PHILIP A. LEWIS, KATE J. HEESOM, TAKESHI TAKARADA, KAZUNOBU SAWAMOTO, MASAKI MATSUSHITA, TOMOO OGI, REBEKA BUTKOVIC, CHRIS DANSON, KEVIN A. WILKINSON, KHALID A. FAKHRO, MAHA S. ZAKI, SHINJI SAITOH, AND PETER J. CULLEN

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Editor’s summary

Ritscher-Schinzel syndrome (RSS) is a congenital malformation syndrome associated with multiorgan dysfunction. Causes of the tissue malformation and dysfunction have not been established, although mutations in the Commander and WASH complex are associated with the syndrome. Kato *et al.* analyzed genetic and proteomic data from patient cohorts to find causative mutations that disrupt Commander/WASH-dependent endosomal recycling of many proteins essential for organ development. Using this information, the authors generated mouse models of the syndrome to replicate the phenotypes of RSS and to demonstrate the mechanistic underpinnings of the tissue dysfunction. Together, the data show that RSS can be considered a recyclinopathy that stems from dysfunctional assembly and stability of the Commander complex and subsequently dysfunctional SNX17/Commander/WASH-mediated endosome recycling. —Brandon Berry

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Article

SOURCE DATA TRANSPARENT PROCESS OPEN ACCESS CHECK FOR UPDATES

THE EMBO JOURNAL

FTO promotes weight gain via altering *Kif1a* splicing and axonal vesicle trafficking in AgRP neurons

Daisuke Kohno[1✉], Reika Kawabata-Iwakawa[2], Sotaro Ichinose[3], Shigetomo Suyama[4,5], Kazuto Ohashi[6], Winda Ariyani[1], Tetsushi Sadakata[7], Hiromi Yokota-Hashimoto[1], Ryosuke Kobayashi[8], Takuro Horii[8], Vina Yanti Susanti[1,9], Ayumu Konno[10,11], Haruka Tsuneoka[1], Chiharu Yoshikawa[1], Sho Matsui[12], Akihiro Harada[13], Toshihiko Yada[5,14,15,16], Izuho Hatada[8,11], Hirokazu Hirai[10,11], Masahiko Nishiyama[17], Tsutomu Sasaki[12] & Tadahiro Kitamura[1]

Abstract

N^6-methyladenosine (m^{6}A) is an abundant chemical RNA modification involved in the regulation of many biological processes. The m^{6}A demethylase FTO (fat mass and obesity-associated protein) is known to affect body weight, but its systemic context and underlying mechanisms remain unclear. Here, we found that mice lacking or overexpressing *Fto* in agouti-related peptide-expressing (AgRP) neurons in the hypothalamus exhibited decreased and increased body weight, respectively. FTO demethylated m^{6}A on mRNAs for proteins associated with membrane trafficking and alternative splicing in AgRP neurons. Downstream, FTO-modulated alternative splicing of the axonal motor protein *Kif1a* affected its hinge region, which is relevant to the structure and function of KIF1A. Notably, *Kif1a* knockdown in AgRP neurons suppressed the weight gain of mice overexpressing *Fto*. In addition, FTO increased the trafficking and secretion of dense-core vesicles containing neuropeptides NPY and AgRP from AgRP neurons. Collectively, these results reveal a novel regulatory FTO-KIF1A axis in the brain affecting appetite-stimulating AgRP neurons and systemic energy homeostasis, via FTO regulation of the epitranscriptome of AgRP neurons.

Keywords AgRP; Alternative Splicing; FTO; KIF1A; Obesity
Subject Categories Membranes & Trafficking; Metabolism; Neuroscience
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Introduction

Obesity rates have increased continuously in most countries over the past several decades (NCD Risk Factor Collaboration, 2016). However, the molecular mechanisms underlying this increase are not fully understood. The obesity epidemic is often attributed to environmental factors, including diet and exercise (Hill, 2006; Pate et al, 2013; Ross et al, 2016), and the mechanisms by which the body responds to these factors may be the basis for the obesity epidemic. Recent studies have shown that chemical epigenetic and epitranscriptomic modifications play an important role in many biological processes, as they respond sensitively to changes in both the internal and external environments (Li et al, 2020; Widagdo et al, 2016). The formation of N^6-methyl-adenosine (m^{6}A) is the most abundant methyl modification of RNA (Zaccara et al, 2019) and directly affects RNA stability, translational efficiency, and alternative splicing (Shi et al, 2019). Furthermore, m^{6}A modifications regulate several physiological processes, including embryonic development, cancer, DNA repair, and brain function (Liu et al, 2023; Widagdo et al, 2016).

m^{6}A modifications are regulated by the methyltransferase-like 3 and 14 (METTL3-METTL14) complex and two demethylases—fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5) (Shi et al, 2019). *Fto* was originally identified through genome-wide association study (GWAS) analyses, which revealed that single-nucleotide polymorphisms (SNPs) in *Fto* intron 1 were strongly associated with obesity (Dina et al, 2007; Frayling et al, 2007). The genotypes associated with these *Fto* SNPs affect the expression level of *Fto* (Berulava and Horsthemke, 2010; Pahl et al, 2023; Stratigopoulos et al, 2016; Zhang et al, 2023) and neighboring genes such as *Rpgrip1l*, *Irx3*, and *Irx5*, which are related to obesity

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[1]Metabolic Signal Research Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan. [2]Division of Integrated Oncology Research, Gunma University Initiative for Advanced Research (GIAR), Maebashi, Japan. [3]Department of Anatomy, Gunma University Graduate School of Medicine, Maebashi, Japan. [4]Department of Physiology, Keio University School of Medicine, Tokyo, Japan. [5]Division of Integrative Physiology, Department of Physiology, Jichi Medical University School of Medicine, Shimotsuke, Japan. [6]Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan. [7]Education and Research Support Center, Gunma University Graduate School of Medicine, Maebashi, Japan. [8]Laboratory of Genome Science, Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan. [9]Internal Medicine Department, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. [10]Department of Neurophysiology & Neural Repair, Gunma University Graduate School of Medicine, Maebashi, Japan. [11]Viral Vector Core, Gunma University, Initiative for Advanced Research (GIAR), Maebashi, Japan. [12]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan. [13]Department of Cell Biology, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan. [14]Division of Integrative Physiology, Center for Integrative Physiology, Kansai Electric Power Medical Research Institute, Kyoto, Japan. [15]Department of Diabetes, Endocrinology and Metabolism/Rheumatology and Clinical Immunology, Gifu University Graduate School of Medicine, Gifu, Japan. [16]Center for One Medicine Innovative Translational Research, Gifu University Institute for Advanced Study, Gifu, Japan. [17]Gunma University, Maebashi, Japan. ✉E-mail: daisuke.kohno@gunma-u.ac.jp

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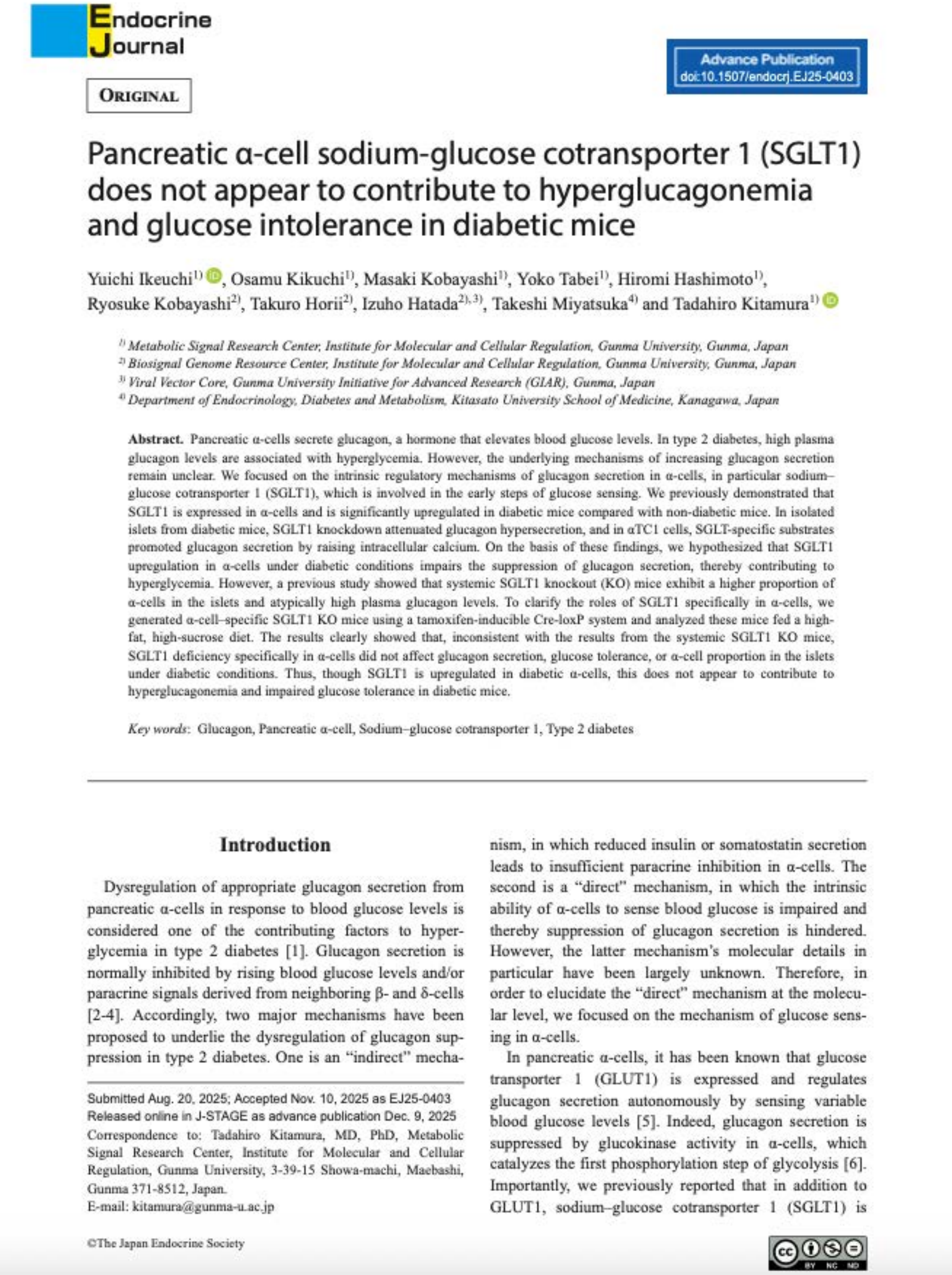

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Pancreatic α-cell sodium-glucose cotransporter 1 (SGLT1) does not appear to contribute to hyperglucagonemia and glucose intolerance in diabetic mice

Yuichi Ikeuchi1), Osamu Kikuchi1), Masaki Kobayashi1), Yoko Tabei1), Hiromi Hashimoto1),
Ryosuke Kobayashi2), Takuro Horii2), Izuho Hatada2), 3), Takeshi Miyatsuka4) and Tadahiro Kitamura1)

1) *Metabolic Signal Research Center, Institute for Molecular and Cellular Regulation, Gunma University, Gunma, Japan*
2) *Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Gunma, Japan*
3) *Viral Vector Core, Gunma University Initiative for Advanced Research (GIAR), Gunma, Japan*
4) *Department of Endocrinology, Diabetes and Metabolism, Kitasato University School of Medicine, Kanagawa, Japan*

Abstract. Pancreatic α-cells secrete glucagon, a hormone that elevates blood glucose levels. In type 2 diabetes, high plasma glucagon levels are associated with hyperglycemia. However, the underlying mechanisms of increasing glucagon secretion remain unclear. We focused on the intrinsic regulatory mechanisms of glucagon secretion in α-cells, in particular sodium–glucose cotransporter 1 (SGLT1), which is involved in the early steps of glucose sensing. We previously demonstrated that SGLT1 is expressed in α-cells and is significantly upregulated in diabetic mice compared with non-diabetic mice. In isolated islets from diabetic mice, SGLT1 knockdown attenuated glucagon hypersecretion, and in αTC1 cells, SGLT-specific substrates promoted glucagon secretion by raising intracellular calcium. On the basis of these findings, we hypothesized that SGLT1 upregulation in α-cells under diabetic conditions impairs the suppression of glucagon secretion, thereby contributing to hyperglycemia. However, a previous study showed that systemic SGLT1 knockout (KO) mice exhibit a higher proportion of α-cells in the islets and atypically high plasma glucagon levels. To clarify the roles of SGLT1 specifically in α-cells, we generated α-cell–specific SGLT1 KO mice using a tamoxifen-inducible Cre-loxP system and analyzed these mice fed a high-fat, high-sucrose diet. The results clearly showed that, inconsistent with the results from the systemic SGLT1 KO mice, SGLT1 deficiency specifically in α-cells did not affect glucagon secretion, glucose tolerance, or α-cell proportion in the islets under diabetic conditions. Thus, though SGLT1 is upregulated in diabetic α-cells, this does not appear to contribute to hyperglucagonemia and impaired glucose tolerance in diabetic mice.

Key words: Glucagon, Pancreatic α-cell, Sodium–glucose cotransporter 1, Type 2 diabetes

Introduction

Dysregulation of appropriate glucagon secretion from pancreatic α-cells in response to blood glucose levels is considered one of the contributing factors to hyperglycemia in type 2 diabetes [1]. Glucagon secretion is normally inhibited by rising blood glucose levels and/or paracrine signals derived from neighboring β- and δ-cells [2-4]. Accordingly, two major mechanisms have been proposed to underlie the dysregulation of glucagon suppression in type 2 diabetes. One is an "indirect" mechanism, in which reduced insulin or somatostatin secretion leads to insufficient paracrine inhibition in α-cells. The second is a "direct" mechanism, in which the intrinsic ability of α-cells to sense blood glucose is impaired and thereby suppression of glucagon secretion is hindered. However, the latter mechanism's molecular details in particular have been largely unknown. Therefore, in order to elucidate the "direct" mechanism at the molecular level, we focused on the mechanism of glucose sensing in α-cells.

In pancreatic α-cells, it has been known that glucose transporter 1 (GLUT1) is expressed and regulates glucagon secretion autonomously by sensing variable blood glucose levels [5]. Indeed, glucagon secretion is suppressed by glucokinase activity in α-cells, which catalyzes the first phosphorylation step of glycolysis [6]. Importantly, we previously reported that in addition to GLUT1, sodium–glucose cotransporter 1 (SGLT1) is

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Correspondence to: Tadahiro Kitamura, MD, PhD, Metabolic Signal Research Center, Institute for Molecular and Cellular Regulation, Gunma University, 3-39-15 Showa-machi, Maebashi, Gunma 371-8512, Japan.
E-mail: kitamura@gunma-u.ac.jp

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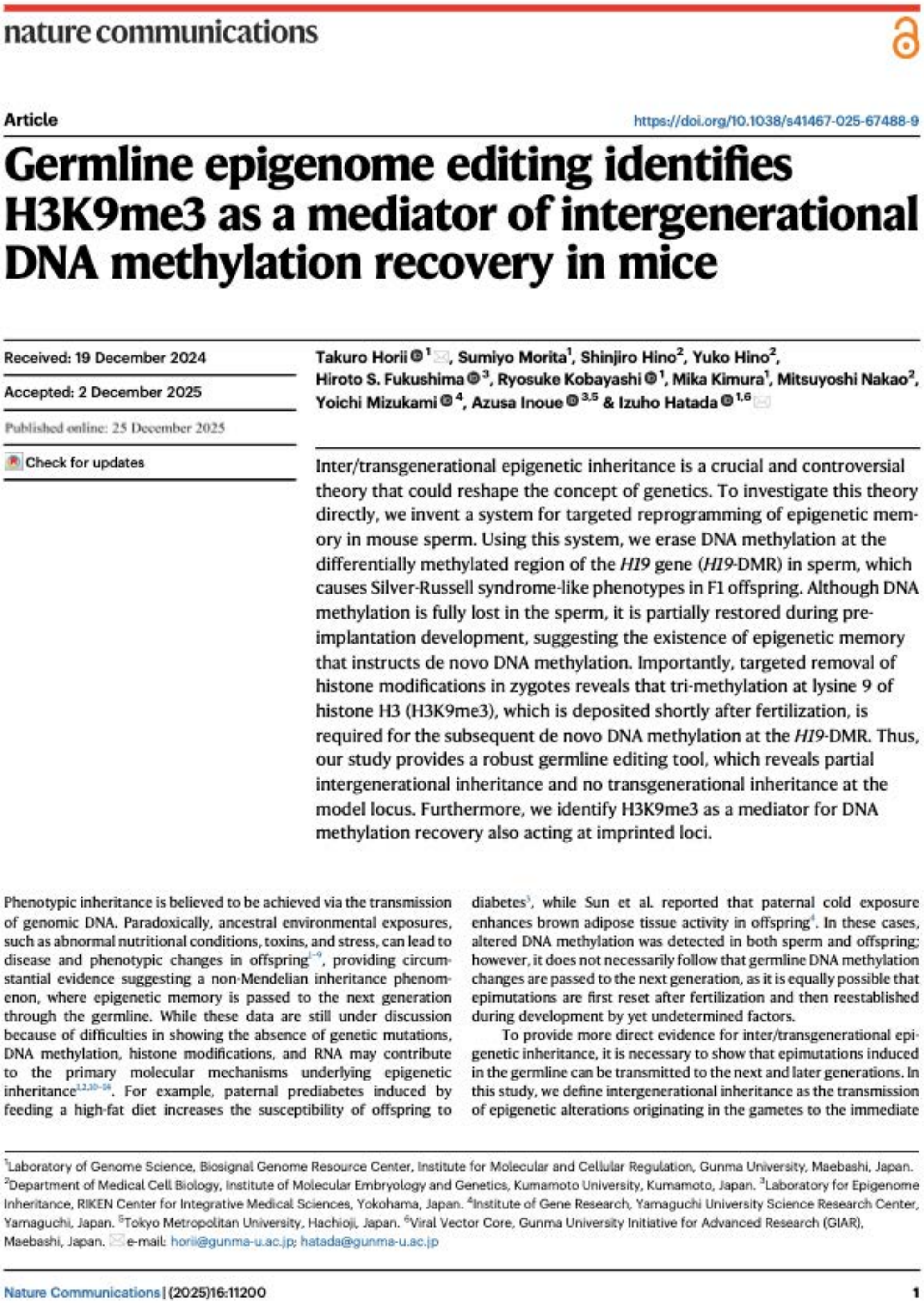
最終責任者　Tadahiro Kitamura (Corresponding Author)



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Article

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Germline epigenome editing identifies H3K9me3 as a mediator of intergenerational DNA methylation recovery in mice

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Takuro Horii[1], **Sumiyo Morita**[1], **Shinjiro Hino**[2], **Yuko Hino**[2], **Hiroto S. Fukushima**[3], **Ryosuke Kobayashi**[1], **Mika Kimura**[1], **Mitsuyoshi Nakao**[2], **Yoichi Mizukami**[4], **Azusa Inoue**[3,5] & **Izuho Hatada**[1,6]

Inter/transgenerational epigenetic inheritance is a crucial and controversial theory that could reshape the concept of genetics. To investigate this theory directly, we invent a system for targeted reprogramming of epigenetic memory in mouse sperm. Using this system, we erase DNA methylation at the differentially methylated region of the *H19* gene (*H19*-DMR) in sperm, which causes Silver-Russell syndrome-like phenotypes in F1 offspring. Although DNA methylation is fully lost in the sperm, it is partially restored during preimplantation development, suggesting the existence of epigenetic memory that instructs de novo DNA methylation. Importantly, targeted removal of histone modifications in zygotes reveals that tri-methylation at lysine 9 of histone H3 (H3K9me3), which is deposited shortly after fertilization, is required for the subsequent de novo DNA methylation at the *H19*-DMR. Thus, our study provides a robust germline editing tool, which reveals partial intergenerational inheritance and no transgenerational inheritance at the model locus. Furthermore, we identify H3K9me3 as a mediator for DNA methylation recovery also acting at imprinted loci.

Phenotypic inheritance is believed to be achieved via the transmission of genomic DNA. Paradoxically, ancestral environmental exposures, such as abnormal nutritional conditions, toxins, and stress, can lead to disease and phenotypic changes in offspring[1–9], providing circumstantial evidence suggesting a non-Mendelian inheritance phenomenon, where epigenetic memory is passed to the next generation through the germline. While these data are still under discussion because of difficulties in showing the absence of genetic mutations, DNA methylation, histone modifications, and RNA may contribute to the primary molecular mechanisms underlying epigenetic inheritance[1,2,10–14]. For example, paternal prediabetes induced by feeding a high-fat diet increases the susceptibility of offspring to diabetes[3], while Sun et al. reported that paternal cold exposure enhances brown adipose tissue activity in offspring[4]. In these cases, altered DNA methylation was detected in both sperm and offspring; however, it does not necessarily follow that germline DNA methylation changes are passed to the next generation, as it is equally possible that epimutations are first reset after fertilization and then reestablished during development by yet undetermined factors.

To provide more direct evidence for inter/transgenerational epigenetic inheritance, it is necessary to show that epimutations induced in the germline can be transmitted to the next and later generations. In this study, we define intergenerational inheritance as the transmission of epigenetic alterations originating in the gametes to the immediate

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[1] Laboratory of Genome Science, Biosignal Genome Resource Center, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan. [2] Department of Medical Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan. [3] Laboratory for Epigenome Inheritance, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. [4] Institute of Gene Research, Yamaguchi University Science Research Center, Yamaguchi, Japan. [5] Tokyo Metropolitan University, Hachioji, Japan. [6] Viral Vector Core, Gunma University Initiative for Advanced Research (GIAR), Maebashi, Japan. ✉ e-mail: horii@gunma-u.ac.jp; hatada@gunma-u.ac.jp

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最終責任者　Takuro Horii and Izuho Hatada (Co-corresponding Authors)

生産動物病態学（Farm Animal Pathophysiology）

Keigo Kosenda

Assistant Professor

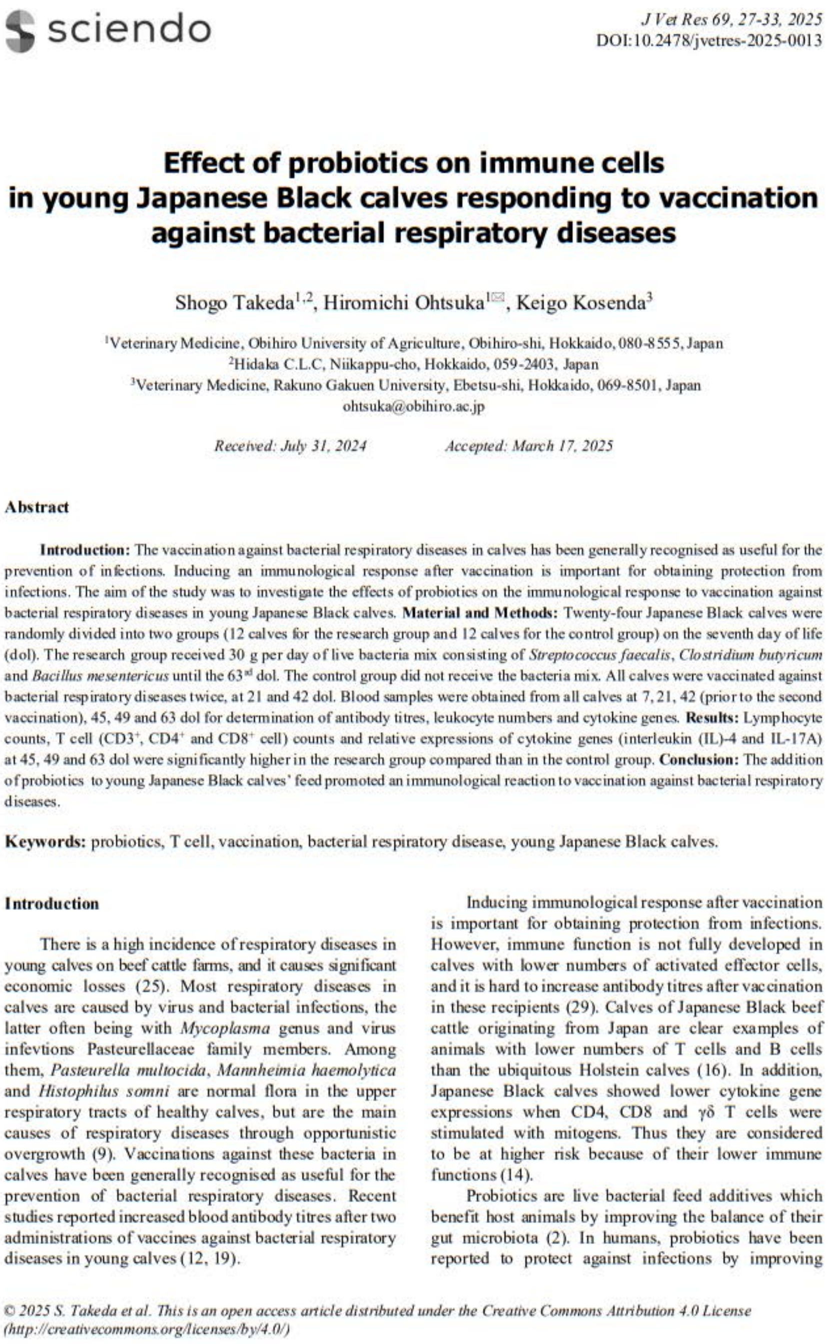
助教　小千田　圭吾

I. 筆頭または責任著者　＜First or Corresponding Author＞

該当なし

II. その他＜Others＞

1） Effect of probiotics on immune cells in young Japanese Black calves responding to vaccination against bacterial respiratory diseases.
Takeda S, Ohtsuka H, **Kosenda K**.
J Vet Res. 69: 27-33. 2025. doi: 10.2478/jvetres-2025-0013.



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Effect of probiotics on immune cells in young Japanese Black calves responding to vaccination against bacterial respiratory diseases

Shogo Takeda[1,2], Hiromichi Ohtsuka[1✉], Keigo Kosenda[3]

[1]Veterinary Medicine, Obihiro University of Agriculture, Obihiro-shi, Hokkaido, 080-8555, Japan
[2]Hidaka C.L.C, Niikappu-cho, Hokkaido, 059-2403, Japan
[3]Veterinary Medicine, Rakuno Gakuen University, Ebetsu-shi, Hokkaido, 069-8501, Japan
ohtsuka@obihiro.ac.jp

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Abstract

Introduction: The vaccination against bacterial respiratory diseases in calves has been generally recognised as useful for the prevention of infections. Inducing an immunological response after vaccination is important for obtaining protection from infections. The aim of the study was to investigate the effects of probiotics on the immunological response to vaccination against bacterial respiratory diseases in young Japanese Black calves. **Material and Methods:** Twenty-four Japanese Black calves were randomly divided into two groups (12 calves for the research group and 12 calves for the control group) on the seventh day of life (dol). The research group received 30 g per day of live bacteria mix consisting of *Streptococcus faecalis*, *Clostridium butyricum* and *Bacillus mesentericus* until the 63rd dol. The control group did not receive the bacteria mix. All calves were vaccinated against bacterial respiratory diseases twice, at 21 and 42 dol. Blood samples were obtained from all calves at 7, 21, 42 (prior to the second vaccination), 45, 49 and 63 dol for determination of antibody titres, leukocyte numbers and cytokine genes. **Results:** Lymphocyte counts, T cell ($CD3^+$, $CD4^+$ and $CD8^+$ cell) counts and relative expressions of cytokine genes (interleukin (IL)-4 and IL-17A) at 45, 49 and 63 dol were significantly higher in the research group compared than in the control group. **Conclusion:** The addition of probiotics to young Japanese Black calves' feed promoted an immunological reaction to vaccination against bacterial respiratory diseases.

Keywords: probiotics, T cell, vaccination, bacterial respiratory disease, young Japanese Black calves.

Introduction

There is a high incidence of respiratory diseases in young calves on beef cattle farms, and it causes significant economic losses (25). Most respiratory diseases in calves are caused by virus and bacterial infections, the latter often being with *Mycoplasma* genus and virus infevtions Pasteurellaceae family members. Among them, *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni* are normal flora in the upper respiratory tracts of healthy calves, but are the main causes of respiratory diseases through opportunistic overgrowth (9). Vaccinations against these bacteria in calves have been generally recognised as useful for the prevention of bacterial respiratory diseases. Recent studies reported increased blood antibody titres after two administrations of vaccines against bacterial respiratory diseases in young calves (12, 19).

Inducing immunological response after vaccination is important for obtaining protection from infections. However, immune function is not fully developed in calves with lower numbers of activated effector cells, and it is hard to increase antibody titres after vaccination in these recipients (29). Calves of Japanese Black beef cattle originating from Japan are clear examples of animals with lower numbers of T cells and B cells than the ubiquitous Holstein calves (16). In addition, Japanese Black calves showed lower cytokine gene expressions when CD4, CD8 and γδ T cells were stimulated with mitogens. Thus they are considered to be at higher risk because of their lower immune functions (14).

Probiotics are live bacterial feed additives which benefit host animals by improving the balance of their gut microbiota (2). In humans, probiotics have been reported to protect against infections by improving

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最終責任者　Hiromichi Ohtsuka (Corresponding Author)

伴侶動物外科学（Companion Animal Surgery）

Toshikazu Sakai

Assistant Professor

助教　酒井　俊和

I. 筆頭または責任著者　＜First or Corresponding Author＞

1）Spontaneous regression of Merkel cell carcinoma at the biopsy site in a cat.
Niwa N, Ishikawa-Niwa M, Obata Y, **Sakai T.**
Vet Rec Case Reports. 13: e70134. 2025. doi.org/10.1002/vrc2.70134

II. その他＜Others＞

1) Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study
Goda Y, Yamamoto S, Miyoshi K, Hanazono K, Morimura M, **Sakai T**, Tamura M, Deguchi T, Endo Y, Kadosawa T, Fujita M, Itami T, Torisu S.
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VetRecord
CaseReports

CASE REPORT

Companion or pet animals

Spontaneous regression of Merkel cell carcinoma at the biopsy site in a cat

Akihiro Niwa[1] | **Mayuko Ishikawa-Niwa**[1] | **Yoshinori Obata**[1] | **Toshikazu Sakai**[2]

[1] Aichi Animal Surgical Hospital, Aichi, Japan

[2] Department of Companion Animal Clinical Science, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

Correspondence
Toshikazu Sakai, Department of Companion Animal Clinical Science, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan.
Email: t-sakai@rakuno.ac.jp

Abstract
Merkel cell carcinoma (MCC) has been observed in the skin of older cats; however, information on this tumour remains limited. Consequently, effective treatment strategies are still being explored. In the present case, a 15-year-old cat developed MCC on a digit. Following a trephine biopsy, the tumour initially increased in size; however, it subsequently regressed and eventually disappeared with only antimicrobial therapy and wound care. However, only the tumour at the digit site regressed, while the disease progressed and metastasised to the lymph nodes and lungs. A previous case study also reported spontaneous regression following biopsy, suggesting a notable biological response. This phenomenon could lead to misinterpretations of treatment efficacy when establishing therapeutic strategies for feline MCC in the future. Therefore, we report this case to raise awareness of the potential for spontaneous regression of MCC following biopsy.

BACKGROUND

Merkel cell carcinoma (MCC) is a rare neuroendocrine tumour in humans, characterised by a high metastatic potential and poor prognosis. In cats, MCC is even rarer, with limited case reports available. Consequently, its pathophysiology and optimal treatment remain poorly understood.[1–4]

In humans, MCC is strongly associated with Merkel cell polyomavirus (MCPyV), which is detected in approximately 80% of MCC cases; due to this trend, MCPyV is suspected to play a key role in MCC tumourigenesis. In contrast, MCPyV has not been identified in feline MCC. However, recent studies in cats have suggested a potential oncogenic role for *Felis catus* papillomavirus type 2 (FcaPV-2) in a subset of feline MCC cases, suggesting that papillomavirus infection may contribute to neoplastic transformation in cats through mechanisms distinct from those in humans.[5,6]

Diagnosis is challenging, as cytology alone is insufficient for definitive identification, necessitating immunohistochemical (IHC) staining for confirmation.[7]

Currently, no standardised treatment protocol exists for feline MCC. Surgical excision, radiation therapy and chemotherapy have been considered; however, its prognosis remains poor.[1–4,7]

In the present case, spontaneous regression of the tumour was observed after biopsy. Although a previous study also reported a similar phenomenon in only one case, the underlying mechanisms remain unclear.[1] This case highlights the need to better understand the biological behaviour of feline MCC and may provide valuable insights into future treatment strategies.

CASE PRESENTATION

A 15-year-old neutered male mixed-breed cat weighing 5.24 kg was referred for further evaluation of a mass on the distal end of the left fourth digit. At the primary care hospital, cytological evaluation suggested lymphoma as a differential diagnosis.

The cat had previously experienced a skin wound on the right forearm, which persisted for approximately 1 year. Despite intermittent treatment with antibiotics and wound cleansing, the lesion did not change significantly, and its progression was continuously monitored. On clinical examination, the overall condition of the cat was unremarkable despite signs of discomfort in the left distal digit.

INVESTIGATIONS

Blood test results were as follows: white blood cell count, 14,600/μL; red blood cell count, 6,870,000/μL; haemoglobin, 10 g/dL; mean corpuscular volume, 46.4 fL; mean corpuscular haemoglobin, 15.3 pg; mean corpuscular haemoglobin concentration, 32.9 g/dL; haematocrit, 32%; platelet count, 380,000/μL; blood urea nitrogen (BUN), 32 mg/dL; creatinine (CRE), 1.9 mg/dL; alanine aminotransferase, 53 IU; alkaline phosphatase, 40 IU; Na, 156 mEq/L; K, 3.6 mEq/L; and Cl, 121 mEq/L.

As the owner declined general anaesthesia, a 3 mm punch biopsy of the mass was performed under local anaesthesia (bupivacaine; 0.48 mg/kg) using a trephine biopsy. The biopsy specimen was placed in 10% formalin and submitted to an external laboratory (IDEXX LAB) for histopathological evaluation. The tumour tissue was histomorphologically

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最終責任者　Toshikazu Sakai (Corresponding Author)

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Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study

Yoshimichi Goda[1#], Shushi Yamamoto[1#], Kenjiro Miyoshi[1], Kiwamu Hanazono[1],
Mai Morimura[1], Toshikazu Sakai[1], Masahiro Tamura[1], Tatsuya Deguchi[1], Yoshifumi Endo[1,2],
Tsuyoshi Kadosawa[1,3], Mayu Fujita[1], Takaharu Itami[1] and Shidow Torisu[1]*

[1]*Department of Companion Animal Clinical Sciences, School of Veterinary Medicine,*
Rakuno Gakuen University, Ebetsu, Japan
[2]*Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, Japan*
[3]*Japan Small Animal Medical Center, Tokorozawa-Shi, Japan*
#Both authors are equally contributed to this work

ABSTRACT

Background: Recently, the usefulness of triple-phase contrast-enhanced computed tomography (CT) scans for liver tumors in canines has been reported. However, detailed information on the CT findings of cholangiocarcinoma (CCA) and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) remains limited.

Aim: This study aimed to retrospectively evaluate triple-phase contrast-enhanced CT findings in canine CCA and cHCC-CCA using objective parameters, such as CT values and morphological characteristics.

Methods: This study included eight dogs that underwent triple-phase contrast-enhanced CT scans and surgical removal and were pathologically diagnosed. Three CCA cases and five cHCC-CCA cases were analyzed. Parameters included CT values and mass sizes, CT values and lymph node sizes, and rim enhancement. "Rim enhancement" was defined as the contrast enhancement effect at the liver parenchyma–mass boundary.

Results: CT values for CCA were as follows: pre-contrast: 43.9 ± 3.6 Hounsfield units (HUs); arterial phase: 76.9 ± 22.5 HU; portal phase: 98.8 ± 37.7 HU; equilibrium phase: 90.9 ± 27.1 HU. For cHCC-CCA, the mean CT values were as follows: pre-contrast: 50.7 ± 6.1 HU; arterial phase: 80.2 ± 19.2 HU; portal phase: 95.0 ± 21.3 HU; equilibrium phase: 86.1 ± 13.4 HU. The peak contrast enhancement for both CCA and cHCC-CCA was in the portal phase. Rim enhancement appeared in the arterial phase in all CCA cases, whereas it appeared in the portal phase in 80% of cHCC-CCA cases, indicating a significant difference.

Conclusion: Rim enhancement may help distinguish between CCA and cHCC-CCA. However, this study has a limited number of cases, and future large-scale, multicenter studies are warranted.

Keywords: Canine, Cholangiocarcinoma, Combined hepatocellular-cholangiocarcinoma, Computed tomography, Dog.

Introduction

Primary hepatic tumors in canines are rare, accounting for 0.6%–1.3% of all canine tumors (Patnaik *et al.*, 1981). The most common malignant liver tumor is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma (CCA) (Liptak *et al.*, 2019). Unlike in humans, the cause of HCC in dogs remains unknown, and the risk of occurrence based on breed or sex is unclear. However, there are reports indicating a higher incidence in male dogs. Infection with trematodes has been reported as a risk factor for the occurrence of CCA, but cases have also been reported outside the infection area, and the relationship remains unclear (Liptak *et al.*, 2019). Combined hepatocellular-cholangiocarcinoma (cHCC-CCA), which shows the histological features of both HCC and CCA, has also been reported (Patnaik *et al.*, 1980; Trigo *et al.*, 1982; Terai *et al.*, 2022). It has been reported that among malignant canine hepatic tumors, the incidence of HCC is 77%, CCA is 9%–41%, and HCC-cCCA is 4.6%, respectively (Liptak *et al.*, 2019; Terai *et al.*, 2022). The diagnosis of HCC and CCA involves blood tests, X-ray, and ultrasonography. As advanced examinations, triple-phase contrast-enhanced computed tomography (CT) and magnetic

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***Corresponding Author:** Shidow Torisu. Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan. Email: *s-torisu@rakuno.ac.jp*

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最終責任者　Shidow Torisu (Corresponding Author)

動物生殖学（Theriogenology）

Tomochika Sugiura

Assistant Professor

助教　杉浦　智親

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1) Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow.
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II. その他＜Others＞

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REVIEWED BY
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Jagmohan Rajput,
Nanaji Deshmukh Veterinary Science
University, India

*CORRESPONDENCE
Tomochika Sugiura
✉ t-sugiura@rakuno.ac.jp

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Case Report: Fetal growth restriction and prolonged gestation associated with umbilical cord torsion and entanglement in a Holstein dairy cow

Koto Ohsaki[1], Yuto Sano[2], Takashi Murakami[3], Yuki Ishiguro[2],
Ayaka Ohtake[3], Ayano Sato[3], Ken Nakada[1] and
Tomochika Sugiura[1]*

[1]Laboratory of Theriogenology, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [2]Laboratory of Veterinary Pathology, Pathobiology, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan, [3]Laboratory of Farm Animal Surgery, Farm Animal Clinical Sciences, Department of Veterinary Medicine, School of Veterinary Medicine, Ebetsu, Hokkaido, Japan

This case report describes a rare instance of a bovine fetus with concurrent umbilical cord torsion and entanglement, which resulted in fetal growth restriction (FGR) and prolonged gestation. A 36-month-old primiparous Holstein cow, 285 days pregnant, was examined after failing to show signs of parturition. Fetal heartbeat was confirmed via abdominal ultrasonography until 295 days post-artificial insemination (AI) but was not detected thereafter, leading to a presumptive diagnosis of fetal death at 313 days post-AI. Following induction, the dead fetus was delivered at 316 days post-AI. Despite a gestational age of approximately 10.5 months, the fetus exhibited severe growth restriction that is equivalent to that of a normal 7-month-old fetus. The umbilical cord measured 40 cm—abnormally long for a 7-month-old fetus—and was tightly wrapped around the right hind limb with more than 360 degrees of torsion along its long axis. Maternal serum analysis revealed persistently high progesterone and markedly low estradiol concentrations before parturition, suggesting that the physiological process of parturition had not been initiated. It is speculated that the FGR resulted from chronic blood flow obstruction likely associated with the umbilical cord abnormalities, which were hypothesized to have occurred during the second trimester (approximately 4 months). The consequent absence of normal fetal signals to initiate parturition and the lack of periparturient endocrine changes contributed to prolonged gestation and underdeveloped mammary glands in the dam. This report represents the first detailed description of intrauterine umbilical cord abnormalities in cattle, demonstrating their potential to cause fetal developmental delay, prolonged gestation, and impaired mammary gland development.

KEYWORDS

bovine fetus, umbilical cord torsion, umbilical cord entanglement, fetal growth restriction, maternal serum analysis, parturition

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最終責任者：Tomochika Sugiura (Corresponding Author)

伴侶動物外科学（Companion Animal Surgery）

Shushi Yamamoto

Assistant Professor

助教　山本　集士

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1）Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study.
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II. その他＜Others＞

1) Impact of Vitamin E-Coated Membrane Hemodiafilter on Serum Albumin Redox State in the Acute Kidney Injury Pig Hemodialysis Model.
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Artif Organs. 9:1076-1081. 2025. doi: 10.1111/aor.14982.
2) Risk factors for complications associated with canine hepatic mass resection: A study of 96 cases.
Konno R, Kaneko Y, Osuga T, Torisu S, **Yamamoto S**, Okadera R, Naganobu K.
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Triple-phase contrast-enhanced computed tomography findings in dogs with cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: Pilot study

Yoshimichi Goda[1#], Shushi Yamamoto[1#], Kenjiro Miyoshi[1], Kiwamu Hanazono[1],
Mai Morimura[1], Toshikazu Sakai[1], Masahiro Tamura[1], Tatsuya Deguchi[1], Yoshifumi Endo[1,2],
Tsuyoshi Kadosawa[1,3], Mayu Fujita[1], Takaharu Itami[1] and Shidow Torisu[1*]

[1]*Department of Companion Animal Clinical Sciences, School of Veterinary Medicine,*
Rakuno Gakuen University, Ebetsu, Japan
[2]*Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, Tottori, Japan*
[3]*Japan Small Animal Medical Center, Tokorozawa-Shi, Japan*
[#]*Both authors are equally contributed to this work*

ABSTRACT

Background: Recently, the usefulness of triple-phase contrast-enhanced computed tomography (CT) scans for liver tumors in canines has been reported. However, detailed information on the CT findings of cholangiocarcinoma (CCA) and combined hepatocellular-cholangiocarcinoma (cHCC-CCA) remains limited.

Aim: This study aimed to retrospectively evaluate triple-phase contrast-enhanced CT findings in canine CCA and cHCC-CCA using objective parameters, such as CT values and morphological characteristics.

Methods: This study included eight dogs that underwent triple-phase contrast-enhanced CT scans and surgical removal and were pathologically diagnosed. Three CCA cases and five cHCC-CCA cases were analyzed. Parameters included CT values and mass sizes, CT values and lymph node sizes, and rim enhancement. "Rim enhancement" was defined as the contrast enhancement effect at the liver parenchyma–mass boundary.

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Conclusion: Rim enhancement may help distinguish between CCA and cHCC-CCA. However, this study has a limited number of cases, and future large-scale, multicenter studies are warranted.

Keywords: Canine, Cholangiocarcinoma, Combined hepatocellular-cholangiocarcinoma, Computed tomography, Dog.

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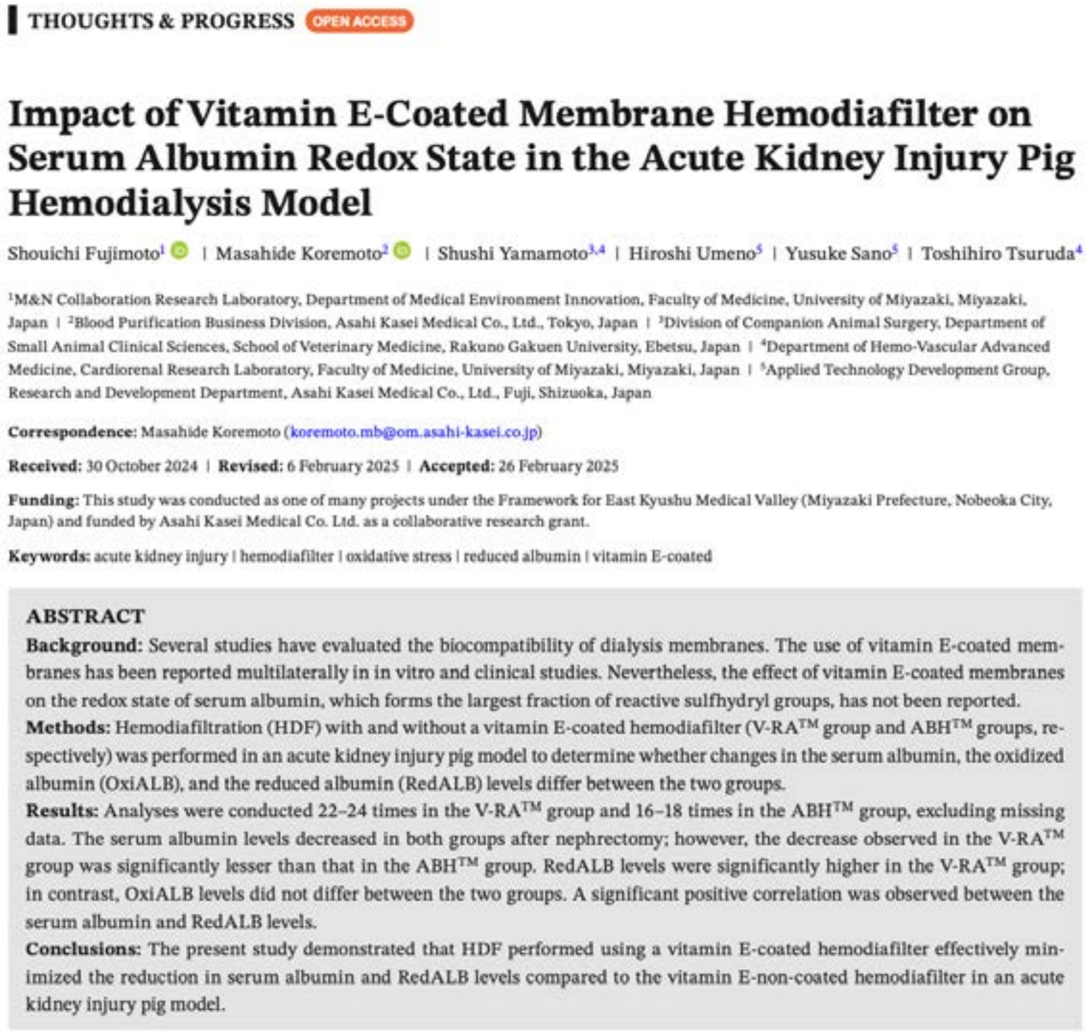
最終責任者 Sidow Torisu (Corresponding Author)



THOUGHTS & PROGRESS OPEN ACCESS

Impact of Vitamin E-Coated Membrane Hemodiafilter on Serum Albumin Redox State in the Acute Kidney Injury Pig Hemodialysis Model

Shouichi Fujimoto[1] | Masahide Koremoto[2] | Shushi Yamamoto[3,4] | Hiroshi Umeno[5] | Yusuke Sano[5] | Toshihiro Tsuruda[4]

[1]M&N Collaboration Research Laboratory, Department of Medical Environment Innovation, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan | [2]Blood Purification Business Division, Asahi Kasei Medical Co., Ltd., Tokyo, Japan | [3]Division of Companion Animal Surgery, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan | [4]Department of Hemo-Vascular Advanced Medicine, Cardiorenal Research Laboratory, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan | [5]Applied Technology Development Group, Research and Development Department, Asahi Kasei Medical Co., Ltd., Fuji, Shizuoka, Japan

Correspondence: Masahide Koremoto (koremoto.mb@om.asahi-kasei.co.jp)

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Keywords: acute kidney injury | hemodiafilter | oxidative stress | reduced albumin | vitamin E-coated

ABSTRACT

Background: Several studies have evaluated the biocompatibility of dialysis membranes. The use of vitamin E-coated membranes has been reported multilaterally in in vitro and clinical studies. Nevertheless, the effect of vitamin E-coated membranes on the redox state of serum albumin, which forms the largest fraction of reactive sulfhydryl groups, has not been reported.

Methods: Hemodiafiltration (HDF) with and without a vitamin E-coated hemodiafilter (V-RA™ group and ABH™ groups, respectively) was performed in an acute kidney injury pig model to determine whether changes in the serum albumin, the oxidized albumin (OxiALB), and the reduced albumin (RedALB) levels differ between the two groups.

Results: Analyses were conducted 22–24 times in the V-RA™ group and 16–18 times in the ABH™ group, excluding missing data. The serum albumin levels decreased in both groups after nephrectomy; however, the decrease observed in the V-RA™ group was significantly lesser than that in the ABH™ group. RedALB levels were significantly higher in the V-RA™ group; in contrast, OxiALB levels did not differ between the two groups. A significant positive correlation was observed between the serum albumin and RedALB levels.

Conclusions: The present study demonstrated that HDF performed using a vitamin E-coated hemodiafilter effectively minimized the reduction in serum albumin and RedALB levels compared to the vitamin E-non-coated hemodiafilter in an acute kidney injury pig model.

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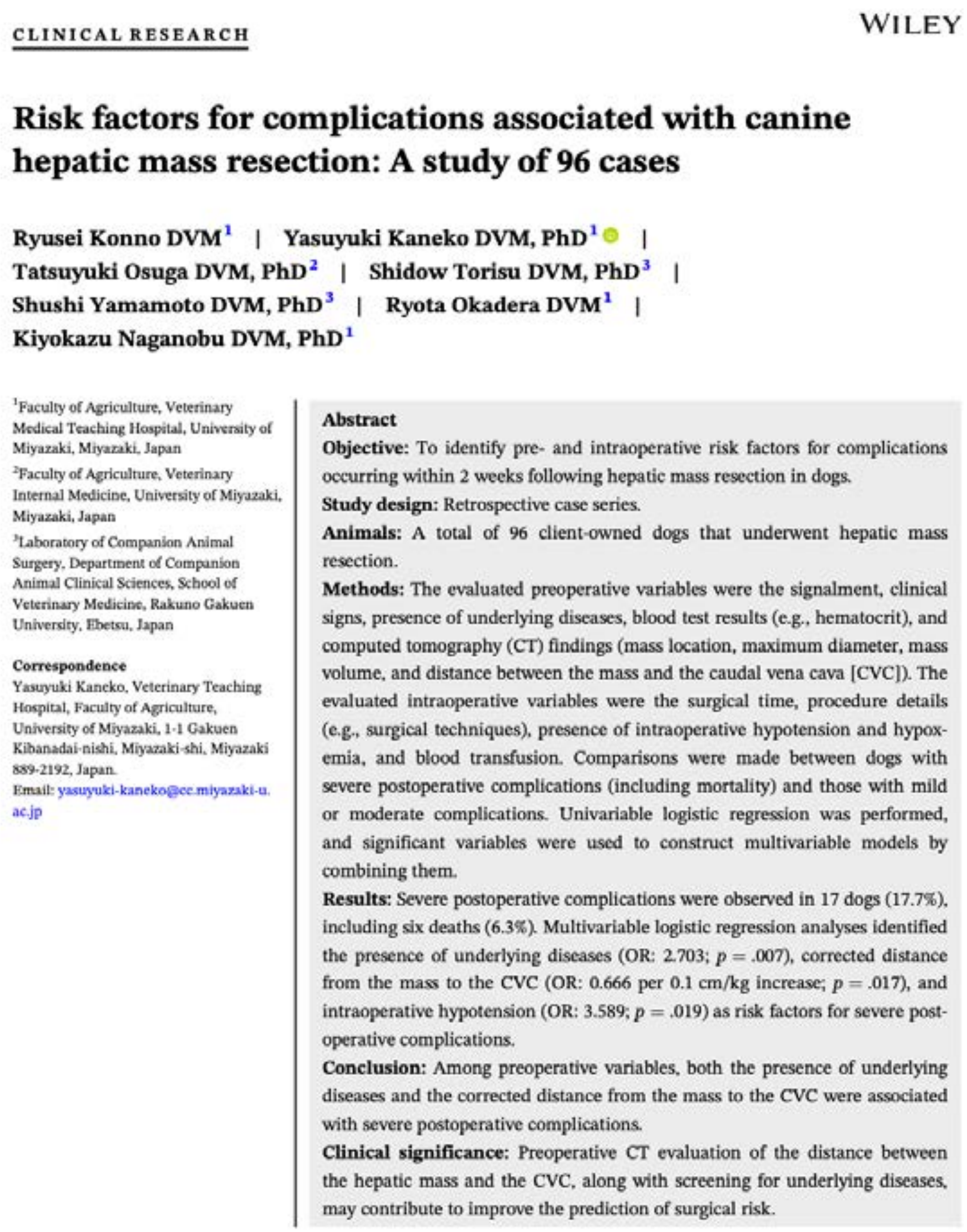
最終責任者　Masahide Koremoto (Corresponding Author)



CLINICAL RESEARCH

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Risk factors for complications associated with canine hepatic mass resection: A study of 96 cases

Ryusei Konno DVM[1] | Yasuyuki Kaneko DVM, PhD[1] | Tatsuyuki Osuga DVM, PhD[2] | Shidow Torisu DVM, PhD[3] | Shushi Yamamoto DVM, PhD[3] | Ryota Okadera DVM[1] | Kiyokazu Naganobu DVM, PhD[1]

[1]Faculty of Agriculture, Veterinary Medical Teaching Hospital, University of Miyazaki, Miyazaki, Japan

[2]Faculty of Agriculture, Veterinary Internal Medicine, University of Miyazaki, Miyazaki, Japan

[3]Laboratory of Companion Animal Surgery, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Correspondence
Yasuyuki Kaneko, Veterinary Teaching Hospital, Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki-shi, Miyazaki 889-2192, Japan.
Email: yasuyuki-kaneko@cc.miyazaki-u.ac.jp

Abstract

Objective: To identify pre- and intraoperative risk factors for complications occurring within 2 weeks following hepatic mass resection in dogs.

Study design: Retrospective case series.

Animals: A total of 96 client-owned dogs that underwent hepatic mass resection.

Methods: The evaluated preoperative variables were the signalment, clinical signs, presence of underlying diseases, blood test results (e.g., hematocrit), and computed tomography (CT) findings (mass location, maximum diameter, mass volume, and distance between the mass and the caudal vena cava [CVC]). The evaluated intraoperative variables were the surgical time, procedure details (e.g., surgical techniques), presence of intraoperative hypotension and hypoxemia, and blood transfusion. Comparisons were made between dogs with severe postoperative complications (including mortality) and those with mild or moderate complications. Univariable logistic regression was performed, and significant variables were used to construct multivariable models by combining them.

Results: Severe postoperative complications were observed in 17 dogs (17.7%), including six deaths (6.3%). Multivariable logistic regression analyses identified the presence of underlying diseases (OR: 2.703; $p = .007$), corrected distance from the mass to the CVC (OR: 0.666 per 0.1 cm/kg increase; $p = .017$), and intraoperative hypotension (OR: 3.589; $p = .019$) as risk factors for severe postoperative complications.

Conclusion: Among preoperative variables, both the presence of underlying diseases and the corrected distance from the mass to the CVC were associated with severe postoperative complications.

Clinical significance: Preoperative CT evaluation of the distance between the hepatic mass and the CVC, along with screening for underlying diseases, may contribute to improve the prediction of surgical risk.

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最終責任者　Yasuyuki Kaneko (Corresponding Author)