Academic Research of
Graduate School of Veterinary Medicine

January – December
2023

Graduate School of Veterinary Medicine

Rakuno Gakuen University

RAKUNO GAKUEN UNIVERSITY
酪農学園大学

Japan Institution for Higher Education Evaluation
JIHEE since 2004
UNIVERSITY
2020.4-2027.3

2023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。

2023年の筆頭著者（FA）および責任著者（CA）の論文合計は51報でした。過去10年間では、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、2013年48報となっており、2017～2021年には右肩上がりで増えていましたが、過去2年間は減少傾向にあります。本学では2021年度より新カリキュラムに移行しました。とくに、獣医学群では、ヨーロッパ獣医学教育機関協会（EAEVE: European Association of Establishments for Veterinary Education）の国際認証取得を目指して獣医学教育の質の保証と国際化に取り組み、学生が卒後1日目の能力（Day One Competencies）を身につけることを保証するために3Rs（Replacement, Reduction, Refinement）の思想を反映したスキルスラボを整備して少人数によるHands On実習やクリニカルローテーションに対応した新カリキュラムをスタートしました。前研究科長が憂慮されていたように、このEAEVE対応の教育負担増が英文業績の公表に影響しているのかもしれません。

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 56 件と増えており（2022年度48件、2021年度37件、2020年度31件）、その配分額（8,164万円）も科研費が配分された私立大学（591大学）では100位と上昇中です（2022年度119位、2021年度129位、2020年度 133 位）。教員個々の研究活動の高まりは持続的に確保されています。獣医学群の新カリキュラムは、獣医保健看護学類で 2024 年度、獣医学類で 2026 年度に完成し、これに対応する教員組織も 2025 年度までに確保される計画のようです。現在の「EAEVE 対応の教育負担増」を教員組織の完成と教員個々の馴化によって切り抜けることができれば、今後の獣医学群教員のFA/CA論文数は2021年を超えていくと期待されます。

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 2023 (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 2023 was 51 reports. In the past 10 years, 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, 51 reports in 2014, and 48 reports in 2013. It was increasing steadily from 2017 to 2021 but has been on a downward trend for the past two years. Our university has transitioned to a new curriculum starting in 2021. In particular, the School of Veterinary Medicine has worked to ensure the quality of veterinary education and internationalize it, aiming to obtain international certification from the European Association of Establishments for Veterinary Education (EAEVE). In the new curriculum, in order to ensure that students acquire Day One Competencies, we have established a skills lab that reflects the philosophy of 3Rs (Replacement, Reduction, Refinement) and have begun small-group hands-on training and clinical rotations. As the former dean of the graduate school was concerned, the increased educational burden associated with EAEVE may be having an impact on the submission and publication of English papers.

On the other hand, the number of projects selected for Grants-in-Aid for Scientific Research (KAKENHI) across the university this year increased to 56 (48 in 2022, 37 in 2021, and 31 in 2020). In addition, the amount allocated this year (81.64 million yen) is also rising to 100th place (out of 591 universities) among private universities in Japan that received KAKENHI (119th in 2022, 129th in 2021, 133rd in 2020). Therefore, a sustained increase in the research activities of individual faculty members is ensured. The new curriculum for the School of Veterinary Medicine will be completed in 2024 for the Department of Veterinary Science and in 2026 for the Department of Veterinary Medicine, and it appears that the plan is to have a corresponding faculty structure in place by 2025. The current "increase in EAEVE's educational burden" will be overcome by the completion of the faculty organization and the acclimatization of individual faculty members, and the number of FA/CA papers in the School of Veterinary Medicine is expected to exceed the record high.

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 2023）

*：大学院担当教員（Supervisor）

■ 獣医学類 Department of Veterinary Medicine

■ **獣医保健看護学類 Department of Veterinary Medicine**

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＞

Ⅱ．その他＜Others＞

1）Tryptanthrin reduces *Campylobacter jejuni* colonization in the chicken gut by a bactericidal mechanism.
Iwata T, Watanabe-Yanai A, Tamamura-Andoh Y, Arai N, **Akiba M**, Kusumoto M.
Appl Environ Microbiol. 89:e0170122. 2023. doi:10.1128/aem.01701-22.

2）Nationwide analysis of antimicrobial resistance in pathogenic *Escherichia coli* strains isolated from diseased swine over 29 years in Japan.
Kusumoto M, Tamamura-Andoh Y, Hikoda-Kogiku Y, Magome A, Okuhama E, Sato K, Mizuno Y, Arai N, Watanabe-Yanai A, Iwata T, Ogura Y, Gotoh Y, Nakamura K, Hayashi T, **Akiba M**.
Front Microbiol. 14:1107566. 2023. doi:10.3389/fmicb.2023.1107566.

3）Occurrence and the potential ecological risk of veterinary antimicrobials in swine farm wastewaters in Japan: Seasonal changes, relation to purchased quantity and after termination of oxytetracycline usage.
Watanabe M, Guruge KS, Uegaki R, Kure K, Yamane I, Kobayashi S, **Akiba M**.
Environ Int. 173: 107812. 2023. doi: 10.1016/j.envint.2023.107812.

4）Characteristics of antimicrobial residues in manure composts from swine farms: Residual patterns, removal efficiencies, and relation to purchased quantities and composting methods in Japan.
Watanabe M, Goswami P, Kure K, Yamane I, Kobayashi S, **Akiba M**, Guruge KS.
J Hazard Mater. 459:132310. 2023. doi:10.1016/j.jhazmat.2023.132310.

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

FOOD MICROBIOLOGY

Tryptanthrin Reduces *Campylobacter jejuni* Colonization in the Chicken Gut by a Bactericidal Mechanism

Taketoshi Iwata,[a] Ayako Watanabe-Yanai,[a] Yukino Tamamura-Andoh,[a] Nobuo Arai,[a] Masato Akiba,[b] Masahiro Kusumoto[a,c]

[a]Division of Zoonosis Research, National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan
[b]Laboratory of Veterinary Bacteriology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[c]Graduate School of Veterinary Science, Osaka Metropolitan University, Izumisano, Osaka, Japan

ABSTRACT *Campylobacter jejuni* is a leading cause of foodborne bacterial gastroenteritis worldwide, and raw or undercooked chicken meat is considered the major source of human campylobacteriosis. In this study, we identified 36 compounds that showed inhibitory effects on *C. jejuni* growth at low concentrations by screening a chemical compound library. Three of the 36 compounds were herbal compounds, including tryptanthrin (TRP), an indoloquinazoline alkaloid. TRP has been reported to have a variety of biological properties, such as antimicrobial, anti-inflammatory, and antitumor activities, but there was previously no information about its anti-*C. jejuni* activity. We further conducted *in vitro* and *in vivo* experiments to evaluate the potential of TRP for the control of *C. jejuni* in chicken farms. The MIC of TRP for *C. jejuni* was much lower than that of 13 other herbal compounds that were previously reported to have anti-*C. jejuni* activities. Time-kill assays under growing and nongrowing conditions demonstrated that TRP has bactericidal activity against *C. jejuni*. In addition, TRP showed a narrow-spectrum antimicrobial effect against *C. jejuni*, and there was little potential for the development of TRP-resistant *C. jejuni* during serially passaged culture. In chick infection experiments, the administration of TRP in drinking water significantly reduced the cecal colonization of *C. jejuni* when TRP was used either before or after *C. jejuni* infection. These data suggest that TRP is effective for the control of *C. jejuni* in chicken farms.

IMPORTANCE *Campylobacter* is a widespread pathogen in the food chain of chickens. Once chickens become infected, large numbers of *Campylobacter* cells are excreted in their feces. The development of an effective material for reducing the amount of *Campylobacter* in the chicken intestinal tract will make it possible to reduce the contamination of the food chain with *Campylobacter* and to produce safe and secure chicken meat. In the present study, *in vivo* experiments revealed that the use of an herbal compound, tryptanthrin, significantly reduced the number of *Campylobacter* cells in the chicken gut by a bactericidal mechanism. Furthermore, our *in vitro* experiments demonstrated that, compared with the other herbal compounds, tryptanthrin achieved antimicrobial activity against *C. jejuni* at the lowest concentration. The use of tryptanthrin may lead to the development of a novel control measure for reducing the colonization of *C. jejuni* in the food chain.

KEYWORDS *Campylobacter jejuni*, chemical compound library, chicken, herbal compound, tryptanthrin

Campylobacter is the most common cause of bacterial foodborne disease in humans worldwide. The World Health Organization (WHO) estimated that more than 95 million cases of foodborne illness caused by *Campylobacter* species occurred worldwide in 2010 (1). In the European Union, the annual cost associated with human campylobacteriosis was estimated to be €2.4 billion (2). Contamination by *Campylobacter jejuni* and

Editor Edward G. Dudley, The Pennsylvania State University

Address correspondence to Masahiro Kusumoto, kusu555@affrc.go.jp.
The authors declare no conflict of interest.
Received 2 October 2022
Accepted 20 December 2022
Published 18 January 2023

出典 doi:10.1128/aem.01701-22.
https://journals.asm.org/doi/full/10.1128/aem.01701-22
最終責任者 Masahiro Kusumoto (Corresponding Author)

frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 17 March 2023
DOI 10.3389/fmicb.2023.1107566

OPEN ACCESS

EDITED BY
Etienne Giraud,
Institut National de la Recherche Agronomique de Toulouse, France

REVIEWED BY
Chang-Wei Lei,
Sichuan University, China
Yuqi Yang,
Northeast Agricultural University, China

*CORRESPONDENCE
Masahiro Kusumoto
kusu555@affrc.go.jp

SPECIALTY SECTION
This article was submitted to Antimicrobials, Resistance and Chemotherapy, a section of the journal Frontiers in Microbiology

RECEIVED 25 November 2022
ACCEPTED 06 March 2023
PUBLISHED 17 March 2023

CITATION
Kusumoto M, Tamamura-Andoh Y, Hikoda-Kogiku Y, Magome A, Okuhama E, Sato K, Mizuno Y, Arai N, Watanabe-Yanai A, Iwata T, Ogura Y, Gotoh Y, Nakamura K, Hayashi T and Akiba M (2023) Nationwide analysis of antimicrobial resistance in pathogenic *Escherichia coli* strains isolated from diseased swine over 29 years in Japan. *Front. Microbiol.* 14:1107566. doi: 10.3389/fmicb.2023.1107566

Nationwide analysis of antimicrobial resistance in pathogenic *Escherichia coli* strains isolated from diseased swine over 29 years in Japan

Masahiro Kusumoto[1,2]*, Yukino Tamamura-Andoh[1], Yuna Hikoda-Kogiku[3], Asami Magome[4], Erina Okuhama[5], Keisuke Sato[6], Yoshino Mizuno[7], Nobuo Arai[1], Ayako Watanabe-Yanai[1], Taketoshi Iwata[1], Yoshitoshi Ogura[8], Yasuhiro Gotoh[9], Keiji Nakamura[9], Tetsuya Hayashi[9] and Masato Akiba[10]

[1]National Institute of Animal Health, National Agriculture and Food Research Organization, Tsukuba, Japan, [2]Graduate School of Veterinary Science, Osaka Metropolitan University, Osaka, Japan, [3]Ehime Chuyo Livestock Hygiene Service Center, Ehime, Japan, [4]Kagoshima Central Livestock Hygiene Service Center, Kagoshima, Japan, [5]Miyazaki Livestock Hygiene Service Center, Miyazaki, Japan, [6]Niigata Chuo Livestock Hygiene Service Center, Niigata, Japan, [7]Kumamoto Chuo Livestock Hygiene Service Center, Kumamoto, Japan, [8]Department of Infectious Medicine, Kurume University School of Medicine, Fukuoka, Japan, [9]Department of Bacteriology, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan, [10]School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Pathogenic *Escherichia coli* strains are important causes of several swine diseases that result in significant economic losses worldwide. In Japan, the use of antimicrobials in swine is much higher than that in other farm animals every year. Antimicrobial resistance in pathogenic *E. coli* strains also heavily impacts the swine industry due to the limited treatment options and an increase in the potential risk of the One Health crisis. In 2016, we investigated 684 Japanese isolates of swine pathogenic *E. coli* belonging to four major serogroups and reported the emergence and increase in the highly multidrug-resistant serogroups O116 and OSB9 and the appearance of colistin-resistant strains. In the present study, by expanding our previous analysis, we determined the serotypes and antimicrobial resistance of 1,708 *E. coli* strains isolated from diseased swine between 1991 and 2019 in Japan and found recent increases in the prevalences of multidrug-resistant strains and minor serogroup strains. Among the antimicrobials examined in this study that have been approved for animal use, a third-generation cephalosporin was found to be effective against the most isolates (resistance rate: 1.2%) but not against highly multidrug-resistant strains. We also analyzed the susceptibilities of the 1,708 isolates to apramycin and bicozamycin, both which are available for treating

出典　doi:10.3389/fmicb.2023.1107566.

https://www.frontiersin.org/articles/10.3389/fmicb.2023.1107566/full

最終責任者　Masahiro Kusumoto (Corresponding Author)

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Occurrence and the potential ecological risk of veterinary antimicrobials in swine farm wastewaters in Japan: Seasonal changes, relation to purchased quantity and after termination of oxytetracycline usage

Mafumi Watanabe [a], Keerthi S. Guruge [a,b,*], Ryuichi Uegaki [a], Katsumasa Kure [c], Itsuro Yamane [a], Sota Kobayashi [a], Masato Akiba [a,1]

[a] National Institute of Animal Health (NIAH), National Agriculture and Food Research Organization (NARO), 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan
[b] Graduate School of Veterinary Science, Osaka Metropolitan University, 1-58 Rinku-oraikita, Izumisano, Osaka 598-8531, Japan
[c] The Japanese Association of Swine Veterinarians (JASV), 1704-3 Nishi-Ooi, Tsukuba, Ibaraki 300-1260, Japan

ARTICLE INFO

Handling Editor: Hefa Cheng

Keywords:
Antimicrobials
Pig
Aerobic wastewater treatment
Activated sludge method
Risk assessment

ABSTRACT

This study provides the first comprehensive investigation of the residual concentrations of eight classes of antimicrobial agents (AMs, 20 compounds) in 13 swine wastewater treatment facilities in Japan. These facilities implemented the aerobic activated sludge (AS) or its alternative methods. The maximum concentrations before treatment were found at the level of 7100, 6900, 6000, 3600, 3400, and 1400 μg/L for tilmicosin, oxytetracycline (OTC), chlortetracycline, lincomycin , sulfamethoxazole, and trimethoprim, respectively. The highest detection rate (96.3%) in influents was noted for the morantel, which was a feed additive. The seasonal difference in residual concentration was much greater for tetracyclines (TCs) and macrolides (MLs) when their residual concentrations were high, especially in the cold season. There was a positive correlation between the purchased quantity of TCs and fluoroquinolones (FQs) and their residue levels detected in the effluents ($p < 0.01$). The estimated removal rate of AMs was greater than 80%. In contrast, on a few occasions, it was diminished due to failing operating conditions, such as water temperature and AS rate in the aeration tank. The estimated ecological risks of AMs in effluents based on risk quotients (RQs) considered to enhance the selection pressure for drug resistance (RQs-AMR) were high for TCs and FQs, whereas ecotoxicological effects (RQs-ENV) to aquatic organisms were higher for sulfonamides and MLs. When OTC usage ceased, its concentration in wastewater decreased rapidly; however, it remained longer period in the effluents, probably due to OTC desorption from the AS. The concentrations (and respective RQs) of TCs were decreased by >99.8% and >92% in the influents and effluents, respectively. This data suggested that it is essential to reduce the amount used and introduce more efficient methods and operating conditions to constantly remove AMs during the treatment to reduce the risk of AM discharge from swine farms.

1. Introduction

Antimicrobials (AMs) are indispensable in controlling infectious diseases in human, veterinary, aquaculture, and agriculture environments. The predicted global AM consumption in the human sector in 2030 could be 200% higher than the 42 billion defined daily doses estimated in 2015 (Klein et al., 2018). In 2018, 1761.4 tons (as active substances) of AMs were sold in Japan (MHLW, 2021). Most of them were purchased as veterinary drugs (646.4 tons) and nutrient ingredients to promote the efficient conversion of feed (feed additives: 216.7 tons) in animal husbandry. Tetracyclines (TCs) as veterinary drugs were the predominant AM class purchased for food-producing animals in Japan, followed by polyethers (as feed additives), penicillins, sulfonamides (SAs), and macrolides (MLs) (MHLW, 2021). In contrast, other classes, such as aminoglycosides, amphenicols, peptides, lincosamides, and fluoroquinolones (FQs), were also used in fewer quantities. It is worth noting that AMs as veterinary drugs have been used more frequently in the pig industry (approximately 60% of total

* Corresponding author at: National Institute of Animal Health (NIAH), National Agriculture and Food Research Organization (NARO), 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan.
E-mail address: guruge@affrc.go.jp (K.S. Guruge).
[1] Current address: School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan.

https://doi.org/10.1016/j.envint.2023.107812
Received 16 September 2022; Received in revised form 7 January 2023; Accepted 7 February 2023
Available online 11 February 2023

出典 doi: 10.1016/j.envint.2023.107812.
https://www.sciencedirect.com/science/article/pii/S0160412023000855
最終責任者 Keerthi S. Guruge (Corresponding Author)

Journal of Hazardous Materials 459 (2023) 132310

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research Paper

Characteristics of antimicrobial residues in manure composts from swine farms: Residual patterns, removal efficiencies, and relation to purchased quantities and composting methods in Japan

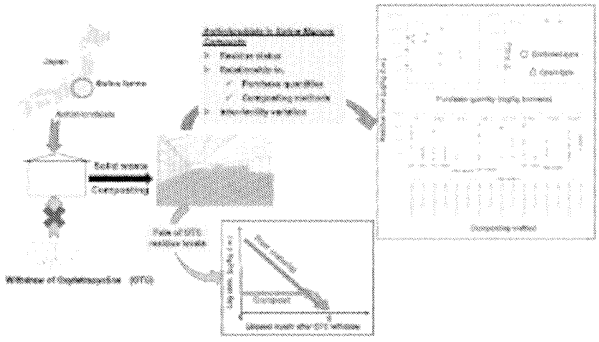
Mafumi Watanabe [a], Prasun Goswami [a], Katsumasa Kure [b], Itsuro Yamane [a], Sota Kobayashi [a], Masato Akiba [c], Keerthi S. Guruge [a, d, *]

[a] National Institute of Animal Health (NIAH), National Agriculture and Food Research Organization (NARO), 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan
[b] The Japanese Association of Swine Veterinarians (JASV), 1704-3 Nishi-Ooi, Tsukuba, Ibaraki 300-1200, Japan
[c] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[d] Graduate School of Veterinary Science, Osaka Metropolitan University, 1-58 Rinku-orai-kita, Izumisano, Osaka 598-8531, Japan

HIGHLIGHTS

- We present the first holistic report on antimicrobial levels in Japanese swine manure compost.
- Tilmicosin and tiamulin concentrations were the highest in composts.
- The removal efficiency depended on antimicrobial types, composting practices, and facility conditions.
- Morantel, a feed additive, was relatively resistant to degradation while composting.
- Once its usage was withdrawn, tetracycline residues in composts dissipated within 6 months.

GRAPHICAL ABSTRACT



Abbreviations: AMs, antimicrobials; AMR, antimicrobial resistance; ABs, antibiotics; ARB, antibiotic-resistant bacteria; TCs, tetracyclines (as AM classes); FQs, fluoroquinolones; SAs, sulfonamides; MLs, macrolides; TC, tetracycline (as an AM compound), OTC, oxytetracycline; CTC, chlortetracycline; DOXY, doxycycline; ERFX, enrofloxacin; CPFX, ciprofloxacin; NFLX, norfloxacin; MBFX, marbofloxacin; DNFX, danofloxacin; OBFX, orbifloxacin; SMX, sulfamethoxazole; SMMX, sulfamonomethoxine; SDMX, sulfadimethoxine; SDZ, sulfadiazine, SMZ, sulfamethazine (sulfadimidine); TS, tylosin; TMS, tilmicosin; LCM, lincomycin; TML, tiamulin; TMP, trimethoprim; MRT, morantel; RMs, raw materials for composts; MCs, matured composts from open-type facilities; ESFs, early stages of composting (nearly RMs) from open-type facilities; ISF, intermediate stages of composting from open-type facilities; SFPs, composts of secondary fermentation in piles (nearly MCs) from the enclosed-type facilities; WWT, wastewater treatment; MDL, method detection limits; MQL, method quantifying limits; EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid disodium salt dihydrate; PP, polypropylene; MHLW, The Ministry of Health, Labour and Welfare, Japan; MAFF, Ministry of Agriculture, Forestry and Fisheries, Japan; NAVL, National Veterinary Assay Laboratory, MAFF, Japan.

* Corresponding author at: National Institute of Animal Health (NIAH), National Agriculture and Food Research Organization (NARO), 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan.
E-mail address: guruge@affrc.go.jp (K.S. Guruge).

https://doi.org/10.1016/j.jhazmat.2023.132310
Received 27 March 2023; Received in revised form 6 August 2023; Accepted 13 August 2023
Available online 15 August 2023

出典　doi:10.1016/j.jhazmat.2023.132310.
https://www.sciencedirect.com/science/article/abs/pii/S0304389423015935
最終責任者　Keerthi S. Guruge (Corresponding Author)

医動物学（Parasitology and Zoology）

Mitsuhiko Asakawa

Professor

教授　浅川　満彦

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

1）Parasites found in a Rough-legged Buzzard, *Buteo lagopus* (Pontoppidan, 1763) collected at Rausu, Hokkaido, Japan.
Yoshino M, **Asakawa, M.**
Jpn J Zoo Wildl Med. 28: 75–78. 2023.
https://www.jstage.jst.go.jp/article/jjzwm/28/2/28_91/_pdf/-char/ja

Ⅱ．その他＜Others＞

Research note *Parasitology*

Parasites Found in a Rough-legged Buzzard, *Buteo lagopus* (Pontoppidan, 1763) Collected at Rausu, Hokkaido, Japan

Tomoo YOSHINO[1, 2], Mitsuhiko ASAKAWA[2)*]

1) Kushiro Zoo, Kushiro, Hokkaido 085-0201, Japan
2) School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

[Received 11 April 2022; accepted 9 June 2022]

ABSTRACT

A young male Rough-legged Buzzard, Buteo lagopus, was found debilitated at Rausu, Hokkaido, Japan on 16 December, 2013 and died shortly after transport to the shelter. A post-mortem revealed emaciation, chest pressure ulceration, sternal fracture, and claw damage suggesting the malnutrition. Nematodes of Capillariidae gen. sp. and Porrocaecum sp., and cestode of Cyclophyllidea fam. gen. sp. were detected in the alimentary tract. Also chewing lice, *Degeeriella fulva*, were found on the body surface. This is the first record of *D. fulva* from *B. lagopus* in eastern Hokkaido, and the first record of Capillariidae gen. sp. and Cyclophyllidea fam. gen. sp. from *B. lagopus* in Japan.

Key words: chewing lice, helminth, malnutrition, Rough-legged Buzzard

— *Jpn J Zoo Wildl Med 28*(2) : 91-94, 2023

The Rough-legged Buzzard, *Buteo lagopus* (Pontoppidan, 1763), is a Holarctic raptor breeding in the tundra and boreal areas of Northern Eurasia and North America, and wintering south of it' s breeding range from eastern Europe in the west to eastern Eurasia and Japan in the east [1]. Subspecies *B. l. menzbieri* Dementiev, 1951 is a scarce winter visitor to Japan, mainly to Hokkaido occasionally further south [2, 3]. Rough-legged Buzzards are among the few raptors to overwinter in Japan, but little biological data is available from Japan [4]. Information on disease causing agents such as viruses, bacteria and parasites, is important for the conservation of birds [5, 6].

On 16 December 2013, a debilitated Rough-legged Buzzard was taken in to captivity, but died shortly thereafter. The carcass was taken to Kushiro Zoo for postmortem examination (Zoo Postmortem No. 13-019). The body surface was examined macroscopically, and the gastrointestinal tract and organs were examined for parasites under a binocular microscope. Parasite specimens were fixed in 70% ethanol and taken to the Wild Animal Medical Center (WAMC) of Rakuno Gakuen University for taxonomical examination. The nematodes were cleared with lacto-phenol solution, cestodes were encapsulated with balsam after staining with aceto-carmine solution, and arthropods were mounted using gum chloral for microscopic observation, and morphological and biometric data were recorded using a Lucida camera (OLYMPUS DP20). The parasite specimens are preserved in Kushiro Zoo and the buzzard specimens is stored at Kushiro City Museum.

The Rough-legged Buzzard weighed only 555.6 g and was emaciated compared with the average of that of this species (1.29 – 1.38 kg [3]), with significantly atrophied pectoral muscles and protruding keels (Table 1). A pressure ulcer had formed on the keel, and a fracture of the sternum was observed. In addition, the claws of the second and third toes of the right foot were broken or missing, but neither blood nor scabs were observed . Examination of the internal organs revealed that each was significantly atrophied, but no pathological conditions were found. A lump of murine hair was observed in the stomach. Neither green staining nor ulceration of the gastric mucosa was observed. A large number of chewing lice were found on the body surface, one nematode each was obtained from the esophagus and stomach, and one tapeworm was obtained from the intestinal tract.

Of the 11 lice obtained, six adult males and five females

* Corresponding author : Mitsuhiko ASAKAWA
(*E-mail*: askam@rakuno.ac.jp)

放射線生物学（Radiation Biology）

Daiji Endoh

Professor

教授　遠藤　大二

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

1）PCR-based gene synthesis with overlapping unisense-oligomers asymmetric extension supported by a simulator for oligonucleotide extension achieved 1 kbp dsDNA.
Nishida Y, Kayama K, Endoh T, Hanazono K, Camer G A, **Endoh D**.
BioTechniques 74:317–332. 2023. doi: 10.2144/btn-2022-0127.

Ⅱ．その他　＜Others＞

1）Satoh S, Tanaka R, Yokono M, **Endoh D**, Yabuki T, Tanaka A. Phylogeny analysis of whole protein-coding genes in metagenomic data detected an environmental gradient for the microbiota.
Plos one 18: e0281288. 2023. doi: 10.1371/journal.pone.0281288.

2）Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan.
Yokokawa A, Wenjing D, Momose K, Iima H, Yoshino T, Izumi K, Kawai Y, Amano T, Nakamura T, Sawada A, **Endoh D**, Nakajima N,　Teraoka H.
Animals (Basel) 13:3167, 2023. doi: 10.3390/ani13203167.

Reports

BioTechniques

PCR-based gene synthesis with overlapping unisense-oligomers asymmetric extension supported by a simulator for oligonucleotide extension achieved 1 kbp dsDNA

Yasunori Nishida[1], Kotetsu Kayama[1], Taichi Endoh[1], Kiwamu Hanazono[1], Gerry Amor Camer[1,2] & Daiji Endoh*,[1]

*[1]Department of Radiation Biology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan; [2]College of Veterinary Medicine, University of Eastern Philippines, Catarman, Northern Samar, 6400, Philippines; *Author for correspondence: dendoh@rakuno.ac.jp*

BioTechniques 74: 317–332 (June 2023) 10.2144/btn-2022-0127
First draft submitted: 30 December 2022; Accepted for publication: 30 May 2023; Published online: 21 June 2023

ABSTRACT

We formulated a method to synthesize 1 kbp DNA fragments using 'oligomer unidirectional joining method' via asymmetric extension supported by a simulator for oligonucleotide extension (AESOE). In this study, trials were conducted on 41 sets of different genomic pieces of ten flaviviral genomes, and 31 bacterial 16s rRNA fragments with sizes ranging from 500 bases to 1.0 kbp. Synthetic gene production was found to be successful in all those sets. The synthesis method has three steps: the first step is a seven-linked AESOE, the second step is the linking of the 400-base fragments from the first step, and the third step is the final amplification. Our present approach is highly reproducible and may no longer require optimization of oligomer design.

出典　doi.org/10.2144/btn-2022-0127.
https://www.future-science.com/doi/full/10.2144/btn-2022-0127

最終責任者　Daiji Endoh (Corresponding Author)

PLOS ONE

RESEARCH ARTICLE

Phylogeny analysis of whole protein-coding genes in metagenomic data detected an environmental gradient for the microbiota

Soichirou Satoh[1,2]*, **Rei Tanaka**[2], **Makio Yokono**[3], **Daiji Endoh**[4], **Tetsuo Yabuki**[5], **Ayumi Tanaka**[6]

1 Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan, **2** Faculty of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan, **3** Division of Environmental Photobiology, National Institute for Basic Biology, Okazaki, Japan, **4** Department of Radiation Biology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, **5** General Education Department, Hokusei Gakuen University, Sapporo, Japan, **6** Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

* s-satoh@kpu.ac.jp

Citation: Satoh S, Tanaka R, Yokono M, Endoh D, Yabuki T, Tanaka A (2023) Phylogeny analysis of whole protein-coding genes in metagenomic data detected an environmental gradient for the microbiota. PLoS ONE 18(2): e0281288. https://doi.org/10.1371/journal.pone.0281288

Editor: Alfonso Esposito, University of Trento, ITALY

Received: July 22, 2022

Accepted: January 20, 2023

Published: February 2, 2023

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0281288

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. Perl script, which used in this study is publicly

Abstract

Environmental factors affect the growth of microorganisms and therefore alter the composition of microbiota. Correlative analysis of the relationship between metagenomic composition and the environmental gradient can help elucidate key environmental factors and establishment principles for microbial communities. However, a reasonable method to quantitatively compare whole metagenomic data and identify the primary environmental factors for the establishment of microbiota has not been reported so far. In this study, we developed a method to compare whole proteomes deduced from metagenomic shotgun sequencing data, and quantitatively display their phylogenetic relationships as metagenomic trees. We called this method Metagenomic Phylogeny by Average Sequence Similarity (MPASS). We also compared one of the metagenomic trees with dendrograms of environmental factors using a comparison tool for phylogenetic trees. The MPASS method correctly constructed metagenomic trees of simulated metagenomes and soil and water samples. The topology of the metagenomic tree of samples from the Kirishima hot springs area in Japan was highly similarity to that of the dendrograms based on previously reported environmental factors for this area. The topology of the metagenomic tree also reflected the dynamics of microbiota at the taxonomic and functional levels. Our results strongly suggest that MPASS can successfully classify metagenomic shotgun sequencing data based on the similarity of whole protein-coding sequences, and will be useful for the identification of principal environmental factors for the establishment of microbial communities. Custom Perl script for the MPASS pipeline is available at https://github.com/s0sat/MPASS.

出典　doi.org/10.1371/journal.pone.0281288
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0281288

最終責任者　Souichirou Sato (Corresponding Author)

animals

MDPI

Article

Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan

Ayaka Yokokawa [1], Wenjing Dong [1], Kunikazu Momose [2], Hiroko Iima [3], Tomoo Yoshino [3], Kenichi Izumi [4], Yusuke Kawai [5], Tomoko Amano [4], Tatsuro Nakamura [1], Akira Sawada [6], Daiji Endoh [1], Nobuyoshi Nakajima [6] and Hiroki Teraoka [1,2,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; yokokawa-ayaka@bozo.co.jp (A.Y.); dongwenjing_2015@163.com (W.D.); t-naka@rakuno.ac.jp (T.N.); dendoh@rakuno.ac.jp (D.E.)
[2] NPO Red-Crowned Crane Conservancy, 9-21 Wakatake-Cho, Kushiro 085-0036, Japan; dzi00244@nifty.com
[3] Kushiro Zoo, 11 Shimoninishibetsu, Kushiro 085-0204, Japan; hiroko.iima@city.kushiro.lg.jp (H.I.); garrulaxcanorus@yahoo.co.jp (T.Y.)
[4] College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan; izmken@rakuno.ac.jp (K.I.); amano@rakuno.ac.jp (T.A.)
[5] Laboratory of Toxicology, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada-cho Nishi, Obihiro 080-8555, Japan; ykawai@obihiro.ac.jp
[6] Biodiversity Division, National Institute for Environmental Studies, Tsukuba 305-8506, Japan; akira.sawada.1312@gmail.com (A.S.); naka-320@nies.go.jp (N.N.)
* Correspondence: hteraoka08@gmail.com

Simple Summary: The red-crowned crane (*Grus japonensis*), which is an endangered and highly protected bird species, is distributed in two populations: a mainland population in far eastern Eurasia and an island population in Hokkaido, Japan. Red-crowned cranes in Japan are resident birds mainly in the eastern part of Hokkaido. As omnivores, they feed on plants, grains, insects, and fish. Most cranes spend the winter around feeding stations in southeastern Hokkaido, where people provide corn. Since most of the cranes in Hokkaido now live near areas inhabited by humans, cases of crop damage caused by cranes have recently been reported. This study showed that the cranes feed on various crops of human origin, mostly outside farmlands.

Abstract: Total DNA extracts from the intestinal contents of 60 flying red-crowned cranes (juveniles, subadults and adults) found dead in 2006–2021, and the feces of 25 chicks collected in June and July of 2016–2018, were used for PCR reactions with primers specific for 16 crops, followed by high-throughput sequencing. The most predominant crop detected was corn in adult and subadult cranes (61.7%). Other grains (barley, wheat, soybean) (5.0–8.3%) and vegetables (tomatoes, Chinese cabbage, etc.) (1.7–6.7%) were also detected in flying cranes. Surprisingly, some of the detected crops were not grown in the Kushiro and Nemuro regions. There was no significant difference in crop intake status in winter and that in other seasons for most of the crops. Corn (28.0%), soybeans (8.0%), wheat and beet (4.0%) were detected in crane chicks in summer, though the detection rates were generally lower than those in flying cranes. Alfalfa, which is not grown in eastern Hokkaido but is used in some cattle feed, was detected in some cranes. Rice, buckwheat, adzuki beans, common beans, potatoes and carrots were not detected at any life stage, indicating the preferences of red-crowned cranes. The results suggest that red-crowned cranes in Hokkaido are dependent on dairy farmers for their feed supply.

Keywords: amplicon sequencing; crop consumption; *Grus japonensis*; Japan; scatological samples

Citation: Yokokawa, A.; Dong, W.; Momose, K.; Iima, H.; Yoshino, T.; Izumi, K.; Kawai, Y.; Amano, T.; Nakamura, T.; Sawada, A.; et al. Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan. *Animals* **2023**, *13*, 3167. https://doi.org/10.3390/ani13203167

Academic Editor: Barry Hartup

Received: 27 July 2023
Revised: 6 October 2023
Accepted: 9 October 2023
Published: 10 October 2023

出典　doi.org/10.3390/ani13203167
https://www.mdpi.com/2076-2615/13/20/3167
最終責任者　Hiroki Teraoka (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）Hepatitis E Virus (HEV) Spreads from Pigs and Sheep in Mongolia.
Batmagnai E, Boldbaatar B, Sodbayasgalan A, Kato-Mori Y, **Hagiwara K**.
Animals. 13:891. 2023. doi: 10.3390/ani13050891.

2）Investigation of oncolytic effect of rrecombinant Newcastle disease virus in primary and metastatic oral melanoma.
Numpadit S, Ito C, Nakaya T, **Hagiwara K**.
Med Oncol. 40:138. 2023. doi: 10.1007/s12032-023-02002-z.

II. その他＜Others＞

1）Decreased immunoreactivity of hepatitis E virus antigen following treatment with Sakhalin spruce (*Picea glehnii*) essential oil.
Maeda, N., Horochi, S., Hasegawa, Y., Iwasaki, T., Nakatani, N., Miyasho, T., **Hagiwara K.,** Yokota H., Funatsu, Y.
Chemistry & Biodiversity 20: e202200924. 2023. doi: 10.1002/cbdv.202200924.

animals

MDPI

Article

Hepatitis E Virus (HEV) Spreads from Pigs and Sheep in Mongolia

Enkhbaatar Batmagnai [1,†], Bazartseren Boldbaatar [2,†], Amarbayasgalan Sodbayasgalan [1], Yuko Kato-Mori [3] and Katsuro Hagiwara [4,*]

[1] Laboratory of Virology, Institute of Veterinary Medicine, Mongolian University of Life Sciences, Ulaanbaatar 17024, Mongolia
[2] Department of Infectious Diseases and Microbiology, School of Veterinary Medicine, Mongolian University of Life Sciences, Ulaanbaatar 17024, Mongolia
[3] Graduate School of Science, Technology and Innovation, Kobe University, 7-1-49 Minatojima Minami-Machi, Chuo-ku, Kobe 650-0047, Japan
[4] Department of Pathobiology, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
* Correspondence: k-hagi@rakuno.ac.jp
† These authors contributed equally to this work.

Simple Summary: Hepatitis E virus (HEV) is a zoonotic pathogen, with an increasing number of cases worldwide. In Asia, including Mongolia, infections are associated with the zoonotic HEV-3 and HEV-4 genotypes, and pigs, deer, and wild boars are the main reservoirs. Recent studies have revealed that sheep are hosts of the virus in several countries. The aim of our study is to diagnose HEV RNA in feces and liver samples of sheep in Mongolia and clarify the origin of the virus and characterize its chain of infection. From our results, we found HEV genotype 4 in sheep and it was closely related to pig HEV genotype 4 in the same region. On Mongolian pig farms, pigs are fed with the raw internal organs of sheep for fattening the pigs as a free resource of protein. There is a concern that the spread of HEV could affect livestock feeding.

Abstract: Hepatitis E is a viral infectious disease in pigs, wild boars, cows, deer, rabbits, camels, and humans as hosts caused by *Paslahepevirus*. Recently, it has been detected in a wide variety of animals including domestic small ruminants. Mongolia is a land of nomadic people living with livestock such as sheep, goats, and cattle. Due to how Mongolian lifestyles have changed, pork has become popular and swine diseases have emerged. Among them, Hepatitis E disease has become a zoonotic infectious disease that needs to be addressed. The HEV problem in pigs is that infected pigs excrete the virus without showing clinical symptoms and it spreads into the environment. We attempted to detect HEV RNA in sheep which had been raised in Mongolia for a long time, and those animals living together with pigs in the same region currently. We also conducted a longitudinal analysis of HEV infection in pigs in the same area and found that they were infected with HEV of the same genotype and cluster. In this study, we examined 400 feces and 120 livers (pigs and sheep) by RT-PCR in Töv Province, Mongolia. HEV detection in fecal samples was 2% (4/200) in sheep and 15% (30/200) in pigs. The results of ORF2 sequence analysis of the HEV RT-PCR-positive pigs and sheep confirmed genotype 4 in both animals. The results suggest that HEV infection is widespread in both pigs and sheep and that urgent measures to prevent infection are needed. This case study points to the changing nature of infectious diseases associated with livestock farming. It will be necessary to reconsider livestock husbandry and public health issues based on these cases.

Keywords: hepatitis E; pig; sheep; prevalence; Mongolia; phylogenetic analysis

Citation: Batmagnai, E.; Boldbaatar, B.; Sodbayasgalan, A.; Kato-Mori, Y.; Hagiwara, K. Hepatitis E Virus (HEV) Spreads from Pigs and Sheep in Mongolia. *Animals* **2023**, *13*, 891. https://doi.org/10.3390/ani13050891

Academic Editor: Fabio Ostanello

Received: 13 February 2023
Revised: 26 February 2023
Accepted: 27 February 2023
Published: 1 March 2023

出典 doi: 10.3390/ani13050891.
https://www.mdpi.com/2076-2615/13/5/891

最終責任者 Katsuro Hagiwara (Corresponding Author)

Medical Oncology (2023) 40:138
https://doi.org/10.1007/s12032-023-02002-z

ORIGINAL PAPER

Investigation of oncolytic effect of recombinant Newcastle disease virus in primary and metastatic oral melanoma

Supaporn Numpadit[1] · Chiaki Ito[1] · Takaaki Nakaya[2] · Katsuro Hagiwara[1]

Received: 7 December 2022 / Accepted: 20 March 2023 / Published online: 6 April 2023

Abstract
Malignant melanoma is aggressive cancer with a high rate of local invasiveness and metastasis. Currently, the treatment options for patients with advanced-stage and metastatic oral melanoma are limited. A promising treatment option is oncolytic viral therapy. This study aimed to evaluate novel therapies for malignant melanoma using a canine model. Oral melanoma, which frequently occurs in dogs is used as a model for human melanoma, was isolated and cultured and used for the evaluation of the tumor lytic effect induced by viral infection. We constructed a recombinant Newcastle disease virus (rNDV) that promotes the extracellular release of IFNγ from the virus-infected melanoma. The expression of oncolytic and apoptosis-related genes, the immune response by lymphocytes, and IFNγ expression were evaluated in virus-infected melanoma cells. The results showed that the rate of rNDV infection varied according to the isolated melanoma cells and the oncolytic effect differed between melanoma cells owing to the infectivity of the virus. The oncolytic effect tended to be greater for the IFNγ-expressing virus than for the GFP-expressing prototype virus. Additionally, lymphocytes co-cultured with the virus showed induced expression of Th1 cytokines. Therefore, recombinant NDV expressing IFNγ is expected to induce cellular immunity and oncolytic activity. This oncolytic treatment shows promise as a therapeutic approach for melanoma treatment once evaluated using clinical samples from humans.

Keywords Malignant melanoma (MM) · rNDV · GFP · cIFNγ · Oncolytic virotherapy

Abbreviations

ALM	Acral lentiginous melanoma
cIFNγ	Canine interferon gamma
FCM	Flow cytometry
GFP	Green Fluorescent protein
Hpi	Hours post-infection
HN	Hemagglutinin-neuraminidase
IFNγ	Interferon gamma
IL-2	Interleukin 2
ICIs	Immune checkpoint inhibitors
ISGs	Interferon-stimulated gene
IRF-1	Interferon regulatory factor 1
LMM	Lentigo maligna melanoma
LDH	Lactate dehydrogenase
MM	Malignant melanoma
MCM	Mucosal melanoma
MHC	Major histocompatibility complex
NM	Nodular melanoma
NDV	Newcastle disease virus
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
QOL	Quality of life
RFS	Recurrence-free survival
rNDV	Recombinant Newcastle disease virus
SSM	Superficial spreading melanoma

✉ Katsuro Hagiwara
k-hagi@rakuno.ac.jp

Supaporn Numpadit
s21941006@stu.rakuno.ac.jp

Chiaki Ito
s21761150@stu.rakuno.ac.jp

Takaaki Nakaya
tnakaya@koto.kpu-m.ac.jp

[1] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

[2] Department of Infectious Disease, Kyoto Prefectural University of Medicine, Kamigyo-ku Kajii-cho, Kawaramachi-Hirokoji, Kyoto-shi 602-8566, Japan

出典 doi: 10.1007/s12032-023-02002-z.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10079733/pdf/12032_2023_Article_2002.pdf

最終責任者 Katsuro Hagiwara (Corresponding Author)

doi.org/10.1002/cbdv.202200924 RESEARCH ARTICLE CHEMISTRY & BIODIVERSITY

Decreased Immunoreactivity of Hepatitis E Virus Antigen Following Treatment with Sakhalin Spruce (*Picea glehnii*) Essential Oil

Naoyuki Maeda,*[a] Shouya Horochi,[a] Yasuhiro Hasegawa,[a] Tomohito Iwasaki,[a] Nobutake Nakatani,[b] Taku Miyasho,[c] Katsuro Hagiwara,[d] Hiroshi Yokota,[e] and Yasuhiro Funatsu[a]

[a] Department of Food Science and Human Wellness, Rakuno Gakuen University, Hokkaido 069-8501, Japan, e-mail: n-maeda@rakuno.ac.jp
[b] Department of Environmental Sciences, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[c] Department of Veterinary Science, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[d] Department of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[e] The General Incorporated Association 'Pine Grace', Hokkaido 069-0834, Japan

The hepatitis E virus (HEV) causes a common infectious disease that infects pigs, wild boars, deer, and humans. In most cases, humans are infected by eating raw meat. Some essential oils have been reported to exhibit antiviral activities. In this study, in order to investigate the anti-HEV properties of essential oils, the immunoreactivities of HEV antigen proteins against the relevant antibodies were analyzed after the HEV antigens underwent treatment with various essential oils. The essential oils extracted from the tea tree, which was previously reported to exhibit antiviral activity, lavender, and lemon had strongly reduced activity. We found that treatment with the essential oil prepared from Sakhalin spruce was associated with the strongest reduction in immunoreactivity of HEV antigen protein(s) among the tested substances. The main volatile constituents of Sakhalin spruce essential oil were found to be bornyl acetate (32.30%), α-pinene (16.66%), camphene (11.14%), camphor (5.52%), β-phellandrene (9.09%), borneol (4.77%), and limonene (4.57%). The anti-HEV properties of the various components of the essential oils were examined: treatment with bornyl acetate, the main component of Sakhalin spruce oil, α-pinene, the main component of tea tree oil, and limonene, the main component of lemon oil, resulted in a strong reduction in HEV antigen immunoreactivity. These results indicate that each main component of the essential oils plays an important role in the reduction of the immunoreactivity of HEV antigen protein(s); they also suggest that Sakhalin spruce essential oil exhibits anti-HEV activity. In a formulation with the potential to eliminate the infectivity of HEV in foodborne infections, this essential oil can be applied as an inactivating agent for meat processing and cooking utensils, such as knives and chopping boards.

Keywords: hepatitis E virus, sakhalin spruce, essential oil, bornyl acetate, enzyme immunoassay.

出典 doi: 10.1002/cbdv.202200924.
https://onlinelibrary.wiley.com/doi/epdf/10.1002/cbdv.202200924

最終責任者 Yasuhiro Funatsu (Corresponding Author)

獣医衛生学(Animal Health)

Hidetoshi Higuchi

Professor

教授　樋口　豪紀

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

1) Characteristics of *Mycoplasma bovis*, *Mycoplasma arginini*, and *Mycoplasma californicum* on immunological response of bovine synovial cells
Nishi K, Okada J, Iwasaki T, Gondaira S, **Higuchi H.**
Vet Immunol Immunopathol. 260:110608. 2023.
doi:10.1016/j.vetimm.2023.110608.

II. その他　＜Others＞

Veterinary Immunology and Immunopathology
Volume 260, June 2023, 110608

Characteristics of *Mycoplasma bovis*, *Mycoplasma arginini*, and *Mycoplasma californicum* on immunological response of bovine synovial cells

Koji Nishi [a b], Julia Okada [a], Tomohito Iwasaki [c], Satoshi Gondaira [a], Hidetoshi Higuchi [a]

https://doi.org/10.1016/j.vetimm.2023.110608

出典　doi: 1 10.1016/j.vetimm.2023.110608.
https://doi.org/10.1016/j.vetimm.2023.110608

最終責任者　Satoshi Gondaira and Hidetoshi Higuchi (Corresponding Author)

伴侶動物外科学（Companion Animal Surgery）

Mitsuhiro Isaka

Professor

教授　井坂　光宏

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

1）Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture..
Isaka M, Konno W, Kokubo D, Udagawa H, Hizuka S, Sakai T, Yamamoto S, Torisu S, Ueno H.
Res Vet Sci. 158:41–43. 2023. doi: 10.1016/j.rvsc.2023.03.006.

Ⅱ．その他＜Others＞

1）Pyruvate kinase deficiency mutant gene carriage in stray cats and rescued cats from animal hoarding in Hokkaido, Japan
Ueno H, Itoh T, Nasuno T, Konno W, Kondo A, Konishi I, Inukai H, Kokubo D, **Isaka M**, Islam MS, Yamato O.
J Vet Med Sci. 85:972–976. 2023. doi: 10.1292/jvms.23-0091.

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture

Mitsuhiro Isaka *, Wataru Konno, Daiki Kokubo, Hiromu Udagawa, Sho Hizuka, Toshikazu Sakai, Shushi Yamamoto, Shidow Torisu, Hiroshi Ueno

Laboratory of Companion Animal Surgery, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Cranial cruciate ligament rupture
Dogs
Osteocrin
Osteotomy

ABSTRACT

The cranial cruciate ligament (CCL) rupture is a common orthopedic disease in dogs that is usually managed with tibial plateau leveling osteotomy (TPLO) or extracapsular lateral suture (ECLS). Osteotomy is generally associated with some complications, including nonunion. The periosteum plays an important role in bone growth and remodeling. Osteocrin (OSTN), which was recently identified and is involved in bone formation and differentiation, is produced in the periosteum and osteoblasts. The aimed to investigate whether the concentrations of serum OSTN change before and after stifle surgery in dogs and compare the OSTN concentrations in the two surgical techniques (TPLO: $n = 20$ vs. ECLS: $n = 36$). The postoperative serum OSTN concentration in the TPLO group was significantly lower than the preoperative value ($p < 0.05$), while serum OSTN concentrations differed statistically between the preoperative and suture-removal periods. In contrast, no significant differences were observed in the ECLS group. In conclusion, osteotomy affects serum OSTN concentrations during the perioperative period in dogs, which may be related to periosteal injury.

The cranial cruciate ligament (CCL) rupture is a common orthopedic condition in dogs (Knight and Danielski, 2018). The surgical procedures for CCL rupture are the tibial plateau leveling osteotomy (TPLO), which is tibial osteotomy with an orthopedic plate, and an expracapsular lateral suture (ECLS), which makes the hole of the tibia passing through the suture without osteotomy techniques (Knight and Danielski, 2018; Krotscheck et al., 2016). Generally the use of osteotomy in small- animal orthopedics may be associated with some complications, including nonunion, implant failure, infection, and revision surgery (Danielski et al., 2022).

The periosteum is a dense and abundantly vascularized connective tissue membrane with fibroblasts, including multipotent mesenchymal stem cells and osteogenic progenitor cells (Allen et al., 2004; Chang and Knothe Tate, 2012). It also covers the surface of the majority of bone, facilitating the attachment of muscles, ligaments, and tendons, and delivering blood, nutrition, and regenerative cells to participate in a healing process (Roberts et al., 2015; Seeman, 2007). A previous study reported that the absence of the periosteum induces a marked decrease in new bone formation and up to 10-fold reduced vascularization (Colnot, 2009; Zhang et al., 2005). Collectively, the periosteum may be an important factor related to the complications of osteotomy.

Osteocrin (OSTN) is a recently discovered natriuretic peptide secreted from periosteum, similar to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (Nishizawa et al., 2004). It is produced in the periosteum and osteoblasts (Thomas et al., 2003) and is thought to be involved in bone formation and differentiation (Thomas et al., 2003; Watanabe-Takano et al., 2021). However, no previous reports have described the effects of osteotomy on serum OSTN concentrations during the perioperative period in veterinary clinical settings. Thus, the aim of this study was to investigate whether the serum OSTN concentrations change before and after operative stifle surgery in dogs and compare the findings between the two surgical techniques.

Fifty-six dogs, including 20 and 36 dogs in the TPLO and ECLS groups, respectively, were included in this study. The body weight and age of the dogs in the TPLO group were 25.77 ± 12.27 kg and 96.65 ± 27.28 months, respectively; the group included two male and nine castrated dogs as well as four female and six spayed dogs. The breeds were as follows: Great Dane, $n = 1$; Labrador Retriever, n = 1; Golden Retriever, $n = 3$; Siberian Husky, n = 3; Shiba Inu, $n = 2$; Welsh Corgi, n = 2; Mongrel, n = 3; Bernese Mountain Dog, $n = 2$; Border Collie, n = 1; and White Shepherd, $n = 2$. Alternatively, the body weight and age of the dogs in the ECLS group were 9.9 ± 7.04 kg and 95.78 ± 36.11

* Corresponding author.
E-mail address: m-isaka@rakuno.ac.jp (M. Isaka).

https://doi.org/10.1016/j.rvsc.2023.03.006
Received 6 October 2022; Received in revised form 15 February 2023; Accepted 5 March 2023
Available online 8 March 2023

出典 doi: 10.1016/j.rvsc.2023.03.006.
https://www.sciencedirect.com/science/article/pii/S0034528823000735?via%3Dihub
最終責任者 Mitsuhiro Isaka (Corresponding Author)

The Journal of Veterinary Medical Science

NOTE

Internal Medicine

Pyruvate kinase deficiency mutant gene carriage in stray cats and rescued cats from animal hoarding in Hokkaido, Japan

Hiroshi UENO[1,2)*], Tomohito ITOH[3)], Toyohiko NASUNO[4)], Wataru KONNO[1)], Atsushi KONDO[5)], Ikuo KONISHI[6)], Hisao INUKAI[7)], Daiki KOKUBO[1)], Mitsuhiro ISAKA[1)], Md Shafiqul ISLAM[8)], Osamu YAMATO[8)]

[1)]Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]National BioResource Project Japanese Macaques, Center for the Evolutionary Origins of Human Behavior, Kyoto University, Aichi, Japan
[3)]Maebashi Institute of Animal Science, Livestock Improvement Association of Japan, Inc., Gumma, Japan
[4)]Nekoiro Clinic, Hokkaido, Japan
[5)]Takahashi Animal Hospital, Hokkaido, Japan
[6)]Palk Animal Hospital, Hokkaido, Japan
[7)]Neko-no-byoin, Hokkaido, Japan
[8)]Laboratory of Clinical Pathology, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan

J. Vet. Med. Sci.
85(9): 972–976, 2023
doi: 10.1292/jvms.23-0091

Received: 22 February 2023
Accepted: 15 July 2023
Advanced Epub:
27 July 2023

ABSTRACT. The mutant allele frequency of the Pyruvate kinase (PK) gene has been investigated mostly in pure breed cats. We investigated the PK mutant gene in stray and animal hoarding mongrel cats in Hokkaido, Japan. We also investigated the kinship of individuals carrying the mutant gene. Genotyping was conducted using the previously reported real-time PCR method. Fourteen microsatellite markers were used to identify the parents and offspring of cats carrying the PK mutant gene, and some kinship such as parent-offspring and siblings was observed. Some stray and animal hoarding cats carried the PK mutation gene and that consanguinity was confirmed among these cats indicated that the PK mutation gene was spread by unregulated interbreeding.

KEYWORDS: animal hoarding cat, microsatellite marker, mutant allele frequency, pyruvate kinase deficiency, stray cat

*Correspondence to: Ueno H: ueno.hiroshi.3u@kyoto-u.ac.jp, National BioResource Project Japanese Macaques, Center for the Evolutionary Origins of Human Behavior, Kyoto University, Kanrin 41-2, Inuyama, Aichi 484-8506, Japan

出典　doi: 10.1292/jvms.23-0091.
https://www.jstage.jst.go.jp/article/jvms/85/9/85_23-0091/_article

最終責任者　Hiroshi Ueno (Corresponding Author)

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) Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy.
Fujiki J, Nakamura K, Nakamura T, **Iwano H.**
Int J Mol Sci. 24:15628. 2023. doi: 10.3390/ijms242115628.

II. その他　＜Others＞

1) Isolation of Streptococcus mutans temperate bacteriophage with broad killing activity to S. mutans clinical isolates.
Sugai K, Kawada-Matsuo M, Le N M, Sugawara Y, Hisatsune J, Fujiki J, **Iwano H**, Tanimoto K, Sugai M, Komatsuzawa H.
iScience. 26: 108465. 2023. doi: 10.1016/j.isci.2023.108465.

2) Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism.
Yamazaki H, Onoyama S, Gotani S, Deguchi T, Tamura M, Ohta H, **Iwano H**, Nishida H, Dickinson P, Akiyoshi H.
Cancers (Basel). 15: 5537. 2023. doi: 10.3390/cancers15235537.

3) Antimicrobial susceptibility of bovine clinical mastitis pathogens in Japan and development of a simplified agar disk diffusion method for clinical practice.
Kawai K., Kueumisawa T., Shinozuka Y., Higuchi, H., **Iwano H.**, Hayashi T., Ozawa M., Koike R., Uchiyama M.
J Vet Med Sci. 85: 143–148. 2023. doi: 10.1292/jvms.21-0450

International Journal of
Molecular Sciences

MDPI

Review

Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy

Jumpei Fujiki [1,2], Keisuke Nakamura [1], Tomohiro Nakamura [1,3,4,5] and Hidetomo Iwano [1,3,*]

[1] Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; j-fujiki@rakuno.ac.jp (J.F.); s21661158@g.rakuno.ac.jp (K.N.); t-nakamura@azabu-u.ac.jp (T.N.)
[2] Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA
[3] Phage Therapy Institute, Waseda University, Tokyo 169-8050, Japan
[4] Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases, Tokyo 208-0011, Japan
[5] Department of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan
* Correspondence: h-iwano@rakuno.ac.jp; Tel./Fax: +81-11-388-4885

Abstract: In recent decades, phage therapy has been overshadowed by the widespread use of antibiotics in Western countries. However, it has been revitalized as a powerful approach due to the increasing prevalence of antimicrobial-resistant bacteria. Although bacterial resistance to phages has been reported in clinical cases, recent studies on the fitness trade-offs between phage and antibiotic resistance have revealed new avenues in the field of phage therapy. This strategy aims to restore the antibiotic susceptibility of antimicrobial-resistant bacteria, even if phage-resistant variants develop. Here, we summarize the basic virological properties of phages and their applications within the context of antimicrobial resistance. In addition, we review the occurrence of phage resistance in clinical cases, and examine fitness trade-offs between phage and antibiotic sensitivity, exploring the potential of an evolutionary fitness cost as a countermeasure against phage resistance in therapy. Finally, we discuss future strategies and directions for phage-based therapy from the aspect of fitness trade-offs. This approach is expected to provide robust options when combined with antibiotics in this era of phage 're'-discovery.

Keywords: bacteriophage; antimicrobial resistance (AMR); fitness cost; phage cocktail; engineered phage; infectious disease; infection control; *P. aeruginosa*; *K. pneumoniae*; *A. baumannii*; *S. aureus*; evolution; arms race

Citation: Fujiki, J.; Nakamura, K.; Nakamura, T.; Iwano, H. Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy. *Int. J. Mol. Sci.* **2023**, *24*, 15628. https://doi.org/10.3390/ijms242115628

Academic Editor: Kosuke Fujimoto

Received: 6 October 2023
Revised: 23 October 2023
Accepted: 23 October 2023
Published: 26 October 2023

1. Introduction

Bacteriophages, also known simply as phages, are prokaryotic viruses that exclusively infect and kill bacteria. While phages were already being explored as antimicrobial agents in the early 1900s [1,2], the rise of antimicrobial chemical agents that became increasingly popular after the discovery of penicillin, overshadowed their use in Western countries [3]. However, after decades of being overlooked, the emergence of bacteria that are resistant to these antibacterial agents has refocused efforts on the use of phages for treating infectious diseases [4–6]. On the other hand, bacterial resistance to phages can also arise through a variety of molecular mechanisms. Several clinical studies on phage therapy have reported the occurrence of phage-resistant variants, which represents a significant concern for the successful development of phage-based therapies [7]. It is therefore important to carefully address phage resistance within the context of developing anti-bacterial treatments and therapies.

Phages have played a significant role in shaping the evolution of bacterial communities and populations through a co-evolutionary mechanism known as an arms race [8]. Recent

出典　doi: 10.1292/jvms.21-0586.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10650657/pdf/ijms-24-15628.pdf

最終責任者　Hidetomo Iwano (Corresponding Author)

iScience

CellPress
OPEN ACCESS

Article

Isolation of *Streptococcus mutans* temperate bacteriophage with broad killing activity to *S. mutans* clinical isolates

Katsuhito Sugai,[1] Miki Kawada-Matsuo,[2,3,*] Mi Nguyen-Tra Le,[2,3] Yo Sugawara,[4] Junzo Hisatsune,[4] Jumpei Fujiki,[5] Hidetomo Iwano,[5] Kotaro Tanimoto,[1] Motoyuki Sugai,[4] and Hitoshi Komatsuzawa[2,3,6,*]

SUMMARY

Bacteriophages are expected to be therapeutic agents against infectious diseases. *Streptococcus mutans* are involved in dental plaque formation related to dental caries and periodontitis. In *S. mutans*, lytic bacteriophages have been isolated previously, but the isolation of temperate bacteriophage has not been reported although their presence in the genome has been confirmed. Here, we report the isolation of temperate bacteriophage, φKSM96, from *S. mutans*. φKSM96 has a circular DNA 39,820 bp long and reveals Siphoviridae morphology. φKSM96 shows a broad range of susceptibility against *S. mutans* strains with different serotypes. By the addition of φKSM96, *S. mutans* growth and biofilm formation were significantly inhibited. In cocultures of *S. mutans* with other bacterial species, the proportion of *S. mutans* significantly decreased in the presence of φKSM96. In summary, φKSM96 shows selective anti-*S. mutans* activity. The isolation of temperate bacteriophage is important for future genetic manipulation to create more efficient bacteriophages.

[1]Department of Orthodontics, Hiroshima University Graduate School of Biomedical and Health Sciences, 1-2-3, Kasumi,Minamiku, Hiroshima-shi, Hiroshima 734-8553, Japan
[2]Department of Bacteriology, Hiroshima University Graduate School of Biomedical and Health Sciences, 1-2-3,Kasumi,Minamiku, Hiroshima-shi, Hiroshima 734-8553, Japan
[3]Project Research Center for Oral Infectious Diseases, Hiroshima University, 1-2-3, Kasumi,Minamiku, Hiroshima-shi, Hiroshima 734-8553, Japan
[4]Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjukuku, Tokyoto, Japan
[5]Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, Rakuno Gakuen University, 568,Bunkyodaimidorimachi, Ebetsu-shi, Hokkaido 069-0836, Japan
[6]Lead contact
*Correspondence: mmatsuo@hiroshima-u.ac.jp (M.K.-M.), komatsuz@hiroshima-u.ac.jp (H.K.)
https://doi.org/10.1016/j.isci.2023.108465

出典　doi: 10.1016/j.isci.2023.108465
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10713843/pdf/main.pdf
最終責任者　Hitoshi Komatsuzawa (Corresponding Author)

cancers

MDPI

Article

Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism

Hiroki Yamazaki [1,*], Seio Onoyama [1], Shunichi Gotani [1], Tatsuya Deguchi [1], Masahiro Tamura [1], Hiroshi Ohta [1], Hidetomo Iwano [2], Hidetaka Nishida [3], Peter J. Dickinson [4] and Hideo Akiyoshi [5]

[1] Laboratory of Veterinary Internal Medicine, Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu 069-0836, Japan; t-deguchi@rakuno.ac.jp (T.D.); m-tamura@rakuno.ac.jp (M.T.); h-ohta@rakuno.ac.jp (H.O.)
[2] Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu 069-0836, Japan; h-iwano@rakuno.ac.jp
[3] Laboratory of Small Animal Clinics, Veterinary Teaching Hospital, Graduate School of Veterinary Science, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara 52-5201, Japan; h-nishida@azabu-u.ac.jp
[4] Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA; pjdickinson@ucdavis.edu
[5] Laboratory of Veterinary Surgery, Graduate School of Life and Environmental Sciences, Osaka Metropolitan University, 1-58 Rinku-Oraikita, Izumisano 598-8531, Japan
* Correspondence: author. hyamazaki@rakuno.ac.jp; Tel.: +81-011-388-4767

Simple Summary: This study investigated the anti-glycolytic effects of evofosfamide (EVO) on three canine glioma (GL)-derived cell lines with activated hypoxia-inducible factor 1α (HIF-1α). Our clinical data showed that glycolytic activity was correlated with poorer outcomes in dogs with spontaneous GL. Our in vitro studies showed that EVO inhibited glycolytic metabolism by targeting HIF-1α-positive cells under hypoxic culture conditions, resulting in the suppression of cellular ATP production. Our in vivo studies showed that EVO significantly decreased tumor development compared to controls or temozolo-mid in orthotopic murine GL models. A metabolic analysis demonstrated that EVO suppressed glycolytic activity by eliminating HIF-1α-positive cells. Our findings suggest that EVO may improve cancer metabolism and restore the microenvironment for both canine and human GL.

Abstract: The transcription factor hypoxia-inducible factor 1α (HIF-1α) drives metabolic reprogramming in gliomas (GLs) under hypoxic conditions, promoting glycolysis for tumor development. Evofosfamide (EVO) releases a DNA-alkylating agent within hypoxic regions, indicating that it may serve as a hypoxia-targeted therapy. The aim of this study was to investigate the glycolytic metabolism and antitumor effects of EVO in a canine GL model. Our clinical data showed that overall survival was significantly decreased in GL dog patients with higher HIF-1α expression compared to that of those with lower HIF-1α expression, and there was a positive correlation between HIF-1α and pyruvate dehydrogenase kinase 1 (PDK1) expression, suggesting that glycolytic activity under hypoxia conditions may contribute to poor outcomes in canine GL. Our glycolysis assay tests showed that the glycolytic ATP level was higher than the mitochondrial ATP level in three types of canine GL cell lines by activating the HIF-1 signal pathway under hypoxia conditions, resulting in an overall increase in total cellular ATP production. However, treatment with EVO inhibited the glycolytic ATP level in the GL cell lines under hypoxia conditions by targeting HIF-1α-positive cells, leading to decrease in total cellular ATP production. Our in vivo tests showed that EVO significantly reduced tumor development compared to controls and temozolomide in murine GL models. A metabolic analysis demonstrated that EVO effectively suppressed glycolytic metabolism by eliminating HIF-1α-positive cells, suggesting that it may restore metabolism in canine GLs. The evidence presented here supports the favorable preclinical evaluation of EVO as a potential improvement in cancer metabolism.

check for updates

Citation: Yamazaki, H.; Onoyama, S.; Gotani, S.; Deguchi, T.; Tamura, M.; Ohta, H.; Iwano, H.; Nishida, H.; Dickinson, P.J.; Akiyoshi, H. Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism. *Cancers* **2023**, *15*, 5537. https://doi.org/10.3390/cancers15235537

Academic Editors: Panagiotis Liakos and Ilias Mylonis

Received: 18 September 2023
Revised: 8 November 2023
Accepted: 16 November 2023
Published: 22 November 2023

出典　doi: 10.3390/cancers15235537.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10705110/pdf/cancers-15-05537.pdf
最終責任者　Hiroki Yamazaki (Corresponding Author)

The Journal of Veterinary Medical Science

FULL PAPER
Bacteriology

Antimicrobial susceptibility of bovine clinical mastitis pathogens in Japan and development of a simplified agar disk diffusion method for clinical practice

Kazuhiro KAWAI[1,2)#], Tomomi KURUMISAWA[1,2)#], Yasunori SHINOZUKA[1,2)*], Hidetoshi HIGUCHI[3)], Hidetomo IWANO[3)], Tomohito HAYASHI[4)], Manao OZAWA[5)], Ryoji KOIKE[5)], Mariko UCHIYAMA[5)]

[1)]School of Veterinary Medicine, Azabu University, Kanagawa, Japan
[2)]Azabu University Mastitis Research Center, Kanagawa, Japan
[3)]School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[4)]National Institute of Animal Health, National Agriculture and Food Research Organization, Hokkaido, Japan
[5)]National Veterinary Assay Laboratory, Ministry of Agriculture Forestry and Fisheries, Tokyo, Japan

ABSTRACT. This study aimed to examine the antimicrobial susceptibility of bovine mastitis pathogens in Japan and develop criteria for testing antimicrobial susceptibility using the simplified agar disk diffusion (ADD) method that is currently being used in clinical practice. Milk samples from 1,349 dairy cows with clinical mastitis were collected and cultured. The minimum inhibitory concentrations (MICs) of the antimicrobials were determined for 504 strains of 28 bacteria. Of the gram-positive bacteria, most *Staphylococcus* spp. were susceptible to penicillin G (PCG), kanamycin (KM), oxytetracycline (OTC), cefazolin (CEZ), pirlimycin, enrofloxacin, and marbofloxacin. *Streptococcus* spp. and Trueperella pyogenes showed resistance to OTC and KM. Most gram-negative bacteria were resistant to OTC and CEZ and particularly susceptible to fluoroquinolones. To develop the criteria for a disk diffusion test of the simplified ADD method, the relationships between MICs and diameters of inhibition zones (DIZs) were analyzed and compared with the conventional method. The susceptibility breakpoints of several antimicrobials were lower for both gram-positive and gram-negative bacteria. Particularly for gram-positive bacteria, the application of the new criteria lowers the breakpoint for PCG, suggesting that the use of PCG instead of CEZ may increase. The results suggest that use of these criteria for the simplified ADD method may lead to appropriate antimicrobial choice and consequently the appropriate use of antimicrobials in clinical practice.

KEYWORDS: antimicrobial resistance, antimicrobial susceptibility testing, bovine mastitis, minimum inhibitory concentration, simplified agar disk diffusion method

J. Vet. Med. Sci.
85(2): 143–148, 2023
doi: 10.1292/jvms.21-0450

Received: 12 August 2021
Accepted: 25 November 2022
Advanced Epub:
20 December 2022

*Correspondence to: Shinozuka T: shinozuka@azabu-u.ac.jp, Laboratory of Veterinary Hygiene, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan
#These authors contributed equally to this work.
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

出典　doi: 10.1292/jvms.21-0450

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10017299/pdf/jvms-85-143.pdf

最終責任者　Kazuhiro Kawai (Corresponding Author)

疾患モデル学（Disease Models）

Hiroshi Kitamura

Professor

教授　北村　浩

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

1）Ubiquitin-specific proteases (USPs) and metabolic disorders.
Kitamura H.
Int J Mo. Sci. 24: 3219. 2023. doi: 10.3390/ijms24043219.

2）Flow cytometric detection of $CD11b^{+}$ $Gr\text{-}1^{+}$ cells in nontumor-bearing mice: a propolis-elicited model.
Kitamura H.
Methods in Cell Biol. in press. doi: 10.1016/bs.mcb.2023.05.010

Ⅱ．その他＜Others＞

International Journal of
Molecular Sciences

MDPI

Review

Ubiquitin-Specific Proteases (USPs) and Metabolic Disorders

Hiroshi Kitamura

Laboratory of Comparative Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; ktmr@rakuno.ac.jp; Tel.: +81-11-388-4781

Abstract: Ubiquitination and deubiquitination are reversible processes that modify the characteristics of target proteins, including stability, intracellular localization, and enzymatic activity. Ubiquitin-specific proteases (USPs) constitute the largest deubiquitinating enzyme family. To date, accumulating evidence indicates that several USPs positively and negatively affect metabolic diseases. USP22 in pancreatic β-cells, USP2 in adipose tissue macrophages, USP9X, 20, and 33 in myocytes, USP4, 7, 10, and 18 in hepatocytes, and USP2 in hypothalamus improve hyperglycemia, whereas USP19 in adipocytes, USP21 in myocytes, and USP2, 14, and 20 in hepatocytes promote hyperglycemia. In contrast, USP1, 5, 9X, 14, 15, 22, 36, and 48 modulate the progression of diabetic nephropathy, neuropathy, and/or retinopathy. USP4, 10, and 18 in hepatocytes ameliorates non-alcoholic fatty liver disease (NAFLD), while hepatic USP2, 11, 14, 19, and 20 exacerbate it. The roles of USP7 and 22 in hepatic disorders are controversial. USP9X, 14, 17, and 20 in vascular cells are postulated to be determinants of atherosclerosis. Moreover, mutations in the *Usp8* and *Usp48* loci in pituitary tumors cause Cushing syndrome. This review summarizes the current knowledge about the modulatory roles of USPs in energy metabolic disorders.

Keywords: ubiquitin-specific protease; metabolic disorder; obesity; diabetes; insulin resistance; non-alcoholic fatty liver disease; atherosclerosis; cardiovascular disease

Citation: Kitamura, H. Ubiquitin-Specific Proteases (USPs) and Metabolic Disorders. *Int. J. Mol. Sci.* **2023**, *24*, 3219. https://doi.org/10.3390/ijms24043219

Academic Editor: Jose Luis Rosa

Received: 26 December 2022
Revised: 2 February 2023
Accepted: 3 February 2023
Published: 6 February 2023

1. Introduction

Age, obesity, and inactivity increase blood pressure, blood glucose, blood triglycerides and cholesterol, and body fat levels. Although the definition of metabolic syndrome differs between groups, the excessive accumulation of lipids in visceral adipose tissue combined with hypertension, hyperglycemia, or hyperlipidemia can be defined as metabolic syndrome [1–3]. Patients with metabolic syndrome exhibit a high prevalence of cardiovascular disease and stroke [4,5], both of which are major causes of death [5–7]. Moreover, metabolic syndrome increases insulin resistance, leading to type 2 diabetes mellitus (T2DM). T2DM worsens metabolic syndrome due to further increases in blood glucose and lipids [8,9]. While the number of people with metabolic disorders, including metabolic syndrome, is increasing due to the COVID-19 pandemic, such an increase was already previously observed in the Western world and has become a worldwide issue due to the spread of Western diets [10]. Metabolic syndrome currently affects over a billion people globally [10]. Metabolic disorders primarily occur due to dysfunction of energy metabolism–competent tissues, such as skeletal muscle, liver, adipose tissues, pancreatic islets, and the hypothalamus [11–13], and subsequent damage to the vascular system and nervous system is observed [11,14]. Local chronic inflammation and/or excessive oxidative stress are proposed to be responsible for the incidence of metabolic disorders [11,15–18]. To date, numerous molecules, including growth factors, cytokines, antioxidants, chaperones, protein kinases, transcriptional regulatory proteins, and histone modifiers, have been identified as key molecules that determine the progression of metabolic disorders [19–25]. Specifically, accumulating evidence indicates that enzymes catalyzing post-translational regulation, such as phosphorylation [26], oxidation [27], O-GlcNacylation (addition of O-linked N-acetylglucosamine

出典 doi: 10.3390/ijms24043219.
https://www.mdpi.com/1422-0067/24/4/3219
最終責任者 Hiroshi Kitamura (Corresponding Author)

Methods in Cell Biology
Available online 11 October 2023
In Press, Corrected Proof What's this?

Flow cytometric detection of CD11b$^+$ Gr-1$^+$ cells in nontumor-bearing mice: A propolis-elicited model

Hiroshi Kitamura

Show more

https://doi.org/10.1016/bs.mcb.2023.05.010 Get rights and content

Abstract

Myeloid-derived suppressor cells (MDSCs) are a heterogenous myeloid lineage population whose conventional surface phenotype is CD11b$^+$ Gr-1$^+$. Due to their rarity and fragility, analyses using primary isolated MDSCs are extremely difficult. However, counting CD11b$^+$ Gr-1$^+$ cells in associated tissues such as tumors and inflammatory lesions provides critical information regarding MDSC involvement in immune disorders in the tissues. Specific MDSC markers have not been identified, limiting our ability to apply histochemical approaches during MDSCs research. However, profiling surface antigens using multi-colorimetric flow cytometry enables us to easily monitor the abundance of MDSCs in vivo. Monitoring of mouse MDSCs and their subpopulations using flow cytometry is well established. In this article, I exemplify a conventional method of monitoring CD11b$^+$ Gr-1$^+$ cells in mouse adipose tissue after administration of Brazilian propolis ethanol extract, which is a strong inducer of MDSCs.

出典 doi: 10.1016/bs.mcb.2023.05.010.
https://www.sciencedirect.com/science/article/abs/pii/S0091679X23001103?via%3Dihub
最終責任者 Hiroshi Kitamura (Corresponding Author)

獣医疫学（Veterinary Epidemiology）

Kohei Makita

Professor

教授　蒔田　浩平

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

1） Structure and milk hygiene of dairy cooperative value chains in an intensive production area of Uganda－ A bottleneck of intervention.Sugino S, Bugeza J, Bahame D, Byaruhanga J, Shimazaki H, Anzai M, Kayano T, Mwebembezi W, Akashaba A, Shimada T, Muramatsu Y, **Makita K.**
Front Sustain Food Syst. 7:1110915. 2023. doi: 10.3389/fsufs.2023.1110915

2） Exploring appropriate strategies for vaccination against classical swine fever under a dynamic change in antibody titer in sows after starting vaccination in a Japanese farm setting.
Ukita M, Kuwata K, Tanaka E, Matsuyama R, Isoda N, Sakoda Y, Yamamoto T, **Makita K.**
Transbound. Emerg. Dis. 5541976. 2023. doi:10.1155/2023/5541976

Ⅱ．その他＜Others＞

1）Role of Plasmids in co-selection of antimicrobial resistances among *Escherichia coli* isolated from pigs.
Ozawa M, Shirakawa T, Moriya K, Furuya Y, Kawanishi M, **Makita K**, Sekiguchi H.
Foodborne Pathog Dis. 20: 435-441. 2023. doi: 10.1089/fpd.2023.0021

frontiers | Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 24 May 2023
DOI 10.3389/fsufs.2023.1110915

OPEN ACCESS

EDITED BY
Hung Nguyen-Viet,
International Livestock Research Institute (ILRI), Kenya

REVIEWED BY
Kebede Amenu,
Addis Ababa University, Ethiopia
Michiu Delia,
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

*CORRESPONDENCE
Kohei Makita
✉ kmakita@rakuno.ac.jp

RECEIVED 29 November 2022
ACCEPTED 05 May 2023
PUBLISHED 24 May 2023

CITATION
Sugino Y, Bugeza J, Bahame D, Byaruhanga J, Shimazaki H, Anzai M, Kayano T, Mwebembezi W, Akashaba A, Shimada T, Muramatsu Y and Makita K (2023) Structure and milk hygiene of dairy cooperative value chains in an intensive production area of Uganda—A bottleneck of intervention.
Front. Sustain. Food Syst. 7:1110915.
doi: 10.3389/fsufs.2023.1110915

Structure and milk hygiene of dairy cooperative value chains in an intensive production area of Uganda—A bottleneck of intervention

Yoshiharu Sugino[1], James Bugeza[2], David Bahame[3], Joseph Byaruhanga[1,4], Haruka Shimazaki[1], Masahiko Anzai[1], Taishi Kayano[1], William Mwebembezi[3], Andrew Akashaba[3], Taku Shimada[3], Yasukazu Muramatsu[5] and Kohei Makita[1]*

[1]Veterinary Epidemiology Unit, WOAH Collaborating Centre for Food Safety, Rakuno Gakuen University, Ebetsu, Japan, [2]Animal Health Research Program, National Livestock Resources Research Institute, Kampala, Uganda, [3]Mbarara District Veterinary Office, Mbarara, Uganda, [4]Research Center for Tropical Diseases and Vector Control (RCT), Department of Veterinary Pharmacy, Clinics and Comparative Medicine, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda, [5]Zoonosis Unit, WOAH Collaborating Centre for Food Safety, Rakuno Gakuen University, Ebetsu, Japan

In Uganda, informal raw milk sales dominate for domestic dairy consumption. This study was implemented to identify the structure of the dairy value chain starting from farms that participated in the Japan International Cooperation Agency Safe Milk Promotion in Mbarara project conducted between 2016 and 2019, to assess the hygiene conditions along the chain, and thereby identify the bottleneck of dairy hygiene intervention. A longitudinal study was conducted in 30 dairy farms in Mbarara District to compare the practice, prevalence of sub-clinical mastitis, and level of milk hygiene in 2016–2017 and 2019, before and after the milking hygiene intervention in 2018. California Mastitis Test was used for diagnosis with sub-clinical mastitis. Bulk milk samples were collected and a checklist was used to examine hygiene practices by observation. A cross-sectional study was conducted in 15 milk collecting centers using a structured questionnaire to quantify the dairy value chain, and to sample milk from cooler tanks in 2020. Microbiological examinations of bulk milk from farms and collection centers were conducted using six-point blood agar scoring and 3M Petri film, respectively. Participatory online appraisals with farmers and dairy cooperatives union were conducted to better understand the overall dairy value chains. The cooperatives sold milk to both formal and informal chains, but the sale of raw milk to Kampala was conducted by independent private traders. Within-herd prevalence of sub-clinical mastitis significantly decreased from 72.3% before the intervention to 25.8% after ($p < 0.001$). However, the farm bulk milk score did not change (3.3 vs. 3.2, $p = 0.418$). A significant increase in the total bacterial count was observed in the milk from collection centers (mean: 6.50 $\log_{10}$ CFU/ml) when compared to farm bulk milk (mean: 3.79 $\log_{10}$ CFU/ml; $p < 0.001$). Only 13.3% of the samples from the centers met the microbiological criteria for processing for human consumption. Our findings suggest that intervention targeted only at mastitis does not lead to better public health due to the low level of hygiene in transportation and milk handling in milk collection centers. Systematic interventions are needed to improve post-harvest dairy hygiene in Uganda.

出典 doi: 10.3389/fsufs.2023.1110915
https://doi.org/10.3389/fsufs.2023.1110915
最終責任者 Kohei Makita (Corresponding Author)

Hindawi
Transboundary and Emerging Diseases
Volume 2023, Article ID 5541976, 15 pages
https://doi.org/10.1155/2023/5541976

WILEY | Hindawi

Research Article

Exploring Appropriate Strategies for Vaccination against Classical Swine Fever under a Dynamic Change in Antibody Titer in Sows after Starting Vaccination in a Japanese Farm Setting

Makoto Ukita,[1] **Keisuke Kuwata**,[2] **Eiji Tanaka**,[3] **Ryota Matsuyama**,[1] **Norikazu Isoda**,[4] **Yoshihiro Sakoda**,[4] **Takehisa Yamamoto**,[5] **and Kohei Makita**[1]

[1]*Veterinary Epidemiology Unit, Graduate School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069–8501, Japan*
[2]*Gifu Prefecture Central Livestock Hygiene Service Center, 1–1 Yanagito, Gifu 501–1112, Japan*
[3]*Livestock Epidemic Prevention and Control Division, Department of Agricultural Policy, Gifu Prefectural Government, 2–1-1 Minami-Yabuta, Gifu 500–8570, Japan*
[4]*Laboratory of Microbiology, Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Kita-Ku, Sapporo, Hokkaido 060–0818, Japan*
[5]*Viral Disease and Epidemiology Research Division, National Institute of Animal Health, National Agriculture Research Organization, 3–1-5 Kannondai, Tsukuba, Ibaraki 305–0856, Japan*

Correspondence should be addressed to Kohei Makita; kmakita@rakuno.ac.jp

Received 26 June 2023; Revised 16 October 2023; Accepted 3 November 2023; Published 30 November 2023

Academic Editor: Lin-Zhu Ren

After 26 years of absence in Japan, a classical swine fever (CSF) outbreak occurred at a domestic pig farm in 2018. Vaccination against the CSF virus with a live attenuated vaccine at pig farms was restarted in October 2019, which was 13 years after the 2006 ban on vaccination. An individual-based simulation model for CSF antibody dynamics was developed to determine an effective CSF vaccination strategy for pig populations. In creating a simulated pig herd, the optimal vaccination age of piglets and the effect of vaccinating piglets twice were evaluated. Additionally, the herd immunity was monitored every 6 months for 4 years after the start of vaccination, and the effects of intensive sow replacement policies were assessed. The simulation results indicated that the vaccination age should be delayed relative to the age used before the 2006 ban on vaccination and shifted earlier, from 8 weeks to 6 weeks, as time elapses. The simulations indicated a tradeoff in protection between the weaning period (i.e., maternally derived antibodies) and the fattening period (i.e., by vaccine-induced antibodies). Mixing sows with high and low antibody titers, particularly sows that received the first vaccination and those born after the start of vaccination, resulted in a high variation in antibody titer among pigs on the farm. This study also clarified the positive effect of intensive sow replacement strategies on shortening the period in which sows show diverse titers. Differences in sow replacement rates among farms and/or the time lag in starting vaccination in different prefectures result in heterogeneity in herd immunity in Japan; thus, herd immunity status should be examined at every farm using this simulation model.

出典　doi: 10.1155/2023/5541976
https://doi.org/10.1155/2023/5541976
最終責任者　Kohei Makita (Corresponding Author)

Role of Plasmids in Co-Selection of Antimicrobial Resistances Among *Escherichia coli* Isolated from Pigs

Manao Ozawa, Takahiro Shirakawa, Konomi Moriya, Yukari Furuya, Michiko Kawanishi, Kohei Makita, and Hideto Sekiguchi

Published Online: 3 Oct 2023 | https://doi.org/10.1089/fpd.2023.0021

Abstract

Co-selection is thought to occur when resistance genes are located on the same mobile genetic element. However, this mechanism is currently poorly understood. In this study, complete circular plasmids from swine-derived *Escherichia coli* were sequenced with short and long reads to confirm that resistance genes involved in co-resistance were co-transferred by the same plasmid. Conjugative transfer tests were performed, and multiple resistance genes were transmitted. The genes possessed by the donor, transconjugant, and plasmid of the donor were highly similar. In addition, the sequences of the plasmid of the donor and the plasmid of the transconjugant were almost identical. Resistance genes associated with statistically significant combinations of antimicrobial use and resistance were co-transmitted by the same plasmid. These results suggest that resistance genes may be involved in co-selection by their transfer between bacteria on the same plasmid.

出典　doi: 10.1089/fpd.2023.0021

https://www.liebertpub.com/doi/10.1089/fpd.2023.0021?url_ver=Z39.882003&rfr_id=ori%3Arid%3 Acrossref.org&rfr_dat=cr_pub++0pubmed

最終責任者　Manao Ozawa (Corresponding Author)

人獣共通感染症学（Zoonotic Diseases）

Yasukazu Muramatsu

Professor

教授　村松　康和

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

Ⅱ．その他　＜Others＞

1）Identification of Three Novel Genes in Phenuiviridae Detected from Aedes Mosquitoes in Hokkaido, Japan.
Uchida L, Sakurai Y, Shimooka M, Morales-Vargas RE, Hagiwara K, **Muramatsu Y.**
*Jpn J Infect Dis.*76:55-63. 2023. doi: 10.7883/yoken.JJID.2022.179.

Jpn. J. Infect. Dis., 76, 55-63, 2023

Original Article

Identification of Three Novel Genes in *Phenuiviridae* Detected from *Aedes* Mosquitoes in Hokkaido, Japan

Leo Uchida[1]*, Yoshimi Sakurai[1], Makoto Shimooka[1], Ronald Enrique Morales-Vargas[2], Katsuro Hagiwara[1], and Yasukazu Muramatsu[1]

[1]*School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan; and*
[2]*Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

ABSTRACT: Mosquitoes are important arthropod vectors of arboviruses. The family *Phenuiviridae* includes several medically important arboviruses, such as the Rift Valley fever phlebovirus and Toscana phlebovirus. Recent comprehensive genetic analyses have identified many novel mosquito-specific viruses that are phylogenetically related to *Phenuiviridae*. We collected mosquitoes from Hokkaido in northern Japan, and conducted reverse transcription polymerase chain reactions (RT-PCRs) targeting the RNA-dependent RNA polymerase (*RdRp*) gene of *Phenuiviridae*. A total of 285 pools, comprising 3,082 mosquitoes from 2 genera and 8 species, were collected. Partial *RdRp* sequences were detected in 97 pools, which allowed us to classify the viruses into 3 clusters provisionally designated as Etutanne virus (ETTV) 1, 2, and 3. The virus most closely related to ETTVs is Narangue virus (family *Phenuiviridae*, genus *Mobuvirus*), which was detected in *Mansonia* mosquitoes; the nucleotide and amino acid sequences of the Narangue virus are 58.4–66.2% and 64.7–86.7% similar, respectively, to those of ETTVs. PCR and RT-PCR using DNA and RNase digestion methods showed that the ETTVs are RNA viruses that do not form non-retroviral integrated RNA virus sequences in the mosquito genome.

INTRODUCTION

Arthropod-borne viruses (also known as arboviruses) are a group of viruses that "replicate in both arthropod vectors and vertebrate hosts and can be transmitted between vertebrate hosts by the arthropod vector" (1). Mosquito-borne viruses belong to a large variety of viral families, such as *Flaviviridae, Phenuiviridae* (order Bunyavirales), *Togaviridae*, and *Reoviridae*, which include several medically and veterinary important viruses; these viruses have accounted for 22.8% of emerging infectious disease events in the latter half of the 20th century (2). A recent study showed that the dengue virus (family *Flaviviridae*) has spread worldwide, with an estimated incidence of 390 million infections, with 96 million individuals showing clinical manifestations annually (3). In the veterinary field, the Rift Valley fever phlebovirus (family *Phenuiviridae*) causes not just human disease but also economic losses in the livestock industry at an estimated USD 5–471 million per outbreak (4). Vaccines and antiviral drugs for most mosquito-borne viral diseases are still under development; therefore, effective vector control strategies are required.

Mosquito-specific viruses are found in mosquitoes and mosquito-derived cell lines and generally do not infect or replicate in vertebrates (5,6). Recent studies have identified several new mosquito-specific viruses that belong to the families *Flaviviridae*, *Phenuiviridae*, *Rhabdoviridae*, and *Mesoniviridae*, as well as unassigned Negeviruses (5). These mosquito-specific viruses show no significant effects in vertebrates, but their potential as control strategies against vector-borne agents as a result of their cooperative or competitive effects on arboviruses has been a focus of interest (7,8). Next-generation sequencing technologies have led to the discovery of several partial viral genes that have been integrated into the arthropod genomes (9,10). These integrated elements are called non-retroviral integrated RNA virus sequences, and partial elements related to *Flaviviridae*, *Phenuiviridae*, *Phasmaviridae*, *Hantaviridae* (order Bunyavirales), and *Rhabdoviridae* have been identified in the mosquito genomes (9,10).

In Japan, approximately 18 genera, including 114 species of mosquitoes, are distributed from Hokkaido in the northeast to Okinawa in the southwest, encompassing an area of approximately 20° latitude

Received March 18, 2022. Accepted September 12, 2022.
J-STAGE Advance Publication September 30, 2022.
DOI: 10.7883/yoken.JJID.2022.179
*Corresponding author: Mailing address: School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu-shi, Hokkaido 069-8501, Japan. Tel: +81-11-388-4828, Fax: +81-11-388-4828, E-mail: uchidaleo@rakuno.ac.jp

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＞

Ⅱ．その他　＜Others＞

1) Efficiency of immunocastration with an anti-gonadotropin-releasing hormone vaccine on cryptorchid bulls.
Goto A, Yoshida N, **Nakada K**, Inoue Y, Hisaeda K, Inaba T, Domoto N, Ishiguro Y, Itoh M, Takahashi E, Sasaki M, Matsui M.
J Vet Med Sci. 85:551–4556. 2023. doi: 10.1292/jvms.22-0571.

The Journal of Veterinary Medical Science

FULL PAPER

Theriogenology

Efficiency of immunocastration with an anti-gonadotropin-releasing hormone vaccine on cryptorchid bulls

Akira GOTO[1)#*], Nanako YOSHIDA[2)#], Ken NAKADA[3)], Yoichi INOUE[1)], Keiichi HISAEDA[1)], Toshio INABA[1)], Nanami DOMOTO[2)], Yuki ISHIGURO[2)], Megumi ITOH[2)], Eiji TAKAHASHI[2)], Motoki SASAKI[2)], Motozumi MATSUI[2)]

[1)]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, Ehime, Japan

[2)]Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan

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

ABSTRACT. Cryptorchid bulls have low economic value owing to the effects of masculinization. Moreover, surgical removal of an ectopic testis is difficult in certain clinical cases. Recently, immunocastration has garnered popularity as a nonsurgical castration method in pig farming; however, the effects of immunocastration on cryptorchid bulls are yet to be yet. Herein, we investigated endocrine changes due to immunocastration in cryptorchid bulls and studied its effectiveness. This study included 13 Holstein bulls diagnosed with cryptorchidism and classified into two groups based on pubertal period: <8 months of age (pregroup) and ≥8 months of age (postgroup). Antigonadotropin-releasing hormone (GnRH) vaccine was used for immunocastration, and two vaccine doses were administered. Blood testosterone and anti-Müllerian hormone (AMH) levels were measured and analyzed for endocrine evaluation. The testosterone levels significantly decreased following the start of immunocastration in both groups, thereby confirming the efficacy of antiGnRH vaccination in cryptorchid bulls. The AMH levels significantly increased in the pregroup with two antiGnRH vaccination, suggesting a compensatory response via the neutralization of GnRH antibodies. The AMH levels did not significantly change in the postgroup, indicating the partial suppression of AMH secretion in Sertoli cells during sexual maturation and failure of Sertoli cell maturation. Thus, we successfully restrained the serum testosterone levels in cryptorchid bulls using antiGnRH vaccine. The testosterone levels are a useful indicator of the immunocastration effect on cryptorchid bulls. Hereafter, a vaccine program that can sustain the castration effect on cryptorchid bulls is necessary.

KEYWORDS: antiGnRH vaccine, anti-Müllerian hormone, bull, cryptorchid, immunocastration

J. Vet. Med. Sci.
85(5): 551–556, 2023
doi: 10.1292/jvms.22-0571

Received: 25 December 2022
Accepted: 3 March 2023
Advanced Epub:
16 March 2023

*Correspondence to: Goto A: a-goto@ous.ac.jp, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science, 1-13 Ikoinooka-oka, Imabari, Ehime 794-0085, Japan

#These authors contributed equally to this work.

出典　doi: 10.1292/jvms.22-0571

https://www.jstage.jst.go.jp/article/jvms/85/5/85_22-0571/_pdf/-char/en

最終責任者　Goto Akira (Corresponding Author)

ハードヘルス学（Veterinary Herd Health）

Shin Oikawa

Professor

教授　及川　伸

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

1）Death-and-culling rates of calves and associated economic losses in the first month of life on dairy farms in eastern Hokkaido, Japan.
Kayasaki S, Chisato K, Fukumori R, **Oikawa S.**
Prev Vet Med. 106072, 2023. doi: 10.1016/j.prevetmed.2023.106072.

2）Epidemiological features of postpartum subclinical ketosis in dairy herds in Hokkaido, Japan.
Chisato K, Yamazaki T, Kayasaki S, Fukumori R, **Oikawa S.**
Animals. 14:144. 2024 (published: 31 December 2023).
doi: 10.3390/ani14010144

3）Ultrasonographic verification of hepatic hydatidosis in a female dromedary camel: a case report.
Tharwat M, El Moghazy HM, **Oikawa S.**
J Vet Med Sci. 85:1286–1290. 2023. doi: 10.1292/jvms.23-0325

Ⅱ．その他　＜Others＞

1）Effects of starch content of calf starter on feed intake, growth performance, and fecal properties in dairy calves under a high plane of milk replacer feeding.
Satoh H, Fukumori R, Osada T, Shimada K, **Oikawa S,** Izumi K.
Anim Sci J. 94:e13911. 2023. doi: 10.1111/asj.13911

2）Effects of Dystocia on the Cardiac Biomarker Troponin I, Acid-Base Balance and Blood Gases Alongside the Hematobiochemical Profiles in Female Dromedary Camels.
Tharwat M, Ali A, Derar D, **Oikawa S,** Almundarij TI.
Int J Vet Sci. 13:115-119. 2024 (published: 31 December 2023).
doi: 10.47278/journal.ijvs/2023.070

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Death-and-culling rates of calves and associated economic losses in the first month of life on dairy farms in eastern Hokkaido, Japan

Shuji Kayasaki [a,b], Kyoko Chisato [b], Rika Fukumori [b], Shin Oikawa [b,*]

[a] Teshikaga Livestock Clinic, Kushiro Central Branch, East Regional Center, Hokkaido Agricultural Mutual Aid Association, 3-10-13 Sakuraoka, Teshikaga-cho, Kawakami-gun, Hokkaido 088-3213, Japan
[b] Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Dairy farm
Calf
Death and culling
Economic loss
Japan

ABSTRACT

The objective of this study was to analyze death and culling (DC) of calves during the first month of life and associated economic losses on dairy farms in eastern Hokkaido, Japan. The DC in the first month of life of 4411 Holstein and Wagyu crossbred calves born in the year 2019–2020 on 39 dairy farms milking Holsteins was investigated. Based on a target DC rate of 6.75%, farms were classified into two groups, those with high DC rates (HDC, 11.68%, n = 18) and those with low DC rates (LDC, 2.67%, n = 21), and analyzed for DC factors (breed, sex, parity of dams, and housing type of dams) and diseases causing DC, their loss estimates, and replenishment of DC calves (birth rate, purchase of heifers, and housing type of dams). Comparisons between groups were made using the Kaplan-Meier method, the Mann-Whitney U test, and chi-square test. The DC rate of Holsteins was significantly higher ($P < 0.001$) for HDC farms than for LDC ones. But, the DC rate of Wagyu crossbreds was not different between the groups, which suggested hybrid vigor. The DC rates for digestive diseases were significantly higher ($P < 0.01$) in the HDC farms, except for congenital diseases and deaths of unknown cause. The overall loss estimates of DC per calf-month for all farms was 8892, JPY/calf-months, 14,726 for HDC farms, and 4065 for LDC farms. The loss estimates of items with significant differences in DC rates were significantly higher ($P < 0.05$) in the HDC farms, with higher loss estimates for Holsteins and digestive diseases being the most common characteristics of HDC farms. In binomial logistic regression analysis with "HDC farms or not" as the response variable and replenishment of DC calves as the explanatory variable, HDC was significantly more likely (OR: 1.10, $P < 0.05$) on farms with a higher birth proportion of Holsteins, and HDC farms supplemented the DC calves by increasing the birth proportion of Holsteins.

1. Introduction

Calf deaths on dairy farms have been reported to be concentrated in the first month of life (Jenny et al., 1981; Agerholm et al., 1993; USDA, 1994; Svensson et al., 2006; Wathes et al., 2008; Azizzadeh et al., 2012; Raboisson et al., 2013; Urie et al., 2018; Santman-Berends et al., 2019). Similarly in eastern Hokkaido, most of the preweaning death and culling occurs during the first month of life, which is a major problem. Calf deaths can lead directly to overall farm losses because they result in the loss of successors on the farm. Examples of economic losses, including herds with high calf mortality having lower milk production (Losinger and Heinrichs, 1997; Torsein et al., 2014), more animals purchased (Torsein et al., 2011, 2014), higher on-farm mortality of cows and a higher incidence of antibiotic treatment (Torsein et al., 2014), have been reported. Based on these findings, formulas for economic losses associated with calf mortality have also been developed (Demir et al., 2019). Buying more animals, especially in large quantities, comes with many risks. For farms, introduced animals are a risk for pathogen invasion (Maunsell and Donovan, 2008; Mee et al., 2012), which may include antibiotic-resistant bacteria (Adhikari et al., 2009; Mee et al., 2012). Also, colostrum from newly introduced late gestation cows may not contain protective antibodies against herd-specific pathogens (Torsein et al., 2011). This increase in the proportion of at-risk purchased cows would increase the mortality of cows (Thomsen et al., 2006; Raboisson et al., 2011), creating a further vicious cycle for farms. In other words, it is safer from a biosecurity standpoint to raise home-bred individuals

* Correspondence to: 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan.
E-mail address: oishin@rakuno.ac.jp (S. Oikawa).

https://doi.org/10.1016/j.prevetmed.2023.106072
Received 10 April 2023; Received in revised form 24 October 2023; Accepted 5 November 2023
Available online 9 November 2023

出典 doi: 10.1016/j.preventmed.2023.106072
https://www.sciencedirect.com/science/article/pii/S0167587723002362?via%3Dihub
最終責任者 Shin Oikawa (Corresponding Author)

animals

MDPI

Article

Epidemiological Features of Postpartum Subclinical Ketosis in Dairy Herds in Hokkaido, Japan

Kyoko Chisato [1], Takerou Yamazaki [2], Shuji Kayasaki [3], Rika Fukumori [1] and Shin Oikawa [1,*]

[1] Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan; k-chisato@rakuno.ac.jp (K.C.); fukumori@rakuno.ac.jp (R.F.)

[2] Memanbetsu Livestock Clinic, Ozora Central Branch, Okhotsk Regional Center, Hokkaido Agricultural Mutual Aid Association, 149-10 Memanbetsu Syouwa, Ozora-cho, Abashiri-gun, Hokkaido 099-2356, Japan; takerou_yamazaki_gb@nosai-do.or.jp

[3] Teshikaga Livestock Clinic, Kushiro Central Branch, East Regional Center, Hokkaido Agricultural Mutual Aid Association, 3-10-13 Sakuraoka, Teshikaga-cho, Kawakami-gun, Hokkaido 088-3213, Japan; s22041006@stu.rakuno.ac.jp

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

Simple Summary: The aim of this study was to investigate the prevalence of subclinical ketosis (SCK) in Hokkaido, Japan, and to assess its characteristics epidemiologically at the individual and herd levels. Blood samples were taken from clinically healthy cows once within 3–88 days in milk (DIM) for blood tests. Cows with SCK were classified as SCK II within 2 weeks postpartum and SCK I from 15 DIM. The prevalence of SCK II (20.2%) tended to be higher than that of SCK I (16.5%, $p = 0.094$). The prevalence peaked around 2 weeks postpartum. The frequency of SCK I was higher at the fourth parity. The number of milking cows in herds with higher SCK (≥25%) was significantly smaller than in herds with lower SCK ($p = 0.004$). The frequency of herds with higher SCK in tie stalls with component feeding was higher than for those in free stalls and free barns fed a total mixed ration ($p = 0.054$ and $p = 0.002$). This study reveals the prevalence of SCK in Hokkaido, Japan, and shows that SCK is associated with parity and the management system.

Citation: Chisato, K.; Yamazaki, T.; Kayasaki, S.; Fukumori, R.; Oikawa, S. Epidemiological Features of Postpartum Subclinical Ketosis in Dairy Herds in Hokkaido, Japan. *Animals* **2024**, *14*, 144. https://doi.org/10.3390/ani14010144

Academic Editors: Robert Kupczyński and Michał Bednarski

Received: 22 November 2023
Revised: 27 December 2023
Accepted: 28 December 2023
Published: 31 December 2023

Abstract: This study was carried out as an observational study in order to determine the prevalence of postpartum subclinical ketosis (SCK) in dairy herds in Hokkaido, Japan. From April 2012 to March 2014, blood β-hydroxybutyrate (BHBA) concentration was measured once within 3–88 days in milk (DIM) in 1394 apparently healthy cows from 108 farms to diagnose SCK (≥1.2 mM). In cows within 14 DIM, this was classified as SCK II, and from 15 DIM, this was classified as SCK I. Herds with a combined percentage of SCK I and SCK II of less than 10% were classified as SCK-negative herds, those with percentages of 10–25%, were classified as alert herds, and those with one of 25% or more, we classified as positive herds. The prevalence of SCK in the entire DIM was 17.6%. The prevalence of SCK II (20.2%) tended to occur more frequently than SCK I (16.5%, $p = 0.094$). The frequency of SCK I was higher at the fourth parity. The number of milking cows in SCK-positive herds was significantly smaller than those of the other two types of herds ($p = 0.004$). The frequency of SCK-positive herds in tie stalls and with component feeding was higher than for free stall or free barn and with total mixed ration ($p = 0.054$ and $p = 0.002$). This study reveals the prevalence of SCK in Hokkaido, Japan, and shows that SCK is associated with parity and the management system.

Keywords: dairy cow; subclinical ketosis; prevalence; risk factor; postpartum

1. Introduction

The three weeks before and after calving are called the "transition period" [1,2], during which dramatic changes occur in the cows. This means that the fetus grows significantly in the three weeks before calving, and after calving, more energy is required for milk synthesis.

出典 doi: 10.3990/ani14010144

https://www.mdpi.com/2076-2615/14/1/144

最終責任者 Shin Oikawa (Corresponding Author)

The Journal of Veterinary Medical Science

NOTE

Internal Medicine

Ultrasonographic verification of hepatic hydatidosis in a female dromedary camel: a case report

Mohamed THARWAT[1], Hazem M EL MOGHAZY[2], Shin OIKAWA[3]*

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia
[2]Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Benha University, Benha, Egypt
[3]Department of Veterinary Herd Health, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. This report describes for the first time, the antemortem diagnosis of hydatidosis in dromedary camel based on the WHO informal working groups on echinococcosis guidelines of echinococcosis classification. The case was admitted at the University Veterinary Hospital, Qassim University, Saudi Arabia, with a history of progressive weight loss and decreased appetite for the past 4 months. Alterations in hematobiochemical parameters included leukopenia, neutropenia, decreased hematocrit percent, red blood cells count and hemoglobin concentration, hypoproteinemia, hypoalbuminemia, hyperglobulinemia, hyperglycemia and azotemia. Ultrasonographically, multiple cysts were imaged within the hepatic tissue containing anechoic fluid and fibrin tags. Ultrasound-guided aspiration of a cyst revealed a clear non-turbid fluid. Ultrasonographic diagnostic is recommended for the work-up of cystic lesions of camel liver suspected to be due to *Echinococcus granulosus*.

KEYWORDS: animal, camel, hydatidosis, pathology, ultrasound

J. Vet. Med. Sci.
85(12): 1286–1290, 2023
doi: 10.1292/jvms.23-0325

Received: 18 August 2023
Accepted: 12 October 2023
Advanced Epub:
26 October 2023

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

出典　doi: 10.1292/jvms.23-0325
https://www.jstage.jst.go.jp/article/jvms/85/12/85_23-0325/_pdf/-char/en
最終責任者　Shin Oikawa (Corresponding Author)

Received: 15 April 2023 | Revised: 26 November 2023 | Accepted: 28 November 2023

DOI: 10.1111/asj.13911

RESEARCH ARTICLE

Animal Science Journal WILEY

Effects of starch content of calf starter on feed intake, growth performance, and fecal properties in dairy calves under a high plane of milk replacer feeding

Hitomi Satoh[1] | Rika Fukumori[1] | Takehiko Osada[2] | Kensuke Shimada[3] | Shin Oikawa[1] | Kenichi Izumi[2]

[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

[3]The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Tokyo, Japan

Correspondence
Kenichi Izumi, Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan.
Email: izmken@rakuno.ac.jp

Funding information
Ito Foundation, Grant/Award Number: 151

Abstract

The objective of this study was to investigate the effects of different starch contents in calf starter on the growth, feed intake, and fecal characteristics in dairy calves fed under a high plane of nutrition. Twenty-seven Holstein calves were assigned equally to one of three calf starter treatment groups in a randomized block design: high (H) starch (41.8%); medium (M) starch (31.9%); and low (L) starch (22.0%). Milk replacer was fed up to 1.2 kg/day as fed, gradually reduced from 6 weeks of age; calves were weaned at 8 weeks of age. Each starter (up to 3.5 kg/day as fed) and chopped hay were provided ad libitum from 5 days of age. Fecal samples, along with body measurements, were collected from 4 to 13 weeks of age. The average daily gain after weaning was greater in the H group than in the L group. The average starter intake for 3 days before weaning was not different among treatments but was greater after weaning in the H group than in the L group. Starter starch content did not affect the number of days with diarrhea or fecal starch concentration. The high-starch starter had no adverse effects during the weaning transition.

KEYWORDS
calf starter, growth performance, starch content, weaning transition

出典　doi: 10.1111/asj.13911

https://onlinelibrary.wiley.com/doi/full/10.1111/asj.13911

最終責任者　Kenichi Izumi (Corresponding Author)

P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.070

Effects of Dystocia on the Cardiac Biomarker Troponin I, Acid-Base Balance and Blood Gases Alongside the Hematobiochemical Profiles in Female Dromedary Camels

Mohamed Tharwat[1], Ahmed Ali[1,2], Derar Derar[1,2], Shin Oikawa[3] and Tariq I. Almundarij[1,*]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt
[3]Department of Veterinary Herd Health, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
***Corresponding author:** tmndrj@qu.edu.sa

Article History: 23-215 Received: 24-May-23 Revised: 26-Jun-23 Accepted: 02-Jul-23

ABSTRACT

This experiment was aimed to document the effect of dystocia in female camels on acid-base elements and blood gas parameters alongside the hemato-biochemical profiles compared to those with eutocia. Eighteen dystotic female dromedary camels were examined. Animals were received within 24, 48, 72, and >72h of birth. Signs included depression, anorexia, distress, colic, straining, and exhaustion. Ten eutocic females were used as controls. Compared to a mean value of 7.37±0.01in the control group, the blood pH in dystotic females was 7.32±0.13. The PO_2 was lower in dystotic females than in controls (61±58mmHg/L versus 183±15mmHg/L). The BE was also lower in dystotic females than controls (-8.8±6.0mmol/L versus -3.7±1.2mmol/L). The HCO_3 was lower in dystotic females than the controls (18±5mmol/L versus 21.4±1.5 mmol/L). The TCO_2 was also lower in dystotic females than in controls (19±5.0mmol/L versus 22.6±1.7mmol/L). The SO_2 decreased significantly in dystotic females than in controls. The PCO_2 and lactate concentrations did not change in a significant manner between the 2 groups. It is concluded that female camels with dystocia have metabolic acidosis compared to those with normal parturition. Changes in blood gases were remarkable that included significant decreases in PO_2, TCO_2, and SO_2 values when compared to eutocic camels.

Key words: Animals; Biomarkers; Blood; Pathophysiology; Ruminant.

Cite This Article as: Tharwat M, Ali A, Derar D, Oikawa S and Almundarij TI, 2024. Effects of dystocia on the cardiac biomarker troponin I, acid-base balance and blood gases alongside the hematobiochemical profiles in female dromedary camels. International Journal of Veterinary Science 13(1): 115-119. https://doi.org/10.47278/journal.ijvs/2023.070

出典 doi: 10.47278/journal.ijvs/2023.070

https://www.ijvets.com/pdf-files/23-215.pdf

最終責任者 Tariq I. Almundarij (Corresponding Author)

獣医生理学（Veterinary Physiology）

Takenori Onaga

Professor

教授　翁長　武紀

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

II. その他　＜Others＞

1）Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina.
Sato Y, Dong W, Nakamura T, Mizoguchi N, Nawaji T, Nishikawa M, **Onaga T**, Ikushiro S, Kobayashi M and Teraoka H
Int J Mol Sci. 24:4013. 2023. doi: org/10.3390/ijms24044013

International Journal of
Molecular Sciences

MDPI

Article

Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina

Yoshinori Sato [1,†], Wenjing Dong [1,†], Tatsuro Nakamura [1], Naohiro Mizoguchi [2], Tasuku Nawaji [2], Miyu Nishikawa [3], Takenori Onaga [1], Shinichi Ikushiro [3], Makoto Kobayashi [4] and Hiroki Teraoka [1,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu 069-8501, Hokkaido, Japan
[2] Chemicals Evaluation and Research Institute, Japan (CERI), 3-2-7, Miyanojin, Kurume 839-0801, Fukuoka, Japan
[3] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180, Kurokawa, Imizu 939-0398, Toyama, Japan
[4] Department of Molecular and Developmental Biology, Institute of Medicine, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan
* Correspondence: hteraoka@rakuno.ac.jp
† These authors contributed equally to this work.

Abstract: Metabolic activation is the primary cause of chemical toxicity including hepatotoxicity. Cytochrome P450 2E (CYP2E) is involved in this process for many hepatotoxicants, including acetaminophen (APAP), one of the most common analgesics and antipyretics. Although the zebrafish is now used as a model for toxicology and toxicity tests, the CYP2E homologue in zebrafish has not been identified yet. In this study, we prepared transgenic zebrafish embryos/larvae expressing rat CYP2E1 and enhanced green fluorescent protein (EGFP) using a β-actin promoter. Rat CYP2E1 activity was confirmed by the fluorescence of 7-hydroxycoumarin (7-HC), a metabolite of 7-methoxycoumarin that was specific for CYP2 in transgenic larvae with EGFP fluorescence (EGFP [+]) but not in transgenic larvae without EGFP fluorescence (EGFP [−]). APAP (2.5 mM) caused reduction in the size of the retina in EGFP [+] larvae but not in EGFP [−] larvae, while APAP similarly reduced pigmentation in both larvae. APAP at even 1 mM reduced the liver size in EGFP [+] larvae but not in EGFP [−] larvae. APAP-induced reduction of liver size was inhibited by *N*-acetylcysteine. These results suggest that rat CYP2E1 is involved in some APAP-induced toxicological endpoints in the retina and liver but not in melanogenesis of the developing zebrafish.

Keywords: acetaminophen; bioactivation; CYP2E1; rat; zebrafish

Citation: Sato, Y.; Dong, W.; Nakamura, T.; Mizoguchi, N.; Nawaji, T.; Nishikawa, M.; Onaga, T.; Ikushiro, S.; Kobayashi, M.; Teraoka, H. Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina. *Int. J. Mol. Sci.* **2023**, *24*, 4013. https://doi.org/10.3390/ijms24044013

Academic Editors: Patrick M. Dansette and Arthur Roberts

Received: 23 December 2022
Revised: 9 February 2023
Accepted: 14 February 2023
Published: 16 February 2023

1. Introduction

Species difference is the most important problem in the use of animal models in pharmacological and toxicological testing. Chemicals absorbed by animals are metabolized by phase I enzymes (oxidation, reduction, hydrolysis) and then phase II enzymes (conjugation reaction) for detoxification. Additionally, it has been established that some active metabolites can be formed through metabolic reactions to exert toxicological and pharmacological responses (bioactivation). Cytochrome P450 (CYP), the most important group of metabolic enzymes, is believed to be involved in bioactivation of chemicals for many toxicological responses [1]. Carcinogenicity is best known as chemical toxicity caused through bioactivation by CYP subtypes (CYPs) as some unstable intermediate products of carcinogenic substances by CYPs can attack genomic DNA [2]. Similarly, the liver is a representative target organ of harmful chemicals through bioactivation by CYPs and other enzymes as the liver contains various species of CYP in large amounts [3].

出典 doi: org/10.3390/ijms24044013
https://doi.org/10.3390/ijms24044013
最終責任者 Hiroki Teraoka (Corresponding Author)

獣医臨床病理学（Veterinary Clinicopathology）

Kazuyuki Suzuki

Professor

教授　鈴木　一由

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

1）Evaluation of blood serum iron concentration as an alternative biomarker for inflammation in dairy cows.
Murakami Y, Tsukano K, Hirata H, **Suzuki K.**
Biol Trace Elem Res. 201:4710–4717. 2023. doi: 10.1007/s12011-022-03544-5.

II. その他　＜Others＞

1）Optimal clinical dose of medetomidine for sedation of young cows during dehorning surgery.
Tsukano K, Yamakawa S, **Suzuki K.**
Acta Vet Hung. 71:142–146. 2023. doi: 10.1556/004.2023.00919.

2）Twenty-year changes in the composition of a mixed stock of foraging green turtles in the Yaeyama islands of Japan.
Hamabata T, Kawata M, Kondo S, Matsuo A, Suyama Y, **Suzuki K**, Kameda K.
Mar Ecol Prog Ser. 716:93–-105. 2023. doi: 10.3354/meps14367

Biological Trace Element Research (2023) 201:4710–4717
https://doi.org/10.1007/s12011-022-03544-5

Evaluation of blood serum iron concentration as an alternative biomarker for inflammation in dairy cows

Yoshiki Murakami[1] · Kenji Tsukano[2] · Haruyuki Hirata[1] · Kazuyuki Suzuki[1]

Received: 25 July 2022 / Accepted: 22 December 2022 / Published online: 9 January 2023

Abstract
This study aimed to clarify the relationship between acute phase protein (APP) concentrations and serum Fe concentrations to determine whether serum iron (Fe) can be clinically applied as a substitute for APPs in cows. One hundred five Holstein–Friesian breed lactating dairy cows were enrolled in this study. Cows with inflammatory diseases were 16 subclinical, and 15 severe mastitis cows, in addition to 15 mild and 16 severe sole ulcer cows. The plasma haptoglobin (HPT), alpha-1 acid glycoprotein (AGP), SAA, serum Fe levels, and other biochemical parameters in the cows were measured. The two-sample *t*-tests and multiple logistic regression analysis were used to compare the control and inflammatory disease groups. ROC analysis was used to evaluate the ability to diagnose inflammation disease. From the results, the proposed diagnostic cutoff value for plasma SAA and serum Fe concentrations to identify dairy cows with inflammatory diseases based on analyses of ROC curves were set at > 3.65 mg/l and < 120.50 μg/dl, respectively. Therefore, instead of using expensive inflammatory markers to evaluate the inflammatory state at the first treatment day for inflammatory diseases in cow, it shows the useful for screening with serum Fe concentration that can be measured easily and inexpensively as alternative inflammatory biomarkers.

Keywords Acute phase proteins · Cows · Inflammation · Blood serum iron

出典 doi: 10.1007/s12011-022-03544-5.
https://link.springer.com/article/10.1007/s12011-022-03544-5

最終責任者 Kazuyuki Suzuki (Corresponding Author)

AKADÉMIAI KIADÓ

Acta Veterinaria Hungarica

71 (2023) 3-4, 142-146

DOI:
10.1556/004.2023.00919

RESEARCH ARTICLE

Optimal clinical dose of medetomidine for sedation of young cows during dehorning surgery

KENJI TSUKANO[1*], SHOHEI YAMAKAWA[2] and KAZUYUKI SUZUKI[3]

[1] Aomori Agricultural Mutual Aid Association, 1-495 Aza Tounosawayama, Touhokumachi, Kamikita-gun, Aomori, 039-2654, Japan

[2] Hokkaido Agricultural Mutual Aid Association, 74-2 Higashimae, Hokuto, Hokkaido, 041-1214, Japan

[3] School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunnkyoudai, Ebetsu, Hokkaido, 069-8501, Japan

Received: 4 April 2023 • Accepted: 12 November 2023
Published online: 24 November 2023

ABSTRACT

The present paper reports on the clinical efficacy and optimal clinical dose of medetomidine for sedation of young cows during dehorning surgery. Medical records of 24 female Holstein cows that underwent dehorning surgery were used in this study. In four groups, the sedation of animals was carried out by one of the four intravenous treatments: 0.1 mg kg^{-1} xylazine (Xyl group, $n = 6$), 5.0 μg kg^{-1} medetomidine (5.0 Med group, $n = 6$), 10.0 μg kg^{-1} medetomidine (10.0 Med group, $n = 6$) or 20.0 μg kg^{-1} medetomidine (20.0 Med group, $n = 6$). The clinical sedation score (CSS) and heart rate (HR) were recorded. The CSSs after intravenous administration of each α_2-adrenergic receptor agonist increased rapidly and peaked at 12.5 (10.0–16.0) at $t = 20$ min in the Xyl group, 11.5 (10.0–15.0) at $t = 10$ min in the 5.0 Med group, 16.0 (14.0–16.0) at $t = 20$ min in the 10.0 Med group and 16.0 (14.0 – 16.0) at $t = 20$ min in the 20.0 Med group. A similar degree of bradycardia was observed after every sedative treatment. We conclude that the intravenous administration of 10.0–20.0 μg kg^{-1} medetomidine is appropriate for sedation of young cows without severe side effects.

KEYWORDS

cow, dehorning, medetomidine, sedation, xylazine

出典　doi: 10.1556/004.2023.00919. PMID: 37999759.
https://akjournals.com/view/journals/004/71/3-4/article-p142.xml
最終責任者　Kenji Tsukano (First and Corresponding Author)

Vol. 716: 93–105, 2023
https://doi.org/10.3354/meps14367

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 10

Twenty-year changes in the composition of a mixed stock of foraging green turtles in the Yaeyama Islands of Japan

Tomoko Hamabata[1,6,*], Masakado Kawata[1], Satomi Kondo[2], Ayumi Matsuo[3], Yoshihisa Suyama[3], Kazuyuki Suzuki[4], Kazunari Kameda[5]

[1]Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi 980-8578, Japan
[2]Ogasawara Marine Center, Everlasting Nature of Asia, Ogasawara, Tokyo 100-2101, Japan
[3]Kawatabi Field Science Center, Graduate School of Agricultural Science, Tohoku University, Osaki, Miyagi 989-6711, Japan
[4]School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[5]Kuroshima Research Station, Sea Turtle Association of Japan, Yaeyama, Okinawa 907-1311, Japan
[6]*Present address:* Graduate School of Information Sciences, Tohoku University, Sendai, Miyagi 980-8579, Japan

出典　doi:org/10.3354/meps14367
https://www.int-res.com/abstracts/meps/v716/p93-105/

最終責任者　Tomoko Hamabata (First and Corresponding Author)

獣医薬理学（Veterinary Pharmacology）

Hiroki Teraoka

Professor

教授　寺岡　宏樹

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

1） Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina.
Sato Y, Dong W, Nakamura T, Mizoguchi N, Nawaji T, Nishikawa M, Onaga T, Ikushiro S, Kobayashi M, **Teraoka H.**
Int J Mol Sci. 24:4013. 2023. doi: 10.3390/ijms24044013.

2） Augmentation of Pectoral Fin Teratogenicity by Thalidomide in Human Cytochrome P450 3A-Expressing Zebrafish.
Dong W, Akasaka I, Komiyama A, Nakamura T, Mizoguchi N, Nawaji T, Ikushiro S, Kobayashi M, **Teraoka H.**
Pharmaceuticals (Basel) 16:368. 2023. doi: 10.3390/ph16030368.

3） Reduced pigmentation and thyroid hormone disruption in zebrafish embryos caused by industrial sludge near Bohai Bay, China.
Dong W, Yin X, Qi C, Wei T, Wei L, Yang J, Mu J, **Teraoka H**, Dong W.
Water Biol Secur. 2:3167. 2023. doi.org/10.1016/j.watbs.2023.100190.
*Co-corresponding author

4） Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan.
Yokokawa A, Dong W, Momose K, Iima H, Yoshino T, Izumi K, Kawai Y, Amano T, Nakamura T, Sawada A, Endoh D, Nakajima N, **Teraoka H.**
Animals (Basel). 13:3167. 2023. doi: 10.3390/ani13203167.

Ⅱ．その他　＜Others＞

International Journal of
Molecular Sciences

MDPI

Article

Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina

Yoshinori Sato [1,†], Wenjing Dong [1,†], Tatsuro Nakamura [1], Naohiro Mizoguchi [2], Tasuku Nawaji [2], Miyu Nishikawa [3], Takenori Onaga [1], Shinichi Ikushiro [3], Makoto Kobayashi [4] and Hiroki Teraoka [1,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu 069-8501, Hokkaido, Japan
[2] Chemicals Evaluation and Research Institute, Japan (CERI), 3-2-7, Miyanojin, Kurume 839-0801, Fukuoka, Japan
[3] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180, Kurokawa, Imizu 939-0398, Toyama, Japan
[4] Department of Molecular and Developmental Biology, Institute of Medicine, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan
* Correspondence: hteraoka@rakuno.ac.jp
† These authors contributed equally to this work.

Abstract: Metabolic activation is the primary cause of chemical toxicity including hepatotoxicity. Cytochrome P450 2E (CYP2E) is involved in this process for many hepatotoxicants, including acetaminophen (APAP), one of the most common analgesics and antipyretics. Although the zebrafish is now used as a model for toxicology and toxicity tests, the CYP2E homologue in zebrafish has not been identified yet. In this study, we prepared transgenic zebrafish embryos/larvae expressing rat CYP2E1 and enhanced green fluorescent protein (EGFP) using a β-actin promoter. Rat CYP2E1 activity was confirmed by the fluorescence of 7-hydroxycoumarin (7-HC), a metabolite of 7-methoxycoumarin that was specific for CYP2 in transgenic larvae with EGFP fluorescence (EGFP [+]) but not in transgenic larvae without EGFP fluorescence (EGFP [−]). APAP (2.5 mM) caused reduction in the size of the retina in EGFP [+] larvae but not in EGFP [−] larvae, while APAP similarly reduced pigmentation in both larvae. APAP at even 1 mM reduced the liver size in EGFP [+] larvae but not in EGFP [−] larvae. APAP-induced reduction of liver size was inhibited by *N*-acetylcysteine. These results suggest that rat CYP2E1 is involved in some APAP-induced toxicological endpoints in the retina and liver but not in melanogenesis of the developing zebrafish.

Keywords: acetaminophen; bioactivation; CYP2E1; rat; zebrafish

Citation: Sato, Y.; Dong, W.; Nakamura, T.; Mizoguchi, N.; Nawaji, T.; Nishikawa, M.; Onaga, T.; Ikushiro, S.; Kobayashi, M.; Teraoka, H. Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina. *Int. J. Mol. Sci.* **2023**, *24*, 4013. https://doi.org/10.3390/ijms24044013

Academic Editors: Patrick M. Dansette and Arthur Roberts

Received: 23 December 2022
Revised: 9 February 2023
Accepted: 14 February 2023
Published: 16 February 2023

1. Introduction

Species difference is the most important problem in the use of animal models in pharmacological and toxicological testing. Chemicals absorbed by animals are metabolized by phase I enzymes (oxidation, reduction, hydrolysis) and then phase II enzymes (conjugation reaction) for detoxification. Additionally, it has been established that some active metabolites can be formed through metabolic reactions to exert toxicological and pharmacological responses (bioactivation). Cytochrome P450 (CYP), the most important group of metabolic enzymes, is believed to be involved in bioactivation of chemicals for many toxicological responses [1]. Carcinogenicity is best known as chemical toxicity caused through bioactivation by CYP subtypes (CYPs) as some unstable intermediate products of carcinogenic substances by CYPs can attack genomic DNA [2]. Similarly, the liver is a representative target organ of harmful chemicals through bioactivation by CYPs and other enzymes as the liver contains various species of CYP in large amounts [3].

出典 doi: 10.3390/ijms24044013.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9968093/

最終責任者 Hiroki Teraoka (Corresponding Author)

pharmaceuticals

MDPI

Article

Augmentation of Pectoral Fin Teratogenicity by Thalidomide in Human Cytochrome P450 3A-Expressing Zebrafish

Wenjing Dong [1], Ippo Akasaka [1], Akifumi Komiyama [1], Tatsuro Nakamura [1], Naohiro Mizoguchi [2], Tasuku Nawaji [2], Shinichi Ikushiro [3], Makoto Kobayashi [4] and Hiroki Teraoka [1,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[2] Chemicals Evaluation and Research Institute, Japan (CERI), 3-2-7, Miyanojin, Kurume 839-0801, Fukuoka, Japan
[3] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180, Kurokawa, Imizu 939-0398, Toyama, Japan
[4] Department of Molecular and Developmental Biology, Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan
* Correspondence: hteraoka@rakuno.ac.jp or hteraoka08@gmail.com

Abstract: The pharmacological and toxicological effects of active metabolites of enzymes including cytochrome P450 (CYP) are important. While it has been believed for a long time that thalidomide causes characteristic limb malformation only in rabbits and primates including humans, the involvement of their CYP3A subtypes (CYP3As) has been suggested. Recently, however, it was reported that zebrafish were sensitive to thalidomide, showing defects of pectoral fins, homologous organs of forelimbs in mammals, as well as other deformities. In this study, we prepared human CYP3A7 (hCYP3A7)-expressing zebrafish (F0) using a transposon system. Thalidomide caused pectoral fin defects and other malformations including pericardial edema in hCYP3A7-expressing embryos/larvae but not in wild-type and hCYP1A1-expressing embryos/larvae. Thalidomide also reduced the expression of fibroblast growth factor 8 in pectoral fin buds in only hCYP3A7-expressing embryos/larvae. The results suggest the involvement of human-type CYP3A in thalidomide teratogenicity.

Keywords: CYP1A1; CYP3A4; CYP3A7; human; teratogenicity; thalidomide; zebrafish

Citation: Dong, W.; Akasaka, I.; Komiyama, A.; Nakamura, T.; Mizoguchi, N.; Nawaji, T.; Ikushiro, S.; Kobayashi, M.; Teraoka, H. Augmentation of Pectoral Fin Teratogenicity by Thalidomide in Human Cytochrome P450 3A-Expressing Zebrafish. *Pharmaceuticals* **2023**, *16*, 368. https://doi.org/10.3390/ph16030368

Academic Editor: Yuhei Nishimura

Received: 30 January 2023
Revised: 21 February 2023
Accepted: 22 February 2023
Published: 28 February 2023

1. Introduction

Zebrafish have been widely used in toxicological and pharmacological fields for about two decades. They were originally used as model animals for developmental biology [1]. Zebrafish embryos are annually available, small in size and transparent for a relatively long time, allowing chemical challenge and phenotype observation under a conventional microscope in a plastic dish for cultured cells. Their small size is also convenient for examining the effects of small amounts of precious chemicals and harmful wastes. According to regulations in the European Union, zebrafish are out of regulation as animal experiments until they have acquired self-feeding ability that is completed by 120 h post fertilization (hpf) [2]. Thus, zebrafish are now being used for the high-throughput screening of chemicals including medical drug candidates and possible environmental pollutants such as pesticides, especially for toxicological testing [3–5]. However, species differences between zebrafish and higher vertebrates should always be considered.

Cytochrome P450 (CYP) enzymes (CYPs) are a superfamily of enzymes that are involved in many metabolic reactions including the detoxication of xenobiotic substances such as medical drugs as well as other active substances such as steroid hormones [6]. It is also known that CYPs are involved in the conversion of prodrugs to active forms and in the bioactivation of promutagens and procarcinogens [7]. Harmful metabolites produced through the bioactivation by CYPs as well as other metabolic enzymes are also

出典　doi: 10.3390/ph16030368.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10055635/

最終責任者　Hiroki Teraoka (Corresponding Author)

Contents lists available at ScienceDirect

Water Biology and Security

journal homepage: www.keaipublishing.com/en/journals/water-biology-and-security

Research Article

Reduced pigmentation and thyroid hormone disruption in zebrafish embryos caused by industrial sludge near Bohai Bay, China

Wenjing Dong [a,b,1], Xiaoyu Yin [a,1], Chelimuge Qi [a], Tingting Wei [a], Lijia Wei [a], Jingfeng Yang [a], Jingli Mu [c,**], Hiroki Teraoka [b,***], Wu Dong [a,*]

[a] College of Animal Science and Technology, Inner Mongolia Minzu University/Inner Mongolia Key Laboratory of Toxicant Monitoring and Toxicology, Tongliao, 028000, China
[b] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan
[c] College of Geography and Oceanography, Minjiang University, Fuzhou, 350108, China

ARTICLE INFO

Keywords:
Bohai bay
PAHs
Pigmentation
Thyroxine
Zebrafish embryos

ABSTRACT

In recent years, pollution caused by the discharge of industrial wastewater into Bohai Bay has posed a potential threat to the health of surrounding residents. Sludge was collected from the outlet of a factory that discharges effluent into Bohai Bay, and alcohol extracts of sludge (SE) were prepared. We confirmed by UPLC-MS/MS analysis that the SE contained PAHs, including fluorene, pyrene, and phenanthrene. Zebrafish embryos as animal models were exposed to 0.1, 0.3, 0.5, 1 and 5 mg/mL SE from 2 to 4 h post-fertilization (hpf) until 120 hpf. The results showed that SE caused a concentration-dependent increase in mortality and a decrease in hatchability. We found that SE significantly reduced eye pigmentation and decreased the movement of embryos and larvae. In addition, SE decreased triiodothyronine (T3) content and down-regulated the mRNA expression of some thyroid hormone-related genes including *TPO* and *Thrβ*, and caused the up-regulation of *Dio2* and *Dio3* at 120 hpf. Exposure to three individual PAHs found in SE, namely fluorene, pyrene, and phenanthrene, caused morphological and transcriptional changes that were similar to those caused by SE exposure. These findings indicate that PAHs in SE can reduce the pigmentation of zebrafish, which may be related to the genetic changes associated with thyroid hormones, and that zebrafish eye pigmentation can be used as an indicator of PAHs exposure.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: jlmu@mju.edu.cn (J. Mu), hteraoka@rakuno.ac.jp (H. Teraoka), dongwu@imun.edu.cn (W. Dong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.watbs.2023.100190
Received 8 March 2023; Received in revised form 10 May 2023; Accepted 1 June 2023
Available online 14 June 2023

出典　doi: 10.1016/j.watbs.2023.100190
https://www.sciencedirect.com/science/article/pii/S2772735123000707
最終責任者　Hiroki Teraoka (Co-corresponding Author)

animals

MDPI

Article

Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan

Ayaka Yokokawa [1], Wenjing Dong [1], Kunikazu Momose [2], Hiroko Iima [3], Tomoo Yoshino [3], Kenichi Izumi [4], Yusuke Kawai [5], Tomoko Amano [4], Tatsuro Nakamura [1], Akira Sawada [6], Daiji Endoh [1], Nobuyoshi Nakajima [6] and Hiroki Teraoka [1,2,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; yokokawa-ayaka@bozo.co.jp (A.Y.); dongwenjing_2015@163.com (W.D.); t-naka@rakuno.ac.jp (T.N.); dendoh@rakuno.ac.jp (D.E.)
[2] NPO Red-Crowned Crane Conservancy, 9-21 Wakatake-Cho, Kushiro 085-0036, Japan; dzi00244@nifty.com
[3] Kushiro Zoo, 11 Shimoninishibetsu, Kushiro 085-0204, Japan; hiroko.iima@city.kushiro.lg.jp (H.I.); garrulaxcanorus@yahoo.co.jp (T.Y.)
[4] College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan; izmken@rakuno.ac.jp (K.I.); amano@rakuno.ac.jp (T.A.)
[5] Laboratory of Toxicology, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada-cho Nishi, Obihiro 080-8555, Japan; ykawai@obihiro.ac.jp
[6] Biodiversity Division, National Institute for Environmental Studies, Tsukuba 305-8506, Japan; akira.sawada.1312@gmail.com (A.S.); naka-320@nies.go.jp (N.N.)
* Correspondence: hteraoka08@gmail.com

Simple Summary: The red-crowned crane (*Grus japonensis*), which is an endangered and highly protected bird species, is distributed in two populations: a mainland population in far eastern Eurasia and an island population in Hokkaido, Japan. Red-crowned cranes in Japan are resident birds mainly in the eastern part of Hokkaido. As omnivores, they feed on plants, grains, insects, and fish. Most cranes spend the winter around feeding stations in southeastern Hokkaido, where people provide corn. Since most of the cranes in Hokkaido now live near areas inhabited by humans, cases of crop damage caused by cranes have recently been reported. This study showed that the cranes feed on various crops of human origin, mostly outside farmlands.

Abstract: Total DNA extracts from the intestinal contents of 60 flying red-crowned cranes (juveniles, subadults and adults) found dead in 2006–2021, and the feces of 25 chicks collected in June and July of 2016–2018, were used for PCR reactions with primers specific for 16 crops, followed by high-throughput sequencing. The most predominant crop detected was corn in adult and subadult cranes (61.7%). Other grains (barley, wheat, soybean) (5.0–8.3%) and vegetables (tomatoes, Chinese cabbage, etc.) (1.7–6.7%) were also detected in flying cranes. Surprisingly, some of the detected crops were not grown in the Kushiro and Nemuro regions. There was no significant difference in crop intake status in winter and that in other seasons for most of the crops. Corn (28.0%), soybeans (8.0%), wheat and beet (4.0%) were detected in crane chicks in summer, though the detection rates were generally lower than those in flying cranes. Alfalfa, which is not grown in eastern Hokkaido but is used in some cattle feed, was detected in some cranes. Rice, buckwheat, adzuki beans, common beans, potatoes and carrots were not detected at any life stage, indicating the preferences of red-crowned cranes. The results suggest that red-crowned cranes in Hokkaido are dependent on dairy farmers for their feed supply.

Keywords: amplicon sequencing; crop consumption; *Grus japonensis*; Japan; scatological samples

Citation: Yokokawa, A.; Dong, W.; Momose, K.; Iima, H.; Yoshino, T.; Izumi, K.; Kawai, Y.; Amano, T.; Nakamura, T.; Sawada, A.; et al. Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan. *Animals* **2023**, *13*, 3167. https://doi.org/10.3390/ani13203167

Academic Editor: Barry Hartup

Received: 27 July 2023
Revised: 6 October 2023
Accepted: 9 October 2023
Published: 10 October 2023

出典　doi: 10.3390/ani13203167.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10603659/

最終責任者　Hiroki Teraoka (Corresponding Author)

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＞

Ⅱ．その他　＜Others＞

1）Computed tomography and magnetic resonance imaging findings with left adrenal pheochromocytoma infiltrating the spinal canal and liver metastases in a dog.
Mizutani S, Mizutani Y, Satoh H, Goda Y, Asanuma T, **Torisu S**.
Jpn J Vet Res. 71:20–26, 2023. doi: 10.57494/jjvr.71.1_20

2）Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture.
Isaka M, Konno W, Kokubo D, Udagawa H, Hizuka S, Sakai T, Yamamoto S, **Torisu S**, Ueno H.
Res Vet Sci. 158:41–43. 2023. doi: 10.1016/j.rvsc.2023.03.006.

3）Use of a gum elastic bougie in a cat with severe upper airway stenosis.
Kato K, Itami T, **Torisu S**, Sakai T, Yamashita K.
Open Vet J. 13:114–118. 2023. doi: 10.5455/OVJ.2023.v13.i1.12.

Japanese Journal of Veterinary Research 71(1): 20-26, 2023

JJVR

SHORT COMMUNICATION Clinical Case Report

Computed tomography and magnetic resonance imaging findings with left adrenal pheochromocytoma infiltrating the spinal canal and liver metastases in a dog

Shinya Mizutani[1,†], Yuko Mizutani[2,†], Hiroyuki Satoh[3,*], Yoshimichi Goda[3], Taketoshi Asanuma[1] and Shidow Torisu[4]

[1] Clinical Veterinary Medicine, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Okayama University of Science Imabari campus, 1-3 Ikoinooka, Imabari City, Ehime 794-8555, Japan.
[2] University of Miyazaki Veterinary Teaching Hospital, 1-1 Gakuen Kibana-dai Nishi, Miyazaki City, Miyazaki 889-2192, Japan.
[3] Laboratory of Veterinary Clinical Radiology, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan.
[4] Companion Animal Surgery, Department of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

Received for publication, February 7, 2022; accepted, December 16, 2022

Abstract

Recently, detection of canine primary adrenal mass has increased owing to progress in diagnostic imaging. Adrenocortical adenoma, adrenocortical carcinoma, and pheochromocytoma are representative primary adrenal masses; the latter two may invade intravascularly and metastasize to other organs. We encountered a dog exhibiting progressive hindlimb paraplegia. Multimodal imaging including computed tomography (CT) and magnetic resonance imaging (MRI) revealed that the cause of the paraplegia was infiltration of a left adrenal pheochromocytoma into the spinal canal. In addition, MRI detected small liver metastatic lesions that were not detected by CT.

Key Words: Dogs, Pheochromocytoma, Spinal canal

* Corresponding author: Hiroyuki Satoh, D.V.M., Ph.D.
Laboratory of Veterinary Clinical Radiology, Department of Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan.
Phone: +81-985-58-7286
E-mail: hsatoh@cc.miyazaki-u.ac.jp
† These authors contributed equally to this work.
doi: 10.57494/jjvr.71.1_20

Research in Veterinary Science 158 (2023) 41–43

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture

Mitsuhiro Isaka *, Wataru Konno, Daiki Kokubo, Hiromu Udagawa, Sho Hizuka, Toshikazu Sakai, Shushi Yamamoto, Shidow Torisu, Hiroshi Ueno

Laboratory of Companion Animal Surgery, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Cranial cruciate ligament rupture
Dogs
Osteocrin
Osteotomy

ABSTRACT

The cranial cruciate ligament (CCL) rupture is a common orthopedic disease in dogs that is usually managed with tibial plateau leveling osteotomy (TPLO) or extracapsular lateral suture (ECLS). Osteotomy is generally associated with some complications, including nonunion. The periosteum plays an important role in bone growth and remodeling. Osteocrin (OSTN), which was recently identified and is involved in bone formation and differentiation, is produced in the periosteum and osteoblasts. The aimed to investigate whether the concentrations of serum OSTN change before and after stifle surgery in dogs and compare the OSTN concentrations in the two surgical techniques (TPLO: $n = 20$ vs. ECLS: $n = 36$). The postoperative serum OSTN concentration in the TPLO group was significantly lower than the preoperative value ($p < 0.05$), while serum OSTN concentrations differed statistically between the preoperative and suture-removal periods. In contrast, no significant differences were observed in the ECLS group. In conclusion, osteotomy affects serum OSTN concentrations during the perioperative period in dogs, which may be related to periosteal injury.

The cranial cruciate ligament (CCL) rupture is a common orthopedic condition in dogs (Knight and Danielski, 2018). The surgical procedures for CCL rupture are the tibial plateau leveling osteotomy (TPLO), which is tibial osteotomy with an orthopedic plate, and an expracapsular lateral suture (ECLS), which makes the hole of the tibia passing through the suture without osteotomy techniques (Knight and Danielski, 2018; Krotscheck et al., 2016). Generally the use of osteotomy in small- animal orthopedics may be associated with some complications, including nonunion, implant failure, infection, and revision surgery (Danielski et al., 2022).

The periosteum is a dense and abundantly vascularized connective tissue membrane with fibroblasts, including multipotent mesenchymal stem cells and osteogenic progenitor cells (Allen et al., 2004; Chang and Knothe Tate, 2012). It also covers the surface of the majority of bone, facilitating the attachment of muscles, ligaments, and tendons, and delivering blood, nutrition, and regenerative cells to participate in a healing process (Roberts et al., 2015; Seeman, 2007). A previous study reported that the absence of the periosteum induces a marked decrease in new bone formation and up to 10-fold reduced vascularization (Colnot, 2009; Zhang et al., 2005). Collectively, the periosteum may be an important factor related to the complications of osteotomy.

Osteocrin (OSTN) is a recently discovered natriuretic peptide secreted from periosteum, similar to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (Nishizawa et al., 2004). It is produced in the periosteum and osteoblasts (Thomas et al., 2003) and is thought to be involved in bone formation and differentiation (Thomas et al., 2003; Watanabe-Takano et al., 2021). However, no previous reports have described the effects of osteotomy on serum OSTN concentrations during the perioperative period in veterinary clinical settings. Thus, the aim of this study was to investigate whether the serum OSTN concentrations change before and after operative stifle surgery in dogs and compare the findings between the two surgical techniques.

Fifty-six dogs, including 20 and 36 dogs in the TPLO and ECLS groups, respectively, were included in this study. The body weight and age of the dogs in the TPLO group were 25.77 ± 12.27 kg and 96.65 ± 27.28 months, respectively; the group included two male and nine castrated dogs as well as four female and six spayed dogs. The breeds were as follows: Great Dane, $n = 1$; Labrador Retriever, n = 1; Golden Retriever, $n = 3$; Siberian Husky, n = 3; Shiba Inu, $n = 2$; Welsh Corgi, n = 2; Mongrel, n = 3; Bernese Mountain Dog, $n = 2$; Border Collie, n = 1; and White Shepherd, $n = 2$. Alternatively, the body weight and age of the dogs in the ECLS group were 9.9 ± 7.04 kg and 95.78 ± 36.11

* Corresponding author.
E-mail address: m-isaka@rakuno.ac.jp (M. Isaka).

https://doi.org/10.1016/j.rvsc.2023.03.006
Received 6 October 2022; Received in revised form 15 February 2023; Accepted 5 March 2023
Available online 8 March 2023

出典 doi: 10.1016/j.rvsc.2023.03.006.
https://www.sciencedirect.com/science/article/pii/S0034528823000735?via%3Dihub
最終責任者 Mitsuhiro Isaka (Corresponding Author)

Open Veterinary Journal, (2023), Vol. 13(1): 114–118
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Case Report
DOI: *10.5455/OVJ.2023.v13.i1.12*

Submitted: 11/07/2022 Accepted: 05/12/2022 Published: 23/01/2023

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*

出典 doi: 10.5455/OVJ.2023.v13.i1.12.

https://www.openveterinaryjournal.com/?mno=56656

最終責任者 Takaharu Itami (Corresponding Author)

食品衛生学 (Food Microbiology and Food Safety)

Masaru Usui

Professor

教授　臼井　優

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

1）Intermittent antibiotic treatment of bacterial biofilms favors the rapid evolution of resistance.
Usui M, Yoshii Y, Thiriet-Rupert S, Ghigo J M, Beloin C.
Commun Biol. 6:275. 2023. doi: 10.1038/s42003-023-04601-y

2）16S rRNA nanopore sequencing for rapid diagnosis of causative bacteria in bovine mastitis.
Usui M, Akiyoshi M, Fukuda A, Iwano H, Kato T.
Res in Vet Sci. 161:45-49. 2023. doi: 10.1016/j.rvsc.2023.06.006

3）Antimicrobial resistant bacteria monitoring in retail raw seafood in Japan: A pilot study focused on Vibrio and Aeromonas
Fukuda A, Tsunashima R, **Usui M**,
Food Saf (Tokyo). 11:65–77. 2023. doi: 10.14252/foodsafetyfscj.D-23-00006

Ⅱ. その他　＜Others＞

1）Characterization of SpsQ from Staphylococcus pseudintermedius as an affinity chromatography ligand for canine therapeutic antibodies.
Takeuchi H, Nakajima C, Konnai S, Maekawa N, Okagawa T, **Usui M,** Tamura Y, Suzuki Y, Murata S, Ohashi K.
Plos One. 18:e0281171. 2023. doi: 10.1371/journal.pone.0281171

2）Inactivation of Antibiotic-resistant Bacteria in Hospital Wastewater by Ozone-Based Advanced Water Treatment Processes.
Azuma T, **Usui M**, Hayashi T.
Sci Total Environ. 906:16743. 2023. doi: 10.1016/j.scitotenv.2023.167432

communications biology

ARTICLE

https://doi.org/10.1038/s42003-023-04601-y OPEN

Intermittent antibiotic treatment of bacterial biofilms favors the rapid evolution of resistance

Masaru Usui[1,2✉], Yutaka Yoshii[2], Stanislas Thiriet-Rupert[2], Jean-Marc Ghigo[2] & Christophe Beloin[2✉]

Bacterial antibiotic resistance is a global health concern of increasing importance and intensive study. Although biofilms are a common source of infections in clinical settings, little is known about the development of antibiotic resistance within biofilms. Here, we use experimental evolution to compare selection of resistance mutations in planktonic and biofilm *Escherichia coli* populations exposed to clinically relevant cycles of lethal treatment with the aminoglycoside amikacin. Consistently, mutations in *sbmA*, encoding an inner membrane peptide transporter, and *fusA*, encoding the essential elongation factor G, are rapidly selected in biofilms, but not in planktonic cells. This is due to a combination of enhanced mutation rate, increased adhesion capacity and protective biofilm-associated tolerance. These results show that the biofilm environment favors rapid evolution of resistance and provide new insights into the dynamic evolution of antibiotic resistance in biofilms.

出典 doi: 10.1038/s42003-023-04601-y

https://www.nature.com/articles/s42003-023-04601-y

最終責任者 Masaru Usui (First Author and Corresponding Author)

Research in Veterinary Science 161 (2023) 45–49

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

16S rRNA nanopore sequencing for rapid diagnosis of causative bacteria in bovine mastitis

Masaru Usui [a,*], Misaki Akiyoshi [a,b], Akira Fukuda [a], Hidetomo Iwano [c], Toshihide Kato [b]

[a] *Laboratory of Food Microbiology and Food Safety, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*

[b] *Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*

[c] *Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan*

ARTICLE INFO

Keywords:
Bovine mastitis
Nanopore sequence
16S rRNA sequencing

ABSTRACT

The rapid identification of specific bacterial pathogens in bovine mastitis is crucial for appropriate antimicrobial treatment. Sequencing of 16S rRNA gene amplicons is a proven, useful strategy for diagnosing bacterial infections. In this study, the use of 16S rRNA analysis with nanopore sequencer for the rapid identification of causative bacteria in bovine mastitis, was evaluated. DNA was extracted from 122 milk samples from cattle with suspected mastitis based on clinical symptoms. 16S rRNA gene amplicon sequencing was conducted using a nanopore sequencer. The efficacy of bacterial identification was verified by comparison with conventional culture methods. Nanopore sequencing identified the causative bacteria with high accuracy within approximately 6 h from the time of sample collection. When the major causative bacteria of bovine mastitis (*Escherichia coli, Streptcoccus uberis, Klebsiella pneumoniae,* and *Staphylococcus aureus*) were detected by nanopore sequencing, 98.3% of the results were consistent with identification through conventional culturing methods. 16S rRNA gene analysis using a nanopore sequencer enabled the rapid and accurate identification of bacterial species in bovine mastitis.

出典 doi: 10.1016/j.rvsc.2023.06.006

https://www.sciencedirect.com/science/article/pii/S003452882300173X

最終責任者 Masaru Usui (First Author and Corresponding Author)

FOOD SAFETY

doi: 10.14252/foodsafetyfscj.D-23-00006
Food Safety 2023; Vol. **, No. **, **–**

Published online in advance by J-STAGE

Original Article

Antimicrobial Resistant Bacteria Monitoring in Raw Seafood Retailed: a Pilot Study Focused on *Vibrio* and *Aeromonas*

Akira Fukuda, Ryu Tsunashima, Masaru Usui

Food Microbiology and Food Safety Unit, Division of Preventive Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

In aquaculture, bacterial infections in sea animals are treated using antimicrobials. As seafood is frequently consumed in its raw form, seafood contaminated with water-borne antimicrobial-resistant bacteria presents a potential transmission route to humans and can influence food safety. In this study, we aimed to determine the abundance of water-borne bacteria in retail raw seafood and to characterize their antimicrobial resistance profiles. In total, 85 retail raw seafood samples (32 fish, 26 shellfish, 25 mollusks, and two crustaceans) were purchased from supermarkets in Japan, and water-borne bacteria were isolated. The isolated bacterial species predominantly included *Vibrio* spp. (54.1%) and *Aeromonas* spp. (34.1%). *Vibrio* or *Aeromonas* spp. were isolated from more than 70% of the seafood samples. Tetracycline-, sulfamethoxazole-, and/or trimethoprim/sulfamethoxazole-resistant *Vibrio* or *Aeromonas* spp. isolates were detected in seven (21.9%) fish samples (two wild-caught and five farm-raised) harboring *tet*, *sul*, and/or *dfr* genes. Sulfamethoxazole- and trimethoprim/sulfamethoxazole-resistant isolates were only detected in farm-raised fish. Tetracycline and sulfamethoxazole are commonly used in aquaculture. These results suggest that water-borne bacteria like *Vibrio* and *Aeromonas* spp. should be the primary focus of antimicrobial-resistant bacteria monitoring to effectively elucidate their spread of bacteria via seafood.

Key word: *Vibrio*, *Aeromonas*, seafood, antimicrobial resistant bacteria, antimicrobial resistance genes

出典 doi: 10.14252/foodsafetyfscj.D-23-00006
https://www.jstage.jst.go.jp/article/foodsafetyfscj/advpub/0/advpub_D-23-00006/_article
最終責任者 Masaru USUI (Corresponding Author)

PLOS ONE

RESEARCH ARTICLE

Characterization of SpsQ from *Staphylococcus pseudintermedius* as an affinity chromatography ligand for canine therapeutic antibodies

Hiroto Takeuchi[1☯], Chie Nakajima[2,3,4☯], Satoru Konnai[1,2,4*], Naoya Maekawa[2], Tomohiro Okagawa[2], Masaru Usui[5], Yutaka Tamura[5], Yasuhiko Suzuki[2,3,4], Shiro Murata[1,2], Kazuhiko Ohashi[1,2,6]

1 Faculty of Veterinary Medicine, Department of Disease Control, Hokkaido University, Sapporo, Japan, **2** Faculty of Veterinary Medicine, Department of Advanced Pharmaceutics, Hokkaido University, Sapporo, Japan, **3** Division of Bioresources, International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, **4** Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Sapporo, Japan, **5** Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, **6** Faculty of Veterinary Medicine, International Affairs Office, Hokkaido University, Sapporo, Japan

☯ These authors contributed equally to this work.
* konnai@vetmed.hokudai.ac.jp

Citation: Takeuchi H, Nakajima C, Konnai S, Maekawa N, Okagawa T, Usui M, et al. (2023) Characterization of SpsQ from *Staphylococcus pseudintermedius* as an affinity chromatography ligand for canine therapeutic antibodies. PLoS ONE 18(1): e0281171. https://doi.org/10.1371/journal.pone.0281171

Editor: Jian Xu, East China Normal University School of Life Sciences, CHINA

Received: October 27, 2022

Accepted: January 17, 2023

Published: January 26, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by Grant-in-Aids from the Japan Society for the Promotion of Science (JSPS) under grant numbers 22H02503 [to SK], 21K1498301 [to NM], and JP22J12766 [to HT], the Japan Agency for Medical Research and Development (AMED) under grant numbers JP223fa627005 [to SK], JP22jk0210005 [to YS],

Abstract

Coagulase-positive Staphylococci express protein A, which binds to host antibodies, to evade the immune system. Taking advantage of its specific binding to antibodies, protein A from *Staphylococcus aureus*, which is called SpA, is commonly used as an affinity chromatography ligand for human therapeutic antibodies. However, among four canine IgG subclasses (A, B, C, and D), only IgG-B binds to SpA strongly and establishing an efficient and robust purification scheme for canine therapeutic antibodies whose IgG subclass is A, C, or D remains difficult and depends on finding a suitable substitute to SpA. *S. pseudintermedius*, a major coagulase-positive Staphylococci found in dogs, expresses *spsQ* gene which is orthologous to *S. aureus spa*. We hypothesized that to serve *S. pseudintermedius* to better adapt to the dog immune system, SpsQ would bind to canine IgGs stronger than SpA, making it a better affinity chromatography ligand for canine therapeutic antibodies. To characterize SpsQ, we first determined the *spsQ* nucleotide sequence from *S. pseudintermedius* isolates. Based on the identified sequence, we prepared recombinant proteins containing the immunoglobulin-binding domains of SpA (r-SpA) and SpsQ (r-SpsQ) and determined their binding capacity for each canine IgG subclass. The binding capacity of r-SpsQ for IgG-B was almost as high as that of r-SpA. Interestingly, while both r-SpsQ and r-SpA showed no binding to IgG-C, the binding capacity of r-SpsQ for IgG-A and IgG-D was significantly higher than that of r-SpA. Finally, we performed affinity chromatography using r-SpsQ- or r-SpA-immobilized resin and revealed that the recovery rates of IgG-A and IgG-D using r-SpsQ were significantly higher than those using r-SpA. Our findings indicate that SpsQ has a strong potential to be used as an affinity chromatography ligand for canine therapeutic antibodies of subclass A, B, and D.

出典 DOI: 10.1371/journal.pone.0281171

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0281171

最終責任者　Satoru Konnai (Corresponding Author)

Science of the Total Environment 906 (2024) 167432

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Inactivation of antibiotic-resistant bacteria in hospital wastewater by ozone-based advanced water treatment processes

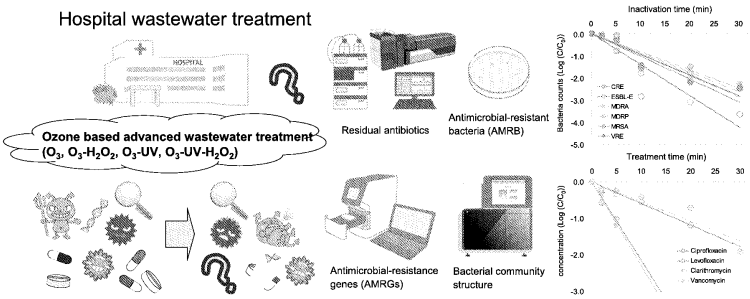
Takashi Azuma [a,*], Masaru Usui [b], Tetsuya Hayashi [a,c]

[a] Department of Pharmacy, Osaka Medical and Pharmaceutical University, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan
[b] Food Microbiology and Food Safety, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan
[c] Faculty of Human Development, Department of Food and Nutrition Management Studies, Soai University, 4-4-1 Nankonaka, Osaka Suminoeku, Osaka 559-0033, Japan

HIGHLIGHTS

- Ozone (O_3)-based advanced wastewater treatment for hospital wastewater was evaluated.
- Direct O_3 treatment of hospital wastewater is effective for disinfection.
- Antimicrobials and antimicrobial-resistant bacteria were effectively inactivated.
- Wastewater treatment with O_3 effectively removed antimicrobial resistance genes.
- O_3/UV and O_3/UV/H_2O_2 showed higher efficiencies for hospital wastewater treatment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Damia Barcelo

Keywords:
Antimicrobial resistance (AMR)
Hospital wastewater
Advanced oxidation process (AOPs)
Ozonation
Antimicrobials
Antimicrobial-resistant bacteria (AMRB)
Antimicrobial resistance genes (AMRGs)
Environmental risk management
Wastewater treatment plant (WWTP)
River environment

ABSTRACT

The emergence and spread of antimicrobial resistance (AMR) continue on a global scale. The impacts of wastewater on the environment and human health have been identified, and understanding the environmental impacts of hospital wastewater and exploring appropriate forms of treatment are major societal challenges. In the present research, we evaluated the efficacy of ozone (O_3)-based advanced wastewater treatment systems (O_3, O_3/H_2O_2, O_3/UV, and O_3/UV/H_2O_2) for the treatment of antimicrobials, antimicrobial-resistant bacteria (AMRB), and antimicrobial resistance genes (AMRGs) in wastewater from medical facilities. Our results indicated that the O_3-based advanced wastewater treatment inactivated multiple antimicrobials (>99.9%) and AMRB after 10–30 min of treatment. Additionally, AMRGs were effectively removed (1.4–6.6 $\log_{10}$) during hospital wastewater treatment. The inactivation and/or removal performances of these pollutants through the O_3/UV and O_3/UV/H_2O_2 treatments were significantly ($P < 0.05$) better than those in the O_3 and O_3/H_2O_2 treatments. Altered taxonomic diversity of microorganisms based on 16S rRNA gene sequencing following the O_3-based treatment showed that advanced wastewater treatments not only removed viable bacteria but also removed genes constituting microorganisms in the wastewater. Consequently, the objective of this study was to apply advanced wastewater treatments to treat wastewater, mitigate environmental pollution, and alleviate potential threats to environmental and human health associated with AMR. Our findings will contribute to enhancing the

出典 doi: 10.1016/j.scitotenv.2023.167432
https://linkinghub.elsevier.com/retrieve/pii/S004896972306059X
最終責任者 Takashi Azuma (First Author and 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＞

1）The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits.
Wei Y, Chen IY, Tamogi H, Sugita C, Daimaruya N, Hirokawa T, Kato K, Itami T, Sano T, **Yamashita K.**
J Vet Med Sci. 85: 471–478. 2023. doi: 10.1292/jvms.22-0484.

2）Sedative and cardiorespiratory effects of intranasal atomized alfaxalone in Japanese White rabbits.
Wei Y, Nakagawa M, Chen IY, Itami T, Sano T, Pasloske K, **Yamashita K.**
Vet Anaesth Analg. 50:255–262. 2023. doi: 10.1016/j.vaa.2023.02.001.

3）ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs.
Chen IY, Sugita C, Wei Y, Daimaruya N, Itami T, Sano T, **Yamashita K.** *Vet Anaesth Analg.* 50:204–210. 2023. doi: 10.1016/j.vaa.2023.02.008.

4）Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs.
Chen IY, Sugita C, Wei Y, Daimaruya N, Itami T, Sano T, **Yamashita K.**
Vet Anaesth Analg. 50:485–491. 2023. doi: 10.1016/j.vaa.2023.08.002.

Ⅱ．その他　＜Others＞

1）Use of a gum elastic bougie in a cat with severe upper airway stenosis.
Kato K, Itami T, Torisu S, Sakai T, **Yamashita K.**
Open Vet J. 13:114–118. doi: 10.5455/OVJ.2023.v13.i1.12.

The Journal of Veterinary Medical Science

FULL PAPER
Surgery

The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits

Yixian WEI[1)], I-Ying CHEN[1)], Haruka TAMOGI[1)], Chihiro SUGITA[1)], Nozomi DAIMARUYA[1)], Taku HIROKAWA[1)], Keiko KATO[1)], Takaharu ITAMI[1)], Tadashi SANO[1)], Kazuto YAMASHITA[1)]*

[1)]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. To prevent aspiration in Japanese White (JW) rabbits, the maximum single volume of medetomidine administered intranasally is 0.3 mL per nostril using a mucosal atomization device (MAD). This study aimed to examine the sedative effect of intranasal administration of medetomidine using MAD in eight healthy female JW rabbits. Each rabbit received intranasal atomization (INA) of saline (Control treatment) along with three doses of 1 mg/mL medetomidine (0.3 mL to one nostril [MED0.3 treatment]; 0.3 mL each to both nostrils [MED0.6 treatment]; 0.3 mL twice to both nostrils [MED1.2 treatment]), with a washout period of at least 7 days between treatments. The actual doses of medetomidine were 82 (75–84) μg/kg (median [25th–75th percentile]), 163 (156–168) μg/kg, and 323 (295–343) μg/kg for the MED0.3, MED0.6, and MED1.2 treatments, respectively. A medetomidine-dose dependent sedative effect was detected, and the loss of righting reflex (LRR) was achieved in one rabbit at 18 min, seven rabbits at 11 (9–18) min, and eight rabbits at 7 (4–18) min after the MED0.3, MED0.6, and MED1.2 treatments, respectively. The LRR was maintained for 63 (29–71) min and 83 (68–101) min after the MED0.6 and MED1.2 treatments, respectively. Additionally, the INA of medetomidine produced a significant dose-dependent cardiorespiratory depression including a decrease in pulse rate, respiratory rate, percutaneous oxygen saturation, and arterial partial pressure of oxygen, and an increase in arterial partial pressure of carbon dioxide in the rabbits.

KEYWORDS: intranasal atomization, medetomidine, mucosal atomization device, rabbit

J. Vet. Med. Sci.
85(4): 471–478, 2023
doi: 10.1292/jvms.22-0484

Received: 25 October 2022
Accepted: 22 February 2023
Advanced Epub:
6 March 2023

*Correspondence to: Yamashita K: yamasita@rakuno.ac.jp, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

出典　doi: 10.1292/jvms.22-0484.

https://www.jstage.jst.go.jp/article/jvms/85/4/85_22-0484/_pdf/-char/en

最終責任者　Kazuto Yamashita (Corresponding Author)

Veterinary Anaesthesia and Analgesia 2023, **50**, 255–262 https://doi.org/10.1016/j.vaa.2023.02.001

RESEARCH PAPER

Sedative and cardiorespiratory effects of intranasal atomized alfaxalone in Japanese White rabbits

Yixian Wei[a], **Mari Nakagawa**[a], **I-Ying Chen**[a], **Takaharu Itami**[a], **Tadashi Sano**[a], **Kirby Pasloske**[b] & **Kazuto Yamashita**[a]

[a]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[b]Jurox Pty Ltd, Rutherford, NSW, Australia

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yumasita@rakuno.ac.jp

Abstract

Objective To investigate the sedative and cardiorespiratory effects of intranasal atomization (INA) of alfaxalone using a mucosal atomization device in Japanese White rabbits.

Study design Randomized, prospective, crossover study.

Animals A total of eight healthy female rabbits, weighing 3.6–4.3 kg and aged 12–24 months.

Methods Each rabbit was randomly assigned to four INA treatments administered 7 days apart: Control treatment, 0.15 mL 0.9% saline in both nostrils; treatment INA0.3, 0.15 mL 4% alfaxalone in both nostrils; treatment INA0.6, 0.3 mL 4% alfaxalone in both nostrils; treatment INA0.9, 0.3 mL 4% alfaxalone in left, then right, then left nostril. Sedation was scored 0–13 using a composite measure scoring system for rabbits. Simultaneously, pulse rate (PR), respiratory rate (f_R), noninvasive mean arterial pressure (MAP), peripheral hemoglobin oxygen saturation (SpO_2) and arterial blood gases were measured until 120 minutes. The rabbits breathed room air during the experiment and were administered flow-by oxygen when hypoxemia (SpO_2 <90% or PaO_2 <60 mmHg; 8.0 kPa) developed. Data were analyzed using the Fisher's exact test and the Friedman test ($p < 0.05$).

Results No rabbit was sedated in treatments Control and INA0.3. All rabbits in treatment INA0.9 developed loss of righting reflex for 15 (10–20) minutes [median (25th–75th percentile)]. Sedation score significantly increased from 5 to 30 minutes in treatments INA0.6 and INA0.9 with maximum scores of 2 (1–4) and 9 (9–9), respectively. f_R decreased in an alfaxalone dose-dependent manner and one rabbit developed hypoxemia in treatment INA0.9. No significant changes were observed in PR and MAP.

Conclusions and clinical relevance INA alfaxalone resulted in dose-dependent sedation and respiratory depression in Japanese White rabbits to values considered not clinically relevant. Further investigation of INA alfaxalone in combination with other drugs is warranted.

Keywords alfaxalone, intranasal mucosal atomization, rabbits, sedative effects.

Introduction

Over the past decade, interest in intranasal drug delivery has been garnering increased attention in both human and veterinary medicine. The theory behind intranasal drug absorption is that the intranasal cavity is lined by highly vascularized mucosa facilitating rapid drug absorption, and the olfactory epithelium provides direct access to the central nervous system (CNS) by bypassing the blood–brain barrier (Grassin-Delyle et al. 2012; Keller et al. 2021; Mignani et al. 2021). Drugs absorbed through the trigeminal and olfactory nerve pathways from the nasal cavity are directly delivered to the brain. Thus intranasal administration is a promising alternative route to oral, intravenous and intramuscular administration of drugs that bypasses the blood–brain barrier (Erdő et al. 2018).

In rabbits, a total volume of 0.3–0.64 mL kg^{-1} of solution containing sedative and anesthetic drugs has been administered intranasally through the nostrils using a catheter-tipped syringe to produce sedation (Santangelo et al. 2016; Weiland et al. 2017; Yanmaz et al. 2022). An intranasal combination of dexmedetomidine, midazolam and butorphanol resulted in deep sedation with moderate hypoxemia and hypercarbia in New Zealand White (NZW) rabbits (Santangelo et al. 2016). In a different study, intranasal combination of dexmedetomidine and midazolam resulted in rapid sedation in NZW rabbits;

出典 doi: 10.1016/j.vaa.2023.02.008.

https://www.sciencedirect.com/science/article/pii/S1467298723000326?via%3Dihub

最終責任者 Kazuto Yamashita (Corresponding Author)

RESEARCH PAPER

ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs

I-Ying Chen, Chihiro Sugita, Yixian Wei, Nozomi Daimaruya, Takaharu Itami, Tadashi Sano & Kazuto Yamashita

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

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasite@rakuno.ac.jp

Abstract

Objective To determine the median effective dose (ED_{50}) and effective dose required to depress the twitch value by 95% (ED_{95}) of rocuronium during alfaxalone anesthesia in dogs.

Study design A randomized, prospective, crossover experimental study.

Animals A total of eight adult Beagle dogs (four female, four male), weighing 10.3–14.6 kg and aged 6–8 years.

Methods The dogs were anesthetized three times with 1.25-fold the individual minimum infusion rate of alfaxalone at intervals of ≥ 14 days. Neuromuscular function was monitored with train-of-four (TOF) stimulation of the peroneal nerve by acceleromyography. After recording the control TOF ratio (TOFRC) and first twitch of TOF (T1C), a single bolus dose of rocuronium 100, 175 or 250 μg kg^{-1} (treatments R100, R175 or R250) was administered intravenously. The maximum suppression of the first twitch of TOF (T1) was recorded and calibrated with T1C to construct the dose–response curve, from which ED_{50} and ED_{95} were calculated. Time from rocuronium administration to TOF ratio/TOFRC > 0.9 (duration TOFR0.9) was recorded.

Results ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia were 175 and 232 μg kg^{-1}, respectively. The median (range) duration TOFR0.9 was longer in treatment R250 [10.1 (9.2–10.9) minutes] than in treatments R100 [3.1 (2.9–4.4) minutes; $p < 0.0001$] and R175 [7.7 (6.9–8.1) minutes; $p < 0.0001$]; and longer in treatment R175 than in treatment R100 ($p < 0.0001$).

Conclusions and clinical relevance The duration of TOFR0.9 correlated positively with the dosage of rocuronium, indicating that recovery time of rocuronium was also dose-dependent in dogs anesthetized with alfaxalone. The duration TOFR0.9 of rocuronium 250 μg kg^{-1} was 10 minutes during alfaxalone anesthesia in dogs.

Keywords administration and dosage, alfaxalone, dose–response relationship, drug, prospective studies, veterinary.

Introduction

Rocuronium bromide is an intermediate-acting non-depolarizing neuromuscular blocking agent (NMBA) in dogs (Marshall et al. 1994; Dugdale et al. 2002). As rocuronium does not produce active metabolites in human plasma (Appiah-Ankam & Hunter 2004), it is now widely used as an intravenous (IV) bolus, for incremental IV doses, and for constant rate infusion in anesthetized dogs (Dugdale et al. 2002; Alderson et al. 2007; Auer et al. 2007; Briganti et al. 2015; Haga et al. 2019). Nevertheless, NMBAs do not have an anesthetic effect and, therefore, should be administered during general anesthesia (Kastrup et al. 2005).

Alfaxalone is a synthetic neuroactive steroid anesthetic agent that is used for induction and maintenance of anesthesia in veterinary practice (Quirós-Carmona et al. 2017; Bennell et al. 2019; Dehuisser et al. 2019; Bustamante et al. 2020). The single-dose rocuronium-induced neuromuscular blockade was longer in alfaxalone than propofol anesthesia in dogs (Chen et al. 2022). However, information to guide veterinary physicians in clinical practice regarding the effective doses of rocuronium with alfaxalone is still insufficient.

The potency of NMBAs can be evaluated by constructing dose–response curves from a linear least squares regression analysis model using the single-dose method (Fuchs-Buder et al. 2007). In this method, the degree of neuromuscular block is measured following administration of different doses of NMBA to determine the median effective dose 50 (ED_{50}) and

出典 doi: 10.1016/j.vaa.2023.02.008.

https://www.sciencedirect.com/science/article/pii/S1467298723000326

最終責任者 Kazuto Yamashita (Corresponding Author)

Veterinary Anaesthesia and Analgesia 2023, **50**, 485–491 https://doi.org/10.1016/j.vaa.2023.08.002

RESEARCH PAPER

Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs

I-Ying Chen, Chihiro Sugita, Yixian Wei, Nozomi Daimaruya, Takaharu Itami, Tadashi Sano & Kazuto Yamashita

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

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasita@rakuno.ac.jp

Abstract

Objective To investigate the reversal effect of sugammadex on neuromuscular blockade induced by a single bolus of rocuronium in dogs under alfaxalone anesthesia.

Study design Randomized, prospective, crossover experimental study.

Animals A group of six adult Beagle dogs (three females and three males), weighing 11.3–15.8 kg and aged 6–8 years, were used.

Methods Dogs were anesthetized twice with a 1.25 times minimum infusion rate of alfaxalone, with a washout period of at least 14 days between experiments. Neuromuscular function was monitored using acceleromyography with train-of-four (TOF) stimulation of the peroneal nerve. After recording the control TOF ratio (TOFRC), rocuronium (0.5 mg kg^{-1}) was administered intravenously. Subsequently, sugammadex (4 mg kg^{-1}) or an equal volume of saline (control treatment) was administered intravenously when the TOF count returned from 0 to 1 after neuromuscular blockade. Time from rocuronium injection to TOF count = 0 (onset time), time from TOF count = 0 to TOF count = 1 (maximum blockade period), time of first twitch amplitude recovery from 0.25 to 0.75 (recovery index), and time from sugammadex or saline administration to TOF ratio/TOFRC ≥ 0.9 (recovery time) were recorded.

Results The onset time and maximum blockade duration did not differ between sugammadex treatment [1.2 (0.7–1.5) minutes and 9.9 (6.3–10.5) minutes, respectively] and control treatment [median (range); 1.0 (0.7–1.1) minutes and 9.9 (8.8–11.5) minutes, respectively] ($p = 0.219$ and 0.844, respectively). Recovery index was 0.5 (0.3–0.7) minutes in sugammadex treatment, which was shorter than that in control treatment [4.5 (3.7–4.9) minutes] ($p = 0.031$). Recovery time was 0.8 (0.5–2.8) minutes in sugammadex treatment, which was shorter than that in control treatment [10.5 (6.8–14.3) minutes] ($p = 0.031$).

Conclusions and clinical relevance Rocuronium-induced neuromuscular blockade was effectively reversed by sugammadex in dogs anesthetized with alfaxalone.

Keywords alfaxalone, dogs, neuromuscular blockade, rocuronium, sugammadex.

Introduction

Sugammadex, a gamma-cyclodextrin, selectively reverses rocuronium-induced neuromuscular blockade via chemical encapsulation (Bom et al. 2002; de Boer et al. 2006a,b). The structure of sugammadex comprises a lipophilic internal cavity that encapsulates aminosteroid neuromuscular-blocking molecules to form an inactive complex, which is then excreted unchanged via the kidneys, thus reversing the neuromuscular blockade (Bom et al. 2002; Peeters et al. 2011). The reversal effect of sugammadex on rocuronium-induced neuromuscular blockade has been evaluated in dogs under isoflurane anesthesia (Mosing et al. 2012). Compared with acetylcholinesterase inhibitors, sugammadex does not have cardiovascular side effects and can reverse profound neuromuscular block (Sacan et al. 2007). However, encapsulation of other steroidal drugs or endogenous steroids may occur with sugammadex administration (Hunter & Flockton 2006).

Alfaxalone, a synthetic neuroactive steroid anesthetic agent, is used to induce and maintain anesthesia in veterinary practice (Bennett et al. 2019). An alfaxalone formulation licensed for clinical use in dogs and cats uses 2-hydroxypropyl-beta cyclodextrin as a solubilizing excipient (Ferré et al. 2006). There is a possibility that alfaxalone, as a synthetic neuroactive

出典 doi: 10.1016/j.vaa.2023.08.002.
https://www.sciencedirect.com/science/article/pii/S1467298723001186

最終責任者 Kazuto Yamashita (Corresponding Author)

Open Veterinary Journal, (2023), Vol. 13(1): 114–118
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Case Report
DOI: *10.5455/OVJ.2023.v13.i1.12*

Submitted: 11/07/2022 Accepted: 05/12/2022 Published: 23/01/2023

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*

出典 doi: 10.5455/OVJ.2023.v13.i1.12.

https://www.openveterinaryjournal.com/?mno=56656

最終責任者 Takaharu Itami (Corresponding Author)

獣医ウイルス学（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＞

Ⅱ．その他　＜Others＞

1） Chimeric Newcastle Disease Virus Vectors Expressing Human IFN-γ Mediate Target Immune Responses and Enable Multifaceted Treatments.
Soliman RM, Nishioka K, **Daidoji T**, Noyori O, Nakaya T.
Biomedicines 11:455. doi: 10.3390/biomedicines11020455.

2） Stimulation of interferon-β responses by aberrant SARS-CoV-2 small viral RNAs acting as retinoic acid-inducible gene-I agonists. Arai Y, Yamanaka I, Okamoto T, Isobe A, Nakai N, Kamimura N, Suzuki T, **Daidoji T**, Ono T, Nakaya T, Matsumoto K, Okuzaki D, Watanabe Y.
iScience. 26:105742. 2023. doi: 10.1016/j.isci.2022.105742.

biomedicines

MDPI

Article

Chimeric Newcastle Disease Virus Vectors Expressing Human IFN-γ Mediate Target Immune Responses and Enable Multifaceted Treatments

Rofaida Mostafa Soliman [1,2,†], Keisuke Nishioka [1,*,†], Tomo Daidoji [1], Osamu Noyori [3] and Takaaki Nakaya [1]

[1] Department of Infectious Diseases, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan
[2] Department of Animal Medicine, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt
[3] Laboratory of Immunology and Microbiology, College of Pharmaceutical Sciences, Ritsumeikan University, Shiga 525-8577, Japan
* Correspondence: din85051@koto.kpu-m.ac.jp; Tel.: +81-75-251-5325
† These authors contributed equally to this work.

Abstract: The therapeutic potential of Newcastle disease virus (NDV) has been reported as both an oncolytic agent and a vaccine vector against many antigens. However, in the individuals already immunized with NDVs, second and subsequent administration does not provide substantial benefits. In this study, two types of recombinant chimeric NDVs using APMV-2 F and HN genes were generated. In rNDV-2HN, the wild-type NDV HN gene was replaced with the APMV-2 HN gene, and in rNDV-2F/2HN, both wild-type F and HN genes were replaced with APMV-2 F and HN genes, respectively. We enhanced the immune responses of these chimeric viruses by inserting the human IFN-γ gene. To examine the escape from NDV antiserum, each virus was treated with diluted NDV antiserum, and HEp-2 cells were infected with these virus particles. The two constructed chimeric viruses indicated notably lower virus-neutralizing titer compared to wild-type NDV and escaped the action of NDV antiserum. These two chimeric viruses infected both respiratory and colon cancer cell lines, indicating their potential as a cancer treatment tool. Chimeric viruses with enhanced immune responses can be considered a novel therapeutic strategy in cancer treatment that can be administered multiple times and used to enhance immune cells interaction.

Keywords: anti-tumor response; chimeric virus; immune response; Newcastle disease virus; recombinant virus vector; vaccination

Citation: Soliman, R.M.; Nishioka, K.; Daidoji, T.; Noyori, O.; Nakaya, T. Chimeric Newcastle Disease Virus Vectors Expressing Human IFN-γ Mediate Target Immune Responses and Enable Multifaceted Treatments. *Biomedicines* **2023**, *11*, 455. https://doi.org/10.3390/biomedicines11020455

Academic Editor: Giuseppe Murdaca

Received: 29 December 2022
Revised: 27 January 2023
Accepted: 2 February 2023
Published: 4 February 2023

1. Introduction

Newcastle disease is a zoonotic disease caused by the Newcastle disease virus (NDV), and countermeasures must be taken to manage it, especially in poultry farms [1]. Chickens are vaccinated in each poultry farm, because the velogenic strain NDV has a high mortality rate, approximately 90% [2]. On the other hand, NDV is known to be less pathogenic to humans, with symptoms limited to conjunctivitis [3]. Although NDV infects normal cells, they are eliminated by host immune responses; however, NDV can replicate efficiently in cancer cells that indicate deficiency in immune responses [4]. Therefore, it is expected to be a useful tool for cancer treatment in humans. There are several strategies. Several isolated strains of NDV that specifically target cancer cells are administered to cancer patients [5]. Cancer cells are taken from patients and killed by NDV infection, and fragments of these cells are used as cancer vaccines [5]. Recombinant NDV is designed by inserting anti-tumor cytokines and is then administrated to cancer patients [5,6]. Some of the treatment strategies using NDV have been highly effective in cancer treatment [5]. Additionally, because NDV infects a wide range of hosts, including humans, it was investigated as a vaccine vector [7,8]. As mentioned above, NDV is used as a treatment for various purposes.

出典　doi: 10.3390/biomedicines11020455.
https://www.mdpi.com/2227-9059/11/2/455
最終責任者　Keisuke Nishioka (Corresponding Author)

iScience

CellPress
OPEN ACCESS

Article

Stimulation of interferon-β responses by aberrant SARS-CoV-2 small viral RNAs acting as retinoic acid-inducible gene-I agonists

Yasuha Arai,[1,6] Itaru Yamanaka,[2,6] Toru Okamoto,[3,6] Ayana Isobe,[1] Naomi Nakai,[2] Naoko Kamimura,[2] Tatsuya Suzuki,[3] Tomo Daidoji,[1] Takao Ono,[4] Takaaki Nakaya,[1] Kazuhiko Matsumoto,[1] Daisuke Okuzaki,[2,5] and Yohei Watanabe[1,7,*]

SUMMARY

Patients with severe COVID-19 exhibit a cytokine storm characterized by greatly elevated levels of cytokines. Despite this, the interferon (IFN) response is delayed, contributing to disease progression. Here, we report that SARS-CoV-2 excessively generates small viral RNAs (svRNAs) encoding exact 5′ ends of positive-sense genes in human cells *in vitro* and *ex vivo*, whereas endemic human coronaviruses (OC43 and 229E) produce significantly fewer similar svRNAs. SARS-CoV-2 5′ end svRNAs are RIG-I agonists and induce the IFN-β response in the later stages of infection. The first 60-nt ends bearing duplex structures and 5′-triphosphates are responsible for immune-stimulation. We propose that RIG-I activation by accumulated SARS-CoV-2 5′ end svRNAs may contribute to later drive over-exuberant IFN production. Additionally, the differences in the amounts of svRNAs produced and the corresponding IFN response among CoV strains suggest that lower svRNA production during replication may correlate with the weaker immune response seen in less pathogenic CoVs.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is an ongoing global health threat. Although the Delta variant (B.1.617.2) dominated previous variants and spread to >180 countries by September 2021,[1] the newly emerging Omicron variants (B.1.1.529.1/BA.1 and B.1.1.529.2/BA.2) are outcompeting Delta and quickly becoming the dominant lineage globally as of February 2022.[2] Patients with severe COVID-19 exhibit a hyperinflammatory response referred to as a "cytokine storm,"[3] which is characterized by excessive levels of cytokines including interleukin (IL)-6 and a variety of different interferons (IFNs).[4–6]

Like most viral RNAs, coronavirus RNA is detected by host RNA sensors, cytosolic retinoic acid-inducible gene-I (RIG-I)-like receptors including RIG-I and melanoma differentiation-associated protein 5 (MDA-5).[7–11] Upon activation, RIG-I and MDA-5 transduce signaling cascades, leading to the activation of interferon regulatory factor 3 (IRF3) and NF-κB that are required for type 1 IFN and pro-inflammatory cytokine production, respectively. During SARS-CoV-2 infection, both RIG-I and MDA5 reportedly sense SARS-CoV-2 RNAs to activate innate immune responses,[12,13] although studies suggest that it is MDA5 that predominantly governs the innate immune response to SARS-CoV-2.[14,15] However, it is not well understood which viral RNA species are sensed by these molecules on infection. Additionally, longitudinal analyses revealed that SARS-CoV-2 does elicit an IFN response, but this is delayed,[16] suspected to contribute to disease progression.[17–19] However, the viral mechanism driving the characteristic signature of delayed IFN induction in COVID-19 is poorly understood.

Abortive RNA production has been understood to be a result of RNA transcription by RNA polymerase of host machinery (e.g. abortive initiation by bacterial and eukaryotic RNA polymerase),[19,20] host machinery during viral infection (e.g. abortive HIV-1 RNA transcripts consisting of TAR hairpin RNA)[21–24] and RNA viruses (double-stranded RNA viruses and single-stranded RNA viruses with both polarities).[25–27] It remains unclear how host and viral machinery are involved in the production of the abortive RNA transcripts. In

[1]Department of Infectious Diseases, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

[2]Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan

[3]Institute for Advanced Co-Creation Studies, Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan

[4]SANKEN, Osaka University, Osaka 567-0047, Japan

[5]Single Cell Genomics, Human Immunology, WPI Immunology Frontier Research Center, Osaka University, Osaka 565-0871, Japan

[6]These authors contributed equally

[7]Lead contact

*Correspondence: [redacted]

https://doi.org/10.1016/j.isci.2022.105742

出典　doi: 10.1016/j.isci.2022.105742.

https://www.sciencedirect.com/science/article/pii/S2589004222020156?via%3Dihub

最終責任者　Yohei Watanabe (Corresponding Author)

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）Measurement of pH in the cow's rumen for highquality dairy product. **Fukumori R,** Hara K, Hiramatsu Y, Ito A, Arai K, Ashibe S, Nagao Y, Sasaki A, Yamamoto H, Otani H, Yasugi M, Yoshiura Y.
2022 Tenth International Symposium on Computing and Networking Workshops (CANDARW) 40-43 (Date Added to IEEE *Xplore*: 17 March 2023). doi: 10.1109/CANDARW57323.2022.00036

Ⅱ．その他＜Others＞

1）Effects of starch content of calf starter on feed intake, growth performance, and fecal properties in dairy calves under a high plane of milk replacer feeding.
Satoh H, **Fukumori R**, Osada T, Shimada K, Oikawa S, Izumi K.
Anim Sci J. 94:e13911. 2023. doi: 10.1111/asj.13911

2）Death-and-culling rates of calves and associated economic losses in the first month of life on dairy farms in eastern Hokkaido, Japan.
Kayasaki S, Chisato K, **Fukumori R**, Oikawa S.
Prev Vet Med. 106072, 2023. doi: 10.1016/j.prevetmed.2023.106072.

3）Epidemiological features of postpartum subclinical ketosis in dairy herds in Hokkaido, Japan.
Chisato K, Yamazaki T, Kayasaki S, **Fukumori R**, Oikawa S.
Animals 14:144. 2023.　doi: 10.3390/ani14010144

Measurement of pH in the cow's rumen for high-quality dairy product

Raka Fukumori
Food and Environment Sciences
Rakunou Gakuen University
Hokkaido, Japan
fukumori@rakuno.ac.jp

Kaori Hara
Faculty of Economics
Chuo University
Tokyo, Japan
a20.d8fp@g.chuo-u.ac.jp

Yuko Hiramatsu
Faculty of Economics
Chuo University
Tokyo, Japan
susana_y@tamacc.chuo-u.ac.jp

Atsushi Ito
Faculty of Economics
Chuo University
Tokyo, Japan
0000-0002-9686-4019

Kento Arai
School of Agriculture
Utsunomiya University
Tochigi, Japan
mc216902@cc.utsunomiya-u.ac.jp

Shiori Ashibe
School of Agriculture
Utsunomiya University
Tochigi, Japan
ashibe@cc.utsunomiya-u.ac.jp

Yoshikazu Nagao
School of Agriculture
Utsunomiya University
Tochigi, Japan
ynagao@cc.utsunomiya-u.ac.jp

Akira Sasaki
GClue Inc.
Fukushima, Japan
akira@gclue.jp

Hirotsugu Yamamoto
Center for Optical Research and Education, Utsunomiya University
Tochigi, Japan
hirotsugu@yamamotolab.science

Yukitoshi Otani
Center for Optical Research and Education, Utsunomiya University
Tochigi, Japan
otani@cc.utsunomiya-u.ac.jp

Masaki Yasugi
Center for Optical Research and Education, Utsunomiya University
Tochigi, Japan
m_yasugi@yamamotolab.science

Yasutoshi Yoshiura
Faculty of Marine Science and Technology
Fukui Prefectural University
Fukui, Japan
yoshiur@fpu.ac.jp

***Abstract*—The consumption of dairy products per person is increasing, especially cheese and fresh cream. In particular, cheese consumption has been steadily increasing by 0.1 kg per person yearly. However, of the total 340,000 tons of cheese produced in Japan and imported by the country in 2018, domestic production was only 44,000 tons and had declined yearly. This situation is undesirable for maintaining the domestic dairy industry and food security. In addition, the government and local governments are currently promoting the sixth industrialization of the dairy industry. In order to meet this demand, it is necessary to increase the domestic production of dairy products. However, since competing with imported products on price is not viable, it is necessary to add value to dairy products. One of the measures to achieve this purpose is to increase the quality of dairy products through pasturage. Grazing allows cows to be managed in a more natural environment with less stress; thus, higher quality raw milk can be expected. Our research focuses on the health status of the cow's rumen, which is directly related to the health status of a cow. The health status of the cow's rumen often deteriorates due to overeating. However, we still do not know many things, such as whether there is a difference in pH immediately after eating. Therefore, we have developed a device that can transmit pH wirelessly from inside the cow's rumen.**

Keywords*—*high-quality dairy product, high quality milk, grazing, pH sensor, cow's rumen, sixth industrialization

I. INTRODUCTION

A. The current situation surrounding the dairy industry

The current situation surrounding the dairy industry is changing. The EU-Japan EPA [1] took effect on February 1, 2019, creating one of the largest free-trade zones in the world, encompassing 30% of global GDP and 40% of global trade. The agreement eliminated tariffs on many products. With this agreement, Japan could obtain lower prices for dairy products. However, this situation is against dairy farmers producing cheese in Japan. While cheese imports have increased after the EU-Japan EPA started, the amount of domestic cheese production has been declining [2]. Under these circumstances, Japanese dairy farmers must improve their production capacity and business conditions regardless of milk prices. The agricultural diversification of dairy products by dairy farmers is one of the solutions. In addition, it is urgent to strengthen the competitiveness and improve the quality of domestic cheese to compete with the increase in cheese imports from the EU.

The Ministry of Agriculture, Forestry, and Fisheries (MAFF) has set a policy goal of increasing the demand for domestic milk for cheese from 403,000 tons in 2019 to 490,000-550,000 tons in 2030 [3]. In addition, Japanese dairy farmers need to improve their business conditions. One solution is for dairy farmers to produce dairy products like cheese. By integrating the entire process from production to distribution and marketing, dairy farmers can set prices for their products, thus providing a more stable income. This paper focuses on raw milk, which largely

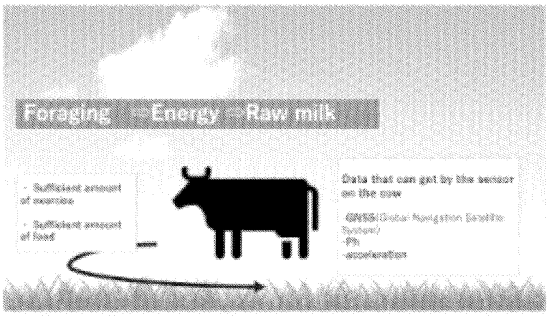


Fig. 1. The relation of cow's behavior and milk quality

The part of the research results was obtained from the commissioned research by the National Institute of Information and Communications Technology (NICT), JAPAN. Also, this research is supported by JSPS Kakenhi (17H02249,18K111849, 20H01278, 20H05702, 22K12598)

出典　doi: 10.1109/CANDARW57323.2022.00036

https://ieeexplore.ieee.org/document/10062703/metrics#metrics

最終責任者　Atsushi Ito (Corresponding Author)

Received: 15 April 2023 | Revised: 26 November 2023 | Accepted: 28 November 2023
DOI: 10.1111/asj.13911

RESEARCH ARTICLE

Animal Science Journal WILEY

Effects of starch content of calf starter on feed intake, growth performance, and fecal properties in dairy calves under a high plane of milk replacer feeding

Hitomi Satoh[1] | Rika Fukumori[1] | Takehiko Osada[2] | Kensuke Shimada[3] | Shin Oikawa[1] | Kenichi Izumi[2]

[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

[3]The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Tokyo, Japan

Correspondence
Kenichi Izumi, Department of Sustainable Agriculture, College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan.
Email: izmken@rakuno.ac.jp

Funding information
Ito Foundation, Grant/Award Number: 151

Abstract

The objective of this study was to investigate the effects of different starch contents in calf starter on the growth, feed intake, and fecal characteristics in dairy calves fed under a high plane of nutrition. Twenty-seven Holstein calves were assigned equally to one of three calf starter treatment groups in a randomized block design: high (H) starch (41.8%); medium (M) starch (31.9%); and low (L) starch (22.0%). Milk replacer was fed up to 1.2 kg/day as fed, gradually reduced from 6 weeks of age; calves were weaned at 8 weeks of age. Each starter (up to 3.5 kg/day as fed) and chopped hay were provided ad libitum from 5 days of age. Fecal samples, along with body measurements, were collected from 4 to 13 weeks of age. The average daily gain after weaning was greater in the H group than in the L group. The average starter intake for 3 days before weaning was not different among treatments but was greater after weaning in the H group than in the L group. Starter starch content did not affect the number of days with diarrhea or fecal starch concentration. The high-starch starter had no adverse effects during the weaning transition.

KEYWORDS
calf starter, growth performance, starch content, weaning transition

出典 doi: 10.1111/asj.13911
https://onlinelibrary.wiley.com/doi/full/10.1111/asj.13911
最終責任者 Kenichi Izumi (Corresponding Author)

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Death-and-culling rates of calves and associated economic losses in the first month of life on dairy farms in eastern Hokkaido, Japan

Shuji Kayasaki [a,b], Kyoko Chisato [b], Rika Fukumori [b], Shin Oikawa [b,*]

[a] Teshikaga Livestock Clinic, Kushiro Central Branch, East Regional Center, Hokkaido Agricultural Mutual Aid Association, 3-10-13 Sakuraoka, Teshikaga-cho, Kawakami-gun, Hokkaido 088-3213, Japan
[b] Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Dairy farm
Calf
Death and culling
Economic loss
Japan

ABSTRACT

The objective of this study was to analyze death and culling (DC) of calves during the first month of life and associated economic losses on dairy farms in eastern Hokkaido, Japan. The DC in the first month of life of 4411 Holstein and Wagyu crossbred calves born in the year 2019–2020 on 39 dairy farms milking Holsteins was investigated. Based on a target DC rate of 6.75%, farms were classified into two groups, those with high DC rates (HDC, 11.68%, n = 18) and those with low DC rates (LDC, 2.67%, n = 21), and analyzed for DC factors (breed, sex, parity of dams, and housing type of dams) and diseases causing DC, their loss estimates, and replenishment of DC calves (birth rate, purchase of heifers, and housing type of dams). Comparisons between groups were made using the Kaplan-Meier method, the Mann-Whitney U test, and chi-square test. The DC rate of Holsteins was significantly higher ($P < 0.001$) for HDC farms than for LDC ones. But, the DC rate of Wagyu crossbreds was not different between the groups, which suggested hybrid vigor. The DC rates for digestive diseases were significantly higher ($P < 0.01$) in the HDC farms, except for congenital diseases and deaths of unknown cause. The overall loss estimates of DC per calf-month for all farms was 8892, JPY/calf-months, 14,726 for HDC farms, and 4065 for LDC farms. The loss estimates of items with significant differences in DC rates were significantly higher ($P < 0.05$) in the HDC farms, with higher loss estimates for Holsteins and digestive diseases being the most common characteristics of HDC farms. In binomial logistic regression analysis with "HDC farms or not" as the response variable and replenishment of DC calves as the explanatory variable, HDC was significantly more likely (OR: 1.10, $P < 0.05$) on farms with a higher birth proportion of Holsteins, and HDC farms supplemented the DC calves by increasing the birth proportion of Holsteins.

1. Introduction

Calf deaths on dairy farms have been reported to be concentrated in the first month of life (Jenny et al., 1981; Agerholm et al., 1993; USDA, 1994; Svensson et al., 2006; Wathes et al., 2008; Azizzadeh et al., 2012; Raboisson et al., 2013; Urie et al., 2018; Santman-Berends et al., 2019). Similarly in eastern Hokkaido, most of the preweaning death and culling occurs during the first month of life, which is a major problem. Calf deaths can lead directly to overall farm losses because they result in the loss of successors on the farm. Examples of economic losses, including herds with high calf mortality having lower milk production (Losinger and Heinrichs, 1997; Torsein et al., 2014), more animals purchased (Torsein et al., 2011, 2014), higher on-farm mortality of cows and a higher incidence of antibiotic treatment (Torsein et al., 2014), have been reported. Based on these findings, formulas for economic losses associated with calf mortality have also been developed (Demir et al., 2019). Buying more animals, especially in large quantities, comes with many risks. For farms, introduced animals are a risk for pathogen invasion (Maunsell and Donovan, 2008; Mee et al., 2012), which may include antibiotic-resistant bacteria (Adhikari et al., 2009; Mee et al., 2012). Also, colostrum from newly introduced late gestation cows may not contain protective antibodies against herd-specific pathogens (Torsein et al., 2011). This increase in the proportion of at-risk purchased cows would increase the mortality of cows (Thomsen et al., 2006; Raboisson et al., 2011), creating a further vicious cycle for farms. In other words, it is safer from a biosecurity standpoint to raise home-bred individuals

* Correspondence to: 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan.
E-mail address: oishin@rakuno.ac.jp (S. Oikawa).

https://doi.org/10.1016/j.prevetmed.2023.106072
Received 10 April 2023; Received in revised form 24 October 2023; Accepted 5 November 2023
Available online 9 November 2023

出典　doi: 10.1016/j.preventmed.2023.106072
https://www.sciencedirect.com/science/article/pii/S1467298723000326
最終責任者　Shin Oikawa (Corresponding Author)

animals

MDPI

Article

Epidemiological Features of Postpartum Subclinical Ketosis in Dairy Herds in Hokkaido, Japan

Kyoko Chisato [1], Takerou Yamazaki [2], Shuji Kayasaki [3], Rika Fukumori [1] and Shin Oikawa [1,*]

[1] Veterinary Herd Health, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan; k-chisato@rakuno.ac.jp (K.C.); fukumori@rakuno.ac.jp (R.F.)

[2] Memanbetsu Livestock Clinic, Ozora Central Branch, Okhotsk Regional Center, Hokkaido Agricultural Mutual Aid Association, 149-10 Memanbetsu Syouwa, Ozora-cho, Abashiri-gun, Hokkaido 099-2356, Japan; takerou_yamazaki_gb@nosai-do.or.jp

[3] Teshikaga Livestock Clinic, Kushiro Central Branch, East Regional Center, Hokkaido Agricultural Mutual Aid Association, 3-10-13 Sakuraoka, Teshikaga-cho, Kawakami-gun, Hokkaido 088-3213, Japan; s22041006@stu.rakuno.ac.jp

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

Simple Summary: The aim of this study was to investigate the prevalence of subclinical ketosis (SCK) in Hokkaido, Japan, and to assess its characteristics epidemiologically at the individual and herd levels. Blood samples were taken from clinically healthy cows once within 3–88 days in milk (DIM) for blood tests. Cows with SCK were classified as SCK II within 2 weeks postpartum and SCK I from 15 DIM. The prevalence of SCK II (20.2%) tended to be higher than that of SCK I (16.5%, $p = 0.094$). The prevalence peaked around 2 weeks postpartum. The frequency of SCK I was higher at the fourth parity. The number of milking cows in herds with higher SCK (≥25%) was significantly smaller than in herds with lower SCK ($p = 0.004$). The frequency of herds with higher SCK in tie stalls with component feeding was higher than for those in free stalls and free barns fed a total mixed ration ($p = 0.054$ and $p = 0.002$). This study reveals the prevalence of SCK in Hokkaido, Japan, and shows that SCK is associated with parity and the management system.

check for updates

Citation: Chisato, K.; Yamazaki, T.; Kayasaki, S.; Fukumori, R.; Oikawa, S. Epidemiological Features of Postpartum Subclinical Ketosis in Dairy Herds in Hokkaido, Japan. *Animals* **2024**, *14*, 144. https://doi.org/10.3390/ani14010144

Academic Editors: Robert Kupczyński and Michał Bednarski

Received: 22 November 2023
Revised: 27 December 2023
Accepted: 28 December 2023
Published: 31 December 2023

Abstract: This study was carried out as an observational study in order to determine the prevalence of postpartum subclinical ketosis (SCK) in dairy herds in Hokkaido, Japan. From April 2012 to March 2014, blood β-hydroxybutyrate (BHBA) concentration was measured once within 3–88 days in milk (DIM) in 1394 apparently healthy cows from 108 farms to diagnose SCK (≥1.2 mM). In cows within 14 DIM, this was classified as SCK II, and from 15 DIM, this was classified as SCK I. Herds with a combined percentage of SCK I and SCK II of less than 10% were classified as SCK-negative herds, those with percentages of 10–25%, were classified as alert herds, and those with one of 25% or more, we classified as positive herds. The prevalence of SCK in the entire DIM was 17.6%. The prevalence of SCK II (20.2%) tended to occur more frequently than SCK I (16.5%, $p = 0.094$). The frequency of SCK I was higher at the fourth parity. The number of milking cows in SCK-positive herds was significantly smaller than those of the other two types of herds ($p = 0.004$). The frequency of SCK-positive herds in tie stalls and with component feeding was higher than for free stall or free barn and with total mixed ration ($p = 0.054$ and $p = 0.002$). This study reveals the prevalence of SCK in Hokkaido, Japan, and shows that SCK is associated with parity and the management system.

Keywords: dairy cow; subclinical ketosis; prevalence; risk factor; postpartum

1. Introduction

The three weeks before and after calving are called the "transition period" [1,2], during which dramatic changes occur in the cows. This means that the fetus grows significantly in the three weeks before calving, and after calving, more energy is required for milk synthesis.

出典 doi: 10.3990/ani14010144

https://www.mdpi.com/2076-2615/14/1/144

最終責任者 Shin Oikawa (Corresponding Author)

Satoshi Gondaira

Associate Professor

准教授　権平　智

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

1）Characteristics of *Mycoplasma bovis*, *Mycoplasma arginini*, and *Mycoplasma californicum* on immunological response of bovine synovial cells.
Nishi K, Okada J, Iwasaki T, **Gondaira S**, Higuchi H.
Vet Immunol Immunopathol. 260:110608. 2023
doi: 10.1016/j.vetimm.2023.110608.

Ⅱ．その他　＜Others＞

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Characteristics of *Mycoplasma bovis*, *Mycoplasma arginini*, and *Mycoplasma californicum* on immunological response of bovine synovial cells

Koji Nishi [a,b], Julia Okada [a], Tomohito Iwasaki [c], Satoshi Gondaira [a,*], Hidetoshi Higuchi [a,*]

[a] Animal Health Laboratory, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[b] Monbetsu Veterinary Clinic, Hokkaido Agricultural Mutual Aid Association, Monbetsu, Hokkaido, Japan
[c] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Hokkaido, Japan

ARTICLE INFO

Keywords:
Mycoplasma bovis
Arthritis
Inflammation
Pathogenicity

ABSTRACT

Mycoplasma arthritis in calves caused by *M. bovis* exhibits joint swelling, lameness, and immobility. In contrast to *M. bovis*, *M. arginini*, and *M. californicum* which were similarly isolated from the affected joints, only induced mild inflammation. The changes in pathogenesis that depended on species, however, remained unknown. This investigation aims to examine the characteristics of immune responses to *M. bovis*, *M. arginini*, and *M. californicum* in synovial cells. Intracellular *M. bovis* was detected by gentamicin assay, but *M. arginini* and *M. californicum* were not detected. *M. bovis*-infected synovial cells were encouraged to proliferate and had their apoptosis suppressed. We suggest that *M. bovis* invaded and inhibited apoptosis of synovial to evade host immunity, which led to long term survival in joints. *M. bovis* infection significantly increased IL-6 mRNA expression compared to control, although *M. arginini* and *M. californicum* infection were comparable to control. We suggest that *M. arginini* and *M. californicum* have low abilities to induce inflammation in joints and therefore do not cause severe pathology. Our findings are the first to show the variations in synovial cell immune responses to *M. bovis*, *M. arginini*, and *M. californicum*, which are thought to be related to the pathogenicity of arthritis.

1. Introduction

Eukaryotes are infected by mycoplasmas, Mollicutes class bacteria that lack a cell wall (Razin et al., 1998). Nineteen species of *Mycoplasma* are pathogenic to cattle, causing mastitis (Fox, 2012), pneumonia (Maunsell et al., 2011), otitis (Lamm et al., 2004), and arthritis (Desrochers and Francoz, 2014; Gagea et al., 2006; Houlihan et al., 2007) that incur substantial economic losses in beef and dairy farms. *Mycoplasma* arthritis in calves manifested as swollen joints, lameness, and immobility (Desrochers and Francoz, 2014). The most common species of *Mycoplasma bovis* (*M. bovis*) found in cattle with arthritis have severe pathology symptoms like synovial hyperplasia and cartilage destruction (Gagea et al., 2006; Mahmood et al., 2017). However, unlike *M. bovis*, *Mycoplasma arginini* (*M. arginini*) and *Mycoplasma californicum* (*M. californicum*) have also been isolated from joints (Deeney et al., 2021; Hewicker-Trautwein et al., 2002) present with mild to moderate inflammation.

M. bovis inhibit the apoptosis of mononuclear cells, whereas promotes the apoptosis of neutrophils (Jimbo et al., 2017; Malongo et al., 2014). Additionally, according to Bürki et al. (2015) and van der Merwe et al. (2010), *M. bovis* invade epithelial cells or peripheral blood mononuclear cells. These characteristics of *M. bovis* were thought to allow for prolonged infection and allow to evade host immunity. Joint tissues are constructed with synovial cells which play a crucial role in immune response (Bartok and Firestein, 2010). Our earlier research showed that *M. bovis* induce inflammatory cytokines such as interleukin (IL)－1β, IL-6, IL-8, IL-17, and tumor necrosis factor-α in bovine synovial tissues, which are linked to causing an inflammatory response in infected joints (Nishi et al., 2019). However, it is unclear how *M. arginini* and *M. californicum* affect the immunological reactions in joints. The purpose of this study is to determine how *M. bovis*, *M. arginini*, and *M. californicum* affected synovial cell proliferation, apoptosis, and the generation of inflammatory cytokines. Furthermore, it was discovered that the pathogenicity of *M. bovis*, *M. arginini*, and *M. californicum* varied depending on how each species invaded synovial cells.

Abbreviations: CFU, Colony-forming units; MOI, Multiplicity of infection; OD, Optical density; SEM, Scanning electron microscope.

* Corresponding authors.
E-mail addresses: s-gondaira@rakuno.ac.jp (S. Gondaira), higuchi@rakuno.ac.jp (H. Higuchi).

https://doi.org/10.1016/j.vetimm.2023.110608
Received 21 November 2022; Received in revised form 2 March 2023; Accepted 9 May 2023
Available online 10 May 2023

出典　doi: 1 10.1016/j.vetimm.2023.110608.
https://doi.org/10.1016/j.vetimm.2023.110608
最終責任者　Satoshi Gondaira and Hidetoshi Higuchi (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＞

Ⅱ．その他　＜Others＞

1）　PCR-based gene synthesis with overlapping unisense-oligomers asymmetric extension supported by a simulator for oligonucleotide extension achieved 1 kbp dsDNA.
Nishida Y, Kayama K, Endoh T, **Hanazono K**, Camer GA, Endoh D.
Biotechniques 74:317–332. 2023. doi: 10.2144/btn-2022-0127.

Reports

BioTechniques

PCR-based gene synthesis with overlapping unisense-oligomers asymmetric extension supported by a simulator for oligonucleotide extension achieved 1 kbp dsDNA

Yasunori Nishida[1], Kotetsu Kayama[1], Taichi Endoh[1], Kiwamu Hanazono[1], Gerry Amor Camer[1,2] & Daiji Endoh*,[1]

*[1]Department of Radiation Biology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 069-8501, Japan; [2]College of Veterinary Medicine, University of Eastern Philippines, Catarman, Northern Samar, 6400, Philippines; *Author for correspondence: dendoh@rakuno.ac.jp*

BioTechniques 74: 317–332 (June 2023) 10.2144/btn-2022-0127

First draft submitted: 30 December 2022; Accepted for publication: 30 May 2023; Published online: 21 June 2023

ABSTRACT

We formulated a method to synthesize 1 kbp DNA fragments using 'oligomer unidirectional joining method' via asymmetric extension supported by a simulator for oligonucleotide extension (AESOE). In this study, trials were conducted on 41 sets of different genomic pieces of ten flaviviral genomes, and 31 bacterial 16s rRNA fragments with sizes ranging from 500 bases to 1.0 kbp. Synthetic gene production was found to be successful in all those sets. The synthesis method has three steps: the first step is a seven-linked AESOE, the second step is the linking of the 400-base fragments from the first step, and the third step is the final amplification. Our present approach is highly reproducible and may no longer require optimization of oligomer design.

出典　doi: 10.2144/btn-2022-0127.

https://www.future-science.com/doi/epub/10.2144/btn-2022-0127

最終責任者　Daiji Endoh (Corresponding Author)

獣医臨床病理学 (Veterinary Clinicopathology)

Haruyuki Hirata

Associate Professor

准教授　平田　晴之

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

Ⅱ．その他　＜Others＞

1）Evaluation of blood serum iron concentration as an alternative biomarker for inflammation in dairy cows.
Murakami Y, Tsukano K, **Hirata H**, Suzuki K.
Biol Trace Elem Res. 201:4710–4717. 2023. doi: 10.1007/s12011-022-03544-5.

Biological Trace Element Research (2023) 201:4710–4717
https://doi.org/10.1007/s12011-022-03544-5

Evaluation of blood serum iron concentration as an alternative biomarker for inflammation in dairy cows

Yoshiki Murakami[1] · Kenji Tsukano[2] · Haruyuki Hirata[1] · Kazuyuki Suzuki[1]

Received: 25 July 2022 / Accepted: 22 December 2022 / Published online: 9 January 2023

Abstract
This study aimed to clarify the relationship between acute phase protein (APP) concentrations and serum Fe concentrations to determine whether serum iron (Fe) can be clinically applied as a substitute for APPs in cows. One hundred five Holstein–Friesian breed lactating dairy cows were enrolled in this study. Cows with inflammatory diseases were 16 subclinical, and 15 severe mastitis cows, in addition to 15 mild and 16 severe sole ulcer cows. The plasma haptoglobin (HPT), alpha-1 acid glycoprotein (AGP), SAA, serum Fe levels, and other biochemical parameters in the cows were measured. The two-sample *t*-tests and multiple logistic regression analysis were used to compare the control and inflammatory disease groups. ROC analysis was used to evaluate the ability to diagnose inflammation disease. From the results, the proposed diagnostic cutoff value for plasma SAA and serum Fe concentrations to identify dairy cows with inflammatory diseases based on analyses of ROC curves were set at > 3.65 mg/l and < 120.50 μg/dl, respectively. Therefore, instead of using expensive inflammatory markers to evaluate the inflammatory state at the first treatment day for inflammatory diseases in cow, it shows the useful for screening with serum Fe concentration that can be measured easily and inexpensively as alternative inflammatory biomarkers.

Keywords Acute phase proteins · Cows · Inflammation · Blood serum iron

出典 doi: 10.1007/s12011-022-03544-5.
https://link.springer.com/article/10.1007/s12011-022-03544-5
最終責任者 Kazuyuki Suzuki (Corresponding Author)

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）Use of a gum elastic bougie in a cat with severe upper airway stenosis.Kato K, **Itami T**, Torisu S, Sakai T, Yamashita K.*Open Vet J.* 13:114–118. doi: 10.5455/OVJ.2023.v13.i1.12.

Ⅱ．その他＜Others＞

1）The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits.
Wei Y, Chen IY, Tamogi H, Sugita C, Daimaruya N, Hirokawa T, Kato K, **Itami T**, Sano T, Yamashita K.
J Vet Med Sci. 85:471–478. 2023. doi: 10.1292/jvms.22-0484.

2）Sedative and cardiorespiratory effects of intranasal atomized alfaxalone in Japanese White rabbits.
Wei Y, Nakagawa M, Chen IY, **Itami T**, Sano T, Pasloske K, Yamashita K.
Vet Anaesth Analg. 50:255–262. 2023. doi: 10.1016/j.vaa.2023.02.001.

3）ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs.
Chen IY, Sugita C, Wei Y, Daimaruya N, **Itami T**, Sano T, Yamashita K.
Vet Anaesth Analg. 50:204–210. 2023. doi: 10.1016/j.vaa.2023.02.008.

4）Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs.
Chen IY, Sugita C, Wei Y, Daimaruya N, **Itami T**, Sano T, Yamashita K.
Vet Anaesth Analg. 50:485–491. 2023. doi: 10.1016/j.vaa.2023.08.002.

Open Veterinary Journal, (2023), Vol. 13(1): 114–118
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Case Report
DOI: *10.5455/OVJ.2023.v13.i1.12*

Submitted: 11/07/2022 Accepted: 05/12/2022 Published: 23/01/2023

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*

出典 doi: 10.5455/OVJ.2023.v13.i1.12.

https://www.openveterinaryjournal.com/?mno=56656

最終責任者 Takaharu Itami (Corresponding Author)

The Journal of Veterinary Medical Science

FULL PAPER
Surgery

The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits

Yixian WEI[1)], I-Ying CHEN[1)], Haruka TAMOGI[1)], Chihiro SUGITA[1)], Nozomi DAIMARUYA[1)], Taku HIROKAWA[1)], Keiko KATO[1)], Takaharu ITAMI[1)], Tadashi SANO[1)], Kazuto YAMASHITA[1)]*

[1)]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. To prevent aspiration in Japanese White (JW) rabbits, the maximum single volume of medetomidine administered intranasally is 0.3 mL per nostril using a mucosal atomization device (MAD). This study aimed to examine the sedative effect of intranasal administration of medetomidine using MAD in eight healthy female JW rabbits. Each rabbit received intranasal atomization (INA) of saline (Control treatment) along with three doses of 1 mg/mL medetomidine (0.3 mL to one nostril [MED0.3 treatment]; 0.3 mL each to both nostrils [MED0.6 treatment]; 0.3 mL twice to both nostrils [MED1.2 treatment]), with a washout period of at least 7 days between treatments. The actual doses of medetomidine were 82 (75–84) µg/kg (median (25th–75th percentile)), 163 (156–168) µg/kg, and 323 (295–343) µg/kg for the MED0.3, MED0.6, and MED1.2 treatments, respectively. A medetomidine-dose dependent sedative effect was detected, and the loss of righting reflex (LRR) was achieved in one rabbit at 18 min, seven rabbits at 11 (9–18) min, and eight rabbits at 7 (4–18) min after the MED0.3, MED0.6, and MED1.2 treatments, respectively. The LRR was maintained for 63 (29–71) min and 83 (68–101) min after the MED0.6 and MED1.2 treatments, respectively. Additionally, the INA of medetomidine produced a significant dose-dependent cardiorespiratory depression including a decrease in pulse rate, respiratory rate, percutaneous oxygen saturation, and arterial partial pressure of oxygen, and an increase in arterial partial pressure of carbon dioxide in the rabbits.

KEYWORDS: intranasal atomization, medetomidine, mucosal atomization device, rabbit

J. Vet. Med. Sci.
85(4): 471–478, 2023
doi: 10.1292/jvms.22-0484

Received: 25 October 2022
Accepted: 22 February 2023
Advanced Epub:
6 March 2023

*Correspondence to: Yamashita K: yamasita@rakuno.ac.jp, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

出典　doi: 10.1292/jvms.22-0484.

https://www.jstage.jst.go.jp/article/jvms/85/4/85_22-0484/_pdf/-char/en

最終責任者　Kazuto Yamashita (Corresponding Author)

Veterinary Anaesthesia and Analgesia 2023, **50**, 255–262 https://doi.org/10.1016/j.vaa.2023.02.001

RESEARCH PAPER

Sedative and cardiorespiratory effects of intranasal atomized alfaxalone in Japanese White rabbits

Yixian Wei[a], **Mari Nakagawa**[a], **I-Ying Chen**[a], **Takaharu Itami**[a], **Tadashi Sano**[a], **Kirby Pasloske**[b] & **Kazuto Yamashita**[a]

[a]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[b]Jurox Pty Ltd, Rutherford, NSW, Australia

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasita@rakuno.ac.jp

Abstract

Objective To investigate the sedative and cardiorespiratory effects of intranasal atomization (INA) of alfaxalone using a mucosal atomization device in Japanese White rabbits.

Study design Randomized, prospective, crossover study.

Animals A total of eight healthy female rabbits, weighing 3.6–4.3 kg and aged 12–24 months.

Methods Each rabbit was randomly assigned to four INA treatments administered 7 days apart: Control treatment, 0.15 mL 0.9% saline in both nostrils; treatment INA0.3, 0.15 mL 4% alfaxalone in both nostrils; treatment INA0.6, 0.3 mL 4% alfaxalone in both nostrils; treatment INA0.9, 0.3 mL 4% alfaxalone in left, then right, then left nostril. Sedation was scored 0–13 using a composite measure scoring system for rabbits. Simultaneously, pulse rate (PR), respiratory rate (f_R), noninvasive mean arterial pressure (MAP), peripheral hemoglobin oxygen saturation (SpO_2) and arterial blood gases were measured until 120 minutes. The rabbits breathed room air during the experiment and were administered flow-by oxygen when hypoxemia (SpO_2 <90% or PaO_2 <60 mmHg; 8.0 kPa) developed. Data were analyzed using the Fisher's exact test and the Friedman test ($p < 0.05$).

Results No rabbit was sedated in treatments Control and INA0.3. All rabbits in treatment INA0.9 developed loss of righting reflex for 15 (10–20) minutes [median (25th–75th percentile)]. Sedation score significantly increased from 5 to 30 minutes in treatments INA0.6 and INA0.9 with maximum scores of 2 (1–4) and 9 (9–9), respectively. f_R decreased in an alfaxalone dose-dependent manner and one rabbit developed hypoxemia in treatment INA0.9. No significant changes were observed in PR and MAP.

Conclusions and clinical relevance INA alfaxalone resulted in dose-dependent sedation and respiratory depression in Japanese White rabbits to values considered not clinically relevant. Further investigation of INA alfaxalone in combination with other drugs is warranted.

Keywords alfaxalone, intranasal mucosal atomization, rabbits, sedative effects.

Introduction

Over the past decade, interest in intranasal drug delivery has been garnering increased attention in both human and veterinary medicine. The theory behind intranasal drug absorption is that the intranasal cavity is lined by highly vascularized mucosa facilitating rapid drug absorption, and the olfactory epithelium provides direct access to the central nervous system (CNS) by bypassing the blood–brain barrier (Grassin-Delyle et al. 2012; Keller et al. 2021; Mignani et al. 2021). Drugs absorbed through the trigeminal and olfactory nerve pathways from the nasal cavity are directly delivered to the brain. Thus intranasal administration is a promising alternative route to oral, intravenous and intramuscular administration of drugs that bypasses the blood–brain barrier (Erdő et al. 2018).

In rabbits, a total volume of 0.3–0.64 mL kg^{-1} of solution containing sedative and anesthetic drugs has been administered intranasally through the nostrils using a catheter-tipped syringe to produce sedation (Santangelo et al. 2016; Weiland et al. 2017; Yanmaz et al. 2022). An intranasal combination of dexmedetomidine, midazolam and butorphanol resulted in deep sedation with moderate hypoxemia and hypercarbia in New Zealand White (NZW) rabbits (Santangelo et al. 2016). In a different study, intranasal combination of dexmedetomidine and midazolam resulted in rapid sedation in NZW rabbits;

出典 doi: 10.1016/j.vaa.2023.02.008.

https://www.sciencedirect.com/science/article/pii/S1467298723000326?via%3Dihub

最終責任者 Kazuto Yamashita (Corresponding Author)

RESEARCH PAPER

ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs

I-Ying Chen, Chihiro Sugita, Yixian Wei, Nozomi Daimaruya, Takaharu Itami, Tadashi Sano & Kazuto Yamashita

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

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasita@rakuno.ac.jp

Abstract

Objective To determine the median effective dose (ED_{50}) and effective dose required to depress the twitch value by 95% (ED_{95}) of rocuronium during alfaxalone anesthesia in dogs.

Study design A randomized, prospective, crossover experimental study.

Animals A total of eight adult Beagle dogs (four female, four male), weighing 10.3–14.6 kg and aged 6–8 years.

Methods The dogs were anesthetized three times with 1.25-fold the individual minimum infusion rate of alfaxalone at intervals of ≥ 14 days. Neuromuscular function was monitored with train-of-four (TOF) stimulation of the peroneal nerve by acceleromyography. After recording the control TOF ratio (TOFRC) and first twitch of TOF (T1C), a single bolus dose of rocuronium 100, 175 or 250 μg kg^{-1} (treatments R100, R175 or R250) was administered intravenously. The maximum suppression of the first twitch of TOF (T1) was recorded and calibrated with T1C to construct the dose–response curve, from which ED_{50} and ED_{95} were calculated. Time from rocuronium administration to TOF ratio/TOFRC > 0.9 (duration TOFR0.9) was recorded.

Results ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia were 175 and 232 μg kg^{-1}, respectively. The median (range) duration TOFR0.9 was longer in treatment R250 [10.1 (9.2–10.9) minutes] than in treatments R100 [3.1 (2.9–4.4) minutes; $p < 0.0001$] and R175 [7.7 (6.9–8.1) minutes; $p < 0.0001$]; and longer in treatment R175 than in treatment R100 ($p < 0.0001$).

Conclusions and clinical relevance The duration of TOFR0.9 correlated positively with the dosage of rocuronium, indicating that recovery time of rocuronium was also dose-dependent in dogs anesthetized with alfaxalone. The duration TOFR0.9 of rocuronium 250 μg kg^{-1} was 10 minutes during alfaxalone anesthesia in dogs.

Keywords administration and dosage, alfaxalone, dose–response relationship, drug, prospective studies, veterinary.

Introduction

Rocuronium bromide is an intermediate-acting non-depolarizing neuromuscular blocking agent (NMBA) in dogs (Marshall et al. 1994; Dugdale et al. 2002). As rocuronium does not produce active metabolites in human plasma (Appiah-Ankam & Hunter 2004), it is now widely used as an intravenous (IV) bolus, for incremental IV doses, and for constant rate infusion in anesthetized dogs (Dugdale et al. 2002; Alderson et al. 2007; Auer et al. 2007; Briganti et al. 2015; Haga et al. 2019). Nevertheless, NMBAs do not have an anesthetic effect and, therefore, should be administered during general anesthesia (Kastrup et al. 2005).

Alfaxalone is a synthetic neuroactive steroid anesthetic agent that is used for induction and maintenance of anesthesia in veterinary practice (Quirós-Carmona et al. 2017; Bennell et al. 2019; Dehuisser et al. 2019; Bustamante et al. 2020). The single-dose rocuronium-induced neuromuscular blockade was longer in alfaxalone than propofol anesthesia in dogs (Chen et al. 2022). However, information to guide veterinary physicians in clinical practice regarding the effective doses of rocuronium with alfaxalone is still insufficient.

The potency of NMBAs can be evaluated by constructing dose–response curves from a linear least squares regression analysis model using the single-dose method (Fuchs-Buder et al. 2007). In this method, the degree of neuromuscular block is measured following administration of different doses of NMBA to determine the median effective dose 50 (ED_{50}) and

出典 doi: 10.1016/j.vaa.2023.02.008.

https://www.sciencedirect.com/science/article/pii/S1467298723000326

最終責任者 Kazuto Yamashita (Corresponding Author)

Veterinary Anaesthesia and Analgesia 2023, **50**, 485–491 https://doi.org/10.1016/j.vaa.2023.08.002

RESEARCH PAPER

Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs

I-Ying Chen, Chihiro Sugita, Yixian Wei, Nozomi Daimaruya, Takaharu Itami, Tadashi Sano & Kazuto Yamashita

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

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamashin@rakuno.ac.jp

Abstract

Objective To investigate the reversal effect of sugammadex on neuromuscular blockade induced by a single bolus of rocuronium in dogs under alfaxalone anesthesia.

Study design Randomized, prospective, crossover experimental study.

Animals A group of six adult Beagle dogs (three females and three males), weighing 11.3–15.8 kg and aged 6–8 years, were used.

Methods Dogs were anesthetized twice with a 1.25 times minimum infusion rate of alfaxalone, with a washout period of at least 14 days between experiments. Neuromuscular function was monitored using acceleromyography with train-of-four (TOF) stimulation of the peroneal nerve. After recording the control TOF ratio (TOFRC), rocuronium (0.5 mg kg^{-1}) was administered intravenously. Subsequently, sugammadex (4 mg kg^{-1}) or an equal volume of saline (control treatment) was administered intravenously when the TOF count returned from 0 to 1 after neuromuscular blockade. Time from rocuronium injection to TOF count = 0 (onset time), time from TOF count = 0 to TOF count = 1 (maximum blockade period), time of first twitch amplitude recovery from 0.25 to 0.75 (recovery index), and time from sugammadex or saline administration to TOF ratio/TOFRC $\geq$ 0.9 (recovery time) were recorded.

Results The onset time and maximum blockade duration did not differ between sugammadex treatment [1.2 (0.7–1.5) minutes and 9.9 (6.3–10.5) minutes, respectively] and control treatment [median (range); 1.0 (0.7–1.1) minutes and 9.9 (8.8–11.5) minutes, respectively] ($p = 0.219$ and 0.844, respectively). Recovery index was 0.5 (0.3–0.7) minutes in sugammadex treatment, which was shorter than that in control treatment [4.5 (3.7–4.9) minutes] ($p = 0.031$). Recovery time was 0.8 (0.5–2.8) minutes in sugammadex treatment, which was shorter than that in control treatment [10.5 (6.8–14.3) minutes] ($p = 0.031$).

Conclusions and clinical relevance Rocuronium-induced neuromuscular blockade was effectively reversed by sugammadex in dogs anesthetized with alfaxalone.

Keywords alfaxalone, dogs, neuromuscular blockade, rocuronium, sugammadex.

Introduction

Sugammadex, a gamma-cyclodextrin, selectively reverses rocuronium-induced neuromuscular blockade via chemical encapsulation (Bom et al. 2002; de Boer et al. 2006a,b). The structure of sugammadex comprises a lipophilic internal cavity that encapsulates aminosteroid neuromuscular-blocking molecules to form an inactive complex, which is then excreted unchanged via the kidneys, thus reversing the neuromuscular blockade (Bom et al. 2002; Peeters et al. 2011). The reversal effect of sugammadex on rocuronium-induced neuromuscular blockade has been evaluated in dogs under isoflurane anesthesia (Mosing et al. 2012). Compared with acetylcholinesterase inhibitors, sugammadex does not have cardiovascular side effects and can reverse profound neuromuscular block (Sacan et al. 2007). However, encapsulation of other steroidal drugs or endogenous steroids may occur with sugammadex administration (Hunter & Flockton 2006).

Alfaxalone, a synthetic neuroactive steroid anesthetic agent, is used to induce and maintain anesthesia in veterinary practice (Bennett et al. 2019). An alfaxalone formulation licensed for clinical use in dogs and cats uses 2-hydroxypropyl-beta cyclodextrin as a solubilizing excipient (Ferré et al. 2006). There is a possibility that alfaxalone, as a synthetic neuroactive

出典 doi: 10.1016/j.vaa.2023.08.002.
https://www.sciencedirect.com/science/article/pii/S1467298723001186

最終責任者 Kazuto Yamashita (Corresponding Author)

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

Hiroshi Ohta

Associate Professor

准教授　大田　寛

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

1）Detecting antegrade urine flow using detective flow imaging in a cat with chronic kidney disease.
Tamura M, **Ohta H**.
J Small Anim Pract. 2023 Oct 31(Online ahead of print.).
doi: 10.1111/jsap.13684.

Ⅱ．その他＜Others＞

1）Influence of the hypoxia-activated prodrug evofosfamide (TH-302) on glycolytic metabolism of canine glioma; prospec-tive improvement in cancer metabolism.
Yamazaki H, Onoyama S, Gotani S, Deguchi T, Tamura M, **Ohta H**, Iwano H, NishidaH, Dickinson PJ, Akiyoshi H.
Cancers 15:5537. 2023. doi: 10.3390/cancers15235537.

2）Diverse genome-wide DNA methylation alterations in canine hepatocellular tumors.
Asari Y, Yamazaki J, Thandar O, Suzuki T, Aoshima K, Takeuchi K, Kinoshita R, Kim S, Hosoya K, Ishizaki T, Kagawa Y, Jelinek J, Yokoyama S, Sasaki N, **Ohta H**, Nakamura K, Takiguchi M.
Vet Med Sci. 9:2006–2014. 2023. doi: 10.1002/vms3.1204.

3）An inflammatory bowel disease-associated SNP increases local thyroglobulin expression to develop inflammation in miniature dachshunds.
Teoh YB, Jiang JJ, Yamasaki T, Nagata N, Sugawara T, Hasebe R, **Ohta H**, Sasaki N, Yokoyama N, Nakamura K, Kagawa Y, Takiguchi M, Murakami M.
Front Vet Sci. 10:1192888. 2023. doi: 10.3389/fvets.2023.1192888.

4）Successful treatment of sclerosing encapsulating peritonitis in a cat using bioresorbable hyaluronate-carboxymethylcellulose membrane after surgical adhesiolysis and long-term prednisolone.

Yokoyama N, Kinoshita R, **Ohta H**, Shimbo G, Sasaoka K, Nagata N, Sasaki N, Morishita K, Nakamura K, Kagawa Y, Takiguchi M.
JFMS Open Rep. 9:20551169231209917. 2023.
doi: 10.1177/20551169231209917.

5) Investigation of the therapeutic effects, predictors, and complications of long-term immunosuppressive therapy in dogs with precursor-targeted immune-mediated anemia.
Sugawara-Suda M, Morishita K, Iwanaga Y, Yamazaki J, Kagawa Y, Yokoyama N, Sasaki N, **Ohta H**, Nakamura K, Takiguchi M.
J Vet Med Sci. 85:695–701. 2023. doi: 10.1292/jvms.23-0010.

6) Effectiveness of 2-dimensional shear wave elastography for noninvasive and reliable estimation of right atrial pressure in dogs with induced volume overload.
Tamura M, **Ohta H**, Osuga T, Takiguchi M.
J Vet Intern Med. 37:866–874. 2023. doi: 10.1111/jvim.16705.

7) Evaluation of responses to immunosuppressive therapy in dogs with suspected non-regenerative immune-mediated anaemia: 11 cases (2012-2018).
Morishita K, Sugawara-Suda M, Yamazaki J, Sasaki N, Nakamura K, **Ohta H**, Takiguchi M.
J Small Anim Pract. 64:527–534. 2023. doi: 10.1111/jsap.13614.

8) Zoobiquity experiments show the importance of the local MMP9-plasminogen axis in inflammatory bowel diseases in both dogs and patients.
Yamasaki T, Nagata N, Atsumi T, Hasebe R, Tanaka Y, Ohki I, Kubota S, Shinohara Y, Teoh YB, Yokoyama N, Sasaki N, Nakamura K, **Ohta H**, Katsurada T, Matsuno Y, Hojyo S, Hashimoto S, Takiguchi M, Murakami M.
Int Immunol. 35:313–326. 2023. doi: 10.1093/intimm/dxad006.

9) Safety and clinical efficacy of an anti-PD-L1 antibody (c4G12) in dogs with advanced malignant tumors.
Maekawa N, Konnai S, Hosoya K, Kim S, Kinoshita R, Deguchi T, Owaki R, Tachibana Y, Yokokawa M, Takeuchi H, Kagawa Y, Takagi S, **Ohta H**, Kato Y, Yamamoto S, Yamamoto K, Suzuki Y, Okagawa T, Murata S, Ohashi K.
PLoS One. 18:e0291727. 2023. doi: 10.1371/journal.pone.0291727.

10) Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors.Maekawa N, Konnai S, Asano Y, Otsuka T, Aoki E, Takeuchi H, Kato Y, Kaneko MK, Yamada S, Kagawa Y, Nishimura M, Takagi S, Deguchi T, **Ohta H**, Nakagawa T, Suzuki Y, Okagawa T, Murata S, Ohashi K.
PLoS One. 18:e0281143. 2023. doi:10.1371/journal.pone.0281143.

Detecting antegrade urine flow using detective flow imaging in a cat with chronic kidney disease

Detective flow imaging represents an innovative advancement in ultrasound Doppler technology, enabling the visualisation of low-velocity flow without contrast. A 14-year-old neutered cat was referred for a screening examination for chronic kidney disease (CKD), which had been diagnosed over 2 months prior. Blood tests revealed elevated levels of blood urea nitrogen (19.64 mmol/L; reference interval, 6.28 to 11.71 mmol/L) and creatinine (398.7 μmol/L; reference interval, 79.6 to 185.6 μmol/L), as well as hypokalaemia (3.1 mmol/L; reference interval, 3.4 to 4.6 mmol/L). Packed cell volume was within normal limits. Urine analysis indicated a decreased urine specific gravity of 1.009, but no other significant abnormalities were noted. The cat was diagnosed with IRIS CKD stage 3. Radiography showed that the left kidney was small, measuring approximately 1.2 times the length of the second lumber vertebra, but the right kidney was normal limits (2.4 times the length of the left kidney). Abdominal ultrasonographic examination (ARIETTA 850; FUJIFILM Medical Co., Ltd., Tokyo, Japan) revealed a small left kidney length measuring 2.1 cm with a mild irregular shape (Fig 1A) and a normal-sized right kidney measuring 4.2 cm. Both kidneys exhibited poor renal architecture. The ultrasound findings were consistent with clinically diagnosed chronic degenerative nephropathy. Detective flow imaging was performed at the level of the ureterovesicular junctions to evaluate the urine production in the small left kidney using an 18 to 5-MHz liner probe (L64; FUJIFILM Medical). The ureteral jet phenomenon was observed as antegrade urine flow from the ureterovesicular junction of the left ureter (Fig 1B) as well as that of the right ureter (Fig 1C). This phenomenon occurred at intervals of once every 10 to 30 s. While not a measure of renal function, detective flow imaging may be used for detection of ureteral jets to confirm urine production and ureteral patency in kidneys with ultrasound findings indicative of chronic degenerative nephropathy.

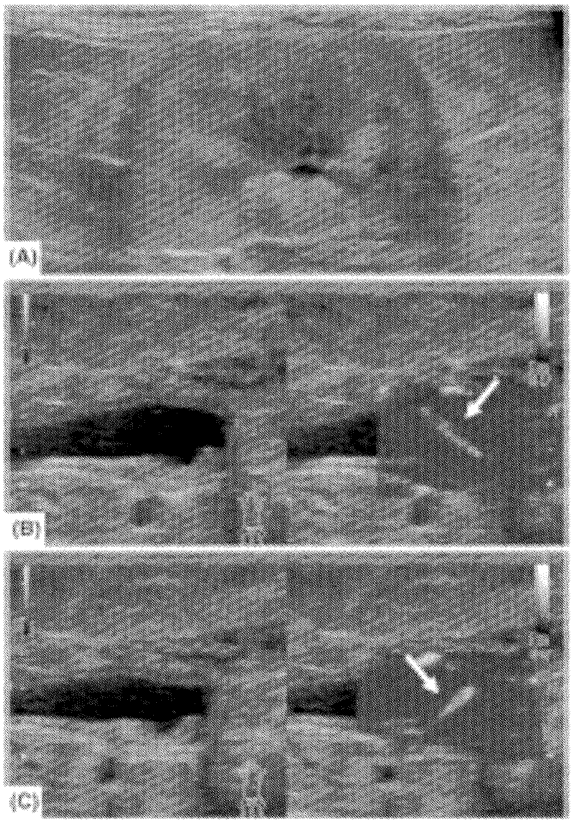


FIG 1. Ultrasonography was used to detect the small size, mild irregular shape and poor renal architecture of the left kidney (A). Detective flow imaging revealed the ureteral jet phenomenon from the ureterovesicular junction of the left ureter (B: white arrow) as well as that of the right ureter (C: white arrow)

M. Tamura and H. Ohta[1]

Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University Ebetsu, Hokkaido 069-8501, Japan

[1]Corresponding author email: h-ohta@rakuno.ac.jp

出典 doi: 10.1111/jsap.13684.

https://onlinelibrary.wiley.com/doi/10.1111/jsap.13684

最終責任者 Hiroshi Ohta (Corresponding Author)

cancers

MDPI

Article

Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism

Hiroki Yamazaki [1,*], Seio Onoyama [1], Shunichi Gotani [1], Tatsuya Deguchi [1], Masahiro Tamura [1], Hiroshi Ohta [1], Hidetomo Iwano [2], Hidetaka Nishida [3], Peter J. Dickinson [4] and Hideo Akiyoshi [5]

[1] Laboratory of Veterinary Internal Medicine, Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu 069-0836, Japan; t-deguchi@rakuno.ac.jp (T.D.); m-tamura@rakuno.ac.jp (M.T.); h-ohta@rakuno.ac.jp (H.O.)

[2] Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu 069-0836, Japan; h-iwano@rakuno.ac.jp

[3] Laboratory of Small Animal Clinics, Veterinary Teaching Hospital, Graduate School of Veterinary Science, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara 52-5201, Japan; h-nishida@azabu-u.ac.jp

[4] Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA; pjdickinson@ucdavis.edu

[5] Laboratory of Veterinary Surgery, Graduate School of Life and Environmental Sciences, Osaka Metropolitan University, 1-58 Rinku-Orai-kita, Izumisano 598-8531, Japan

* Correspondence: author. hyamazaki@rakuno.ac.jp; Tel.: +81-011-388-4767

Simple Summary: This study investigated the anti-glycolytic effects of evofosfamide (EVO) on three canine glioma (GL)-derived cell lines with activated hypoxia-inducible factor 1α (HIF-1α). Our clinical data showed that glycolytic activity was correlated with poorer outcomes in dogs with spontaneous GL. Our in vitro studies showed that EVO inhibited glycolytic metabolism by targeting HIF-1α-positive cells under hypoxic culture conditions, resulting in the suppression of cellular ATP production. Our in vivo studies showed that EVO significantly decreased tumor development compared to controls or temozolomide in orthotopic murine GL models. A metabolic analysis demonstrated that EVO suppressed glycolytic activity by eliminating HIF-1α-positive cells. Our findings suggest that EVO may improve cancer metabolism and restore the microenvironment for both canine and human GL.

Abstract: The transcription factor hypoxia-inducible factor 1α (HIF-1α) drives metabolic reprogramming in gliomas (GLs) under hypoxic conditions, promoting glycolysis for tumor development. Evofosfamide (EVO) releases a DNA-alkylating agent within hypoxic regions, indicating that it may serve as a hypoxia-targeted therapy. The aim of this study was to investigate the glycolytic metabolism and antitumor effects of EVO in a canine GL model. Our clinical data showed that overall survival was significantly decreased in GL dog patients with higher HIF-1α expression compared to that of those with lower HIF-1α expression, and there was a positive correlation between HIF-1α and pyruvate dehydrogenase kinase 1 (PDK1) expression, suggesting that glycolytic activity under hypoxia conditions may contribute to poor outcomes in canine GL. Our glycolysis assay tests showed that the glycolytic ATP level was higher than the mitochondrial ATP level in three types of canine GL cell lines by activating the HIF-1 signal pathway under hypoxia conditions, resulting in an overall increase in total cellular ATP production. However, treatment with EVO inhibited the glycolytic ATP level in the GL cell lines under hypoxia conditions by targeting HIF-1α-positive cells, leading to decrease in total cellular ATP production. Our in vivo tests showed that EVO significantly reduced tumor development compared to controls and temozolomide in murine GL models. A metabolic analysis demonstrated that EVO effectively suppressed glycolytic metabolism by eliminating HIF-1α-positive cells, suggesting that it may restore metabolism in canine GLs. The evidence presented here supports the favorable preclinical evaluation of EVO as a potential improvement in cancer metabolism.

check for updates

Citation: Yamazaki, H.; Onoyama, S.; Gotani, S.; Deguchi, T.; Tamura, M.; Ohta, H.; Iwano, H.; Nishida, H.; Dickinson, P.J.; Akiyoshi, H. Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism. Cancers 2023, 15, 5537. https://doi.org/10.3390/cancers15235537

Academic Editors: Panagiotis Lisboa and Ilias Mylonis

Received: 18 September 2023
Revised: 8 November 2023
Accepted: 16 November 2023
Published: 22 November 2023

出典 doi: 10.3390/cancers15235537.

https://www.mdpi.com/2072-6694/15/23/5537

最終責任者 Hiroki Yamazaki (Corresponding Author)

DOI: 10.1002/vms3.1204

ORIGINAL ARTICLE

WILEY

Diverse genome-wide DNA methylation alterations in canine hepatocellular tumours

Yu Asari[1] | Jumpei Yamazaki[2,3,4] | Oo Thandar[1] | Tamami Suzuki[5] | Keisuke Aoshima[4,5] | Kyosuke Takeuchi[2] | Ryohei Kinoshita[2,4] | Sangho Kim[4,6] | Kenji Hosoya[4,6] | Teita Ishizaki[2,5,7] | Yumiko Kagawa[7] | Jaroslav Jelinek[8] | Shoko Yokoyama[2,3,4] | Noboru Sasaki[1] | Hiroshi Ohta[1] | Kensuke Nakamura[1] | Mitsuyoshi Takiguchi[1]

[1]Laboratory of Veterinary Internal Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

[2]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

[3]Translational Research Unit, Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

[4]One Health Research Center, Cancer Research Unit, Hokkaido University, Sapporo, Japan

[5]Laboratory of Comparative Pathology, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

[6]Laboratory of Veterinary Surgery, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

[7]North Lab, Sapporo, Japan

[8]Coriell Institute for Medical Research, Camden, New Jersey, USA

Correspondence
Jumpei Yamazaki, Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.
Email: j.yamazaki@vetmed.hokudai.ac.jp

Mitsuyoshi Takiguchi, Laboratory of Veterinary Internal Medicine, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan.
Email: m_taki@vetmed.hokudai.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Numbers: 18K05964, 21H02351, 20H03139, 23H02374

Abstract

Background: Canine hepatocellular tumours (HCTs) are common primary liver tumours. However, the exact mechanisms of tumourigenesis remain unclear. Although some genetic mutations have been reported, DNA methylation alterations in canine HCT have not been well studied.

Objectives: In this study, we aimed to analyse the DNA methylation status of canine HCT.

Methods: Tissues from 33 hepatocellular carcinomas, 3 hepatocellular adenomas, 1 nodular hyperplasia, 21 non-tumour livers from the patients and normal livers from 5 healthy dogs were used. We analysed the DNA methylation levels of 72,367 cytosine–guanine dinucleotides (CpG sites) in all 63 samples.

livers varied among tumour cases, suggesting various DNA methylation patterns in different tumour groups. However, patient and clinical parameters, such as age, were not associated with DNA methylation status. In conclusion, we found that HCTs undergo aberrant and diverse patterns of genome-wide DNA methylation compared with normal liver tissue, suggesting a complex epigenetic mechanism in canine HCT.

KEYWORDS
canine hepatocellular carcinoma, DNA methylation, epigenetics

出典　doi: 10.1002/vms3.1204.
https://onlinelibrary.wiley.com/doi/10.1002/vms3.1204

最終責任者　Jumpei Yamazaki and Mitsuyoshi Takiguchi (Corresponding Author)

frontiers | Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 14 July 2023
DOI 10.3389/fvets.2023.1192888

OPEN ACCESS

EDITED BY
Ibo Ozaydin,
Kafkas University, Türkiye

REVIEWED BY
Hirotaka Igarashi,
Azabu University, Japan
Michelle Wai Cheng Fong,
Putra Malaysia University, Malaysia

*CORRESPONDENCE
Masaaki Murakami
murakami@igm.hokudai.ac.jp
Mitsuyoshi Takiguchi
mtaki@vetmed.hokudai.ac.jp

†These authors have contributed equally to this work

RECEIVED 24 March 2023
ACCEPTED 21 June 2023
PUBLISHED 14 July 2023

CITATION
Teoh YB, Jiang J-J, Yamasaki T, Nagata N, Sugawara T, Hasebe R, Ohta H, Sasaki N, Yokoyama N, Nakamura K, Kagawa Y, Takiguchi M and Murakami M (2023) An inflammatory bowel disease-associated SNP increases local thyroglobulin expression to develop inflammation in miniature dachshunds. *Front. Vet. Sci.* 10:1192888. doi: 10.3389/fvets.2023.1192888

An inflammatory bowel disease-associated SNP increases local thyroglobulin expression to develop inflammation in miniature dachshunds

Yong Bin Teoh[1,2†], Jing-Jing Jiang[1†], Takeshi Yamasaki[1,3†], Noriyuki Nagata[1,4], Toshiki Sugawara[1], Rie Hasebe[1,3], Hiroshi Ohta[5], Noboru Sasaki[4], Nozomu Yokoyama[2], Kensuke Nakamura[2], Yumiko Kagawa[6], Mitsuyoshi Takiguchi[2]* and Masaaki Murakami[1,3,7,8]*

[1]Division of Molecular Psychoneuroimmunology, Institute for Genetic Medicine, Graduate School of Medicine, Hokkaido University, Sapporo, Japan, [2]Laboratory of Veterinary Internal Medicine, Department of Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [3]Division of Molecular Neuroimmunology, Department of Homeostatic Regulation, National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan, [4]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [5]Laboratory of Veterinary Internal Medicine, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, [6]North Lab, Sapporo, Japan, [7]Group of Quantum Immunology, Institute for Quantum Life Science, National Institute for Quantum and Radiological Science and Technology (QST), Chiba, Japan, [8]Institute for Vaccine Research and Development (HU-MIaID), Hokkaido University, Sapporo, Japan

Inflammatory colorectal polyp (ICRP) in miniature dachshunds (MDs) is a chronic inflammatory bowel disease (IBD) characterized by granulomatous inflammation that consists of neutrophil infiltration and goblet cell hyperplasia in the colon. Recently, we identified five MD-associated single-nucleotide polymorphisms (SNPs), namely *PLG*, *TCOF1*, *TG*, *COL9A2*, and *COL4A4*, by whole-exome sequencing. Here, we investigated whether *TG* c.4567C>T (p.R1523W) is associated with the ICRP pathology. We found that the frequency of the T/T SNP risk allele was significantly increased in MDs with ICRP. *In vitro* experiments showed that TG expression in non-immune cells was increased by inducing the IL-6 amplifier with IL-6 and TNF-α. On the other hand, a deficiency of *TG* suppressed the IL-6 amplifier. Moreover, recombinant TG treatment enhanced the activation of the IL-6 amplifier, suggesting that TG is both a positive regulator and a target of the IL-6 amplifier. We also found that *TG* expression together with two NF-κB targets, *IL6* and *CCL2*, was increased in colon samples isolated from MDs with the T/T risk allele compared to those with the C/C non-risk allele, but serum TG was not increased. Cumulatively, these results suggest that the T/T SNP is an expression quantitative trait locus (eQTL) of *TG* mRNA in the colon, and local *TG* expression triggered by this SNP increases the risk of ICRP in MDs via the IL-6 amplifier. Therefore, *TG* c.4567C>T is a diagnostic target for ICRP in MDs, and *TG*-mediated IL-6 amplifier activation in the colon is a possible therapeutic target for ICRP.

出典 doi: 10.3389/fvets.2023.1192888.

https://www.frontiersin.org/articles/10.3389/fvets.2023.1192888/full

最終責任者 Mitsuyoshi Takiguchi and Masaaki Murakami (Corresponding Author)

Case Report

JFMS OPEN REPORTS

Journal of Feline Medicine and Surgery Open Reports
1–7

DOI: 10.1177/20551169231209917
journals.sagepub.com/home/jfmsopenreports

This paper was handled and processed by the European Editorial Office (ISFM) for publication in JFMS Open Reports

Sage

Successful treatment of sclerosing encapsulating peritonitis in a cat using bioresorbable hyaluronate-carboxymethylcellulose membrane after surgical adhesiolysis and long-term prednisolone

Nozomu Yokoyama[1], Ryohei Kinoshita[2], Hiroshi Ohta[3], Kazuki Okada[4], Genya Shimbo[2], Kazuyoshi Sasaoka[2], Noriyuki Nagata[2], Noboru Sasaki[2], Keitaro Morishita[2], Kensuke Nakamura[1], Yumiko Kagawa[4] and Mitsuyoshi Takiguchi[1]

Abstract

Case summary An 8-year-old neutered male domestic shorthair indoor cat was presented with an 8-week history of intermittent vomiting, anorexia and weight loss that had been unresponsive to supportive treatment. Abdominal ultrasound revealed plication of the small intestine and fluid accumulation proximal to the lesion, and a linear foreign body was suspected. An exploratory celiotomy showed cocoon-like encapsulation of the entire intestine. Surgical adhesiolysis and full-thickness biopsy were performed, and histopathologic examination revealed mild thickening of the visceral peritoneum with fibrin deposition, as well as mild neutrophil and lymphocyte infiltration. These findings were compatible with sclerosing encapsulating peritonitis (SEP). The cat recovered well postoperatively and was discharged the next day. Prednisolone was administered for 7 weeks to prevent recurrence of SEP. Five months after surgery, the cat was re-presented with anorexia and chronic vomiting. Based on the clinical examination findings, recurrent SEP was suspected. At the second surgery, surgical adhesiolysis was repeated and a bioresorbable hyaluronate-carboxymethylcellulose membrane was used to cover the serosal surface and thus prevent adhesion formation. Histopathologic findings of the peritoneal biopsy specimen confirmed SEP. Long-term prednisolone treatment (1 mg/kg for the first dose and 0.5 mg/kg every 48 h for maintenance) was administered postoperatively. The cat survived for more than 1239 days without recurrence.

Relevance and novel information To our knowledge, this is the first report of SEP in a cat with long-term survival. The use of a bioresorbable hyaluronate-carboxymethylcellulose membrane and long-term prednisolone treatment may have prevented short-term and long-term recurrence, respectively, in this case.

出典　doi: 10.1177/20551169231209917.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10676633/

最終責任者　Mitsuyoshi Takiguchi (Corresponding Author)

The Journal of Veterinary Medical Science

FULL PAPER
Internal Medicine

Investigation of the therapeutic effects, predictors, and complications of long-term immunosuppressive therapy in dogs with precursor-targeted immune-mediated anemia

Mei SUGAWARA-SUDA[1)], Keitaro MORISHITA[2)]*, Yuto IWANAGA[2)], Jumpei YAMAZAKI[3,4)], Yumiko KAGAWA[5)], Nozomu YOKOYAMA[1)], Noboru SASAKI[2)], Hiroshi OHTA[6)], Kensuke NAKAMURA[1)], Mitsuyoshi TAKIGUCHI[1)]

[1)]Laboratory of Veterinary Internal Medicine, Department of Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[2)]Veterinary Teaching Hospital, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[3)]Translational Research Unit, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Hokkaido, Japan
[4)]One Health Research Center, Hokkaido University, Hokkaido, Japan
[5)]North Lab, Hokkaido, Japan
[6)]Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuan University, Hokkaido, Japan

ABSTRACT. Dogs with precursor-targeted immune-mediated anemia (PIMA) are commonly treated with immunosuppressive therapy, but information on predictors of treatment response and response time is limited. Therefore, we retrospectively investigated predictive factors that influenced the treatment response and duration required to observe a response in dogs with PIMA receiving continuous immunosuppressive therapies for more than 105 days. Of 50 client-owned dogs that developed PIMA, 27 were included in this study, of which 18 were responders and 9 were non-responders to immunosuppressive therapies. Sixteen of the 18 responders responded to treatment within 60 days and the remaining 2 responded at 93 and 126 days, respectively. We found that an erythroid-maturation ratio of <0.17 may be a useful predictor for treatment response. In addition, complications of immunosuppressive therapies were investigated further in 50 dogs. Pancreatitis (n=4) and pneumonia (3) occurred over the entire treatment period, and infections such as abscesses (3) tended to be more common in dogs on an extended period of immunosuppressive therapy. These findings may be helpful when planning for the initial treatment and may provide evidence for informed consent about potential comorbidities throughout the treatment course.

KEYWORDS: canine, erythroid-maturation ratio, immunosuppressive therapy, non-regenerative immune-mediated anemia

J. Vet. Med. Sci.
85(7): 695–701, 2023
doi: 10.1292/jvms.23-0010

Received: 6 January 2023
Accepted: 1 May 2023
Advanced Epub:
29 May 2023

出典　doi: 10.1292/jvms.23-0010.
https://www.jstage.jst.go.jp/article/jvms/85/7/85_23-0010/_article

最終責任者　Keitaro Morishita (Corresponding Author)

Received: 12 May 2022 | Accepted: 24 March 2023
DOI: 10.1111/jvim.16705

STANDARD ARTICLE

Journal of Veterinary Internal Medicine ACVIM

Effectiveness of 2-dimensional shear wave elastography for noninvasive and reliable estimation of right atrial pressure in dogs with induced volume overload

Masahiro Tamura[1,2] | Hiroshi Ohta[1] | Tatsuyuki Osuga[3] | Mitsuyoshi Takiguchi[2]

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

[2]Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

[3]Laboratory of Veterinary Internal Medicine, Department of Veterinary Sciences, University of Miyazaki, Miyazaki, Miyazaki, Japan

Correspondence
Mitsuyoshi Takiguchi, Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9, Sapporo, Hokkaido 060-0818, Japan.
Email: mtaki@vetmed.hokudai.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: JP22K15084; Promotion and Mutual Aid Corporation for Private Schools of Japan

Abstract

Background: Two-dimensional shear wave elastography (2D-SWE) provides information on hepatic elastic modulus as shear wave velocity (SWV).

Hypothesis/Objectives: To assess SWV using 2D-SWE in dogs with induced volume overload, investigate the relationship between this information and right atrial pressure (RAP) measured by invasive right heart catheterization, and also evaluate the difference in SWV before and after diuretic administration.

Animals: Six healthy beagles.

Methods: Prospective experimental study. Right heart catheterization and 2D-SWE were performed in 6 anesthetized beagles at baseline and after the induction of volume overload. Volume overload was induced by IV hydroxyethyl starch 70/0.5 infusion (100 mL/kg/h). Furosemide (4-6 mg/kg, IV) was administered, and the SWVs were measured.

Results: Shear wave velocity showed a significant gradual increase during acute volume overload compared to baseline. SWV was significantly positively correlated with RAP ($P < .0001$, $\rho = 0.9729$). The area under the curve of SWV to predict RAP at >10, >15, and >20 mm Hg was 0.9896 (95% confidence interval [95% CI], 0.9690-1.000), 0.9907 (95% CI, 0.9701-1.000), and 0.9722 (95% CI, 0.9280-1.000), respectively. The SWV after diuretic use decreased significantly.

Conclusions and Clinical Importance: Two-dimensional shear wave elastography might be useful for noninvasive and reliable estimation of RAP in dogs with acute volume overload and has potential as a quantitative biomarker for evaluating therapeutic response in dogs with right sided congestive heart failure.

KEYWORDS
2D-SWE, cardiac, right atrial pressure, SWV

出典 doi: 10.1111/jvim.16705.
https://onlinelibrary.wiley.com/doi/10.1111/jvim.16705

最終責任者 Mitsuyoshi Takiguchi (Corresponding Author)

JOURNAL OF SMALL ANIMAL PRACTICE BSAVA

ORIGINAL ARTICLE

Evaluation of responses to immunosuppressive therapy in dogs with suspected non-regenerative immune-mediated anaemia: 11 cases (2012-2018)

K. Morishita [*,§], M. Sugahara-Suda [†], J. Yamazaki [*], N. Sasaki [‡], K. Nakamura [‡], H. Ohta [‡] and M. Takeuchi [*]

[*]Veterinary Teaching Hospital, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan
[†]Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan
[‡]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan
[§]Corresponding author email: kmorishi@vetmed.hokudai.ac.jp

Objectives: We aimed to determine the response time to immunosuppressive therapy and time required to achieve a 5% increase in haematocrit among dogs with non-regenerative immune-mediated anaemia.
Materials and Methods: Client-owned dogs diagnosed with non-regenerative immune-mediated anaemia in Hokkaido University Veterinary Teaching Hospital between December 2012 and May 2018 were enrolled. The first treatment regimen included prednisolone (2 mg/kg/day) and ciclosporin (up to 10 mg/kg/day) for 8 weeks. Dogs that did not respond to the first regimen proceeded to the second regimen comprising prednisolone and mycophenolate mofetil (15 mg/kg, twice a day). Reticulocyte count and haematocrit were monitored every 1 to 2 weeks. Treatment response was defined as an absolute reticulocyte count more than $60\times10^3/\mu L$ or increasing haematocrit.
Results: During the study period, 23 dogs fulfilled the inclusion criteria for non-regenerative immune-mediated anaemia. Twelve dogs were excluded from this study for various reasons and response to therapy was evaluated in the remaining 11 dogs. Treatment responses were observed in 8 of 11 dogs, and the median time to response was 39.5 days (range 8 to 92 days). Two responders were unable to continue the first treatment regimen and were switched to the second regimen owing to anorexia and nausea, possibly induced by ciclosporin; withdrawal of ciclosporin improved their symptoms. The time required to achieve a 5% increase in haematocrit was assessed in the other six dogs, with a median of 56.5 days (range 8 to 135 days).
Clinical Significance: Here we report the response to a standardised treatment protocol in dogs with non-regenerative immune-mediated anaemia. Knowledge of potential side effects and expected therapeutic outcomes may be of use for veterinary practitioners treating this condition.

Journal of Small Animal Practice (2022), 1–8
DOI: 10.1111/jsap.13614
Accepted: 5 March 2022

出典　doi: 10.1111/jsap.13614.
https://onlinelibrary.wiley.com/doi/10.1111/jsap.13614
最終責任者　Keitaro Morishita (Corresponding Author)

International Immunology, Vol. 35, No. 7, pp. 313–325
https://doi.org/10.1093/intimm/dxad006
Advance Access publication 18 March 2023

Zoobiquity experiments show the importance of the local MMP9-plasminogen axis in inflammatory bowel diseases in both dogs and patients

Takeshi Yamasaki[1,2,*], Noriyuki Nagata[3,*], Toru Atsumi[1,*], Rie Hasebe[3,4,*], Yuki Tanaka[1,4,*], Izuru Ohki[1,4], Shimpei Kubota[1], Yuta Shinohara[1], Yong Bin Teoh[1], Nozomu Yokoyama[5], Noboru Sasaki[3], Kensuke Nakamura[3], Hiroshi Ohta[3], Takehiko Katsurada[3], Yoshihiro Matsuno[6], Shintaro Hojyo[7], Shigeru Hashimoto[1], Mitsuyoshi Takiguchi[3,*] and Masaaki Murakami[1,2,4,8,9]

[1]Division of Molecular Psychoimmunology, Institute for Genetic Medicine, Graduate School of Medicine, Hokkaido University, Sapporo, Japan
[2]Division of Molecular Neuroimmunology, National Institute for Physiological Sciences, National Institute of Natural Sciences, Okazaki, Japan
[3]Laboratory of Veterinary Internal Medicine, Department of Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan
[4]Center for Infectious Cancers, Institute for Genetic Medicine, Graduate School of Medicine, Hokkaido University, Sapporo, Japan
[5]Group of Quantum Immunology, Institute for Quantum Life Science, National Institute for Quantum and Radiological Science and Technology (QST), Inage, Japan
[6]Veterinary Teaching Hospital, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan
[7]Laboratory of Veterinary Internal Medicine, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan
[8]Department of Gastroenterology and Hepatology, Graduate School of Medicine, Hokkaido University, Sapporo, Japan
[9]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan
[10]Institute for Vaccine Research and Development (IVReD), Hokkaido University, Sapporo, Japan

Correspondence to: Masaaki Murakami; E-mail: murakami@igm.hokudai.ac.jp or Mitsuyoshi Takiguchi; E-mail: mtaki@vetmed.hokudai.ac.jp

* These authors contributed equally to this study

Received 16 November 2022; editorial decision 15 March 2023; accepted 16 March 2023

Abstract

Using a zoobiquity concept, we directly connect animal phenotypes to a human disease mechanism: the reduction of local plasminogen levels caused by matrix metalloproteinase-9 (MMP9) activity is associated with the development of inflammation in the intestines of dogs and patients with inflammatory bowel disease. We first investigated inflammatory colorectal polyps (ICRPs), which are a canine gastrointestinal disease characterized by the presence of idiopathic chronic inflammation, in Miniature Dachshund (MD) and found 31 missense disease-associated SNPs by whole-exome sequencing. We sequenced them in 19 other dog breeds and found five, *PLG*, *TCOF1*, *TG*, *COL9A2* and *COL4A4*, only in MD. We then investigated two rare and breed-specific missense SNPs (T/T SNPs), *PLG*: c.477G > T and c.478A > T, and found that ICRPs with the T/T SNP risk alleles showed less intact plasminogen and plasmin activity in the lesions compared to ICRPs without the risk alleles but no differences in serum. Moreover, we show that MMP9, which is an NF-κB target, caused the plasminogen reduction and that intestinal epithelial cells expressing plasminogen molecules were co-localized with epithelial cells expressing MMP9 in normal colons with the risk alleles. Importantly, MMP9 expression in patients with ulcerous colitis or Crohn's disease also co-localized with epithelial cells showing enhanced NF-κB activation and less plasminogen expression. Overall, our zoobiquity experiments showed that MMP9 induces the plasminogen reduction in the intestine, contributing to the development of local inflammation and suggesting the local MMP9-plasminogen axis is a therapeutic target in both dogs and patients. Therefore, zoobiquity-type experiments could bring new perspectives for biomarkers and therapeutic targets.

出典　doi: 10.1093/intimm/dxad006.

https://academic.oup.com/intimm/articleabstract/35/7/313/7080519?redirectedFrom=fulltext&login=false

最終責任者　Mitsuyoshi Takiguchi and Masaaki Murakami(Corresponding Author)

PLOS ONE

RESEARCH ARTICLE

Safety and clinical efficacy of an anti-PD-L1 antibody (c4G12) in dogs with advanced malignant tumours

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

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

* konnai@vetmed.hokudai.ac.jp

Citation: Maekawa N, Konnai S, Hosoya K, Kim S, Kinoshita R, Deguchi T, et al. (2023) Safety and clinical efficacy of an anti-PD-L1 antibody (c4G12) in dogs with advanced malignant tumours. PLoS ONE 18(10): e0291727. https://doi.org/10.1371/journal.pone.0291727

Editor: Alessandro Poiaa, IRCCS Giovanni Paolo II Cancer Hospital, ITALY

Received: July 20, 2023

Accepted: September 2, 2023

Published: October 4, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting information files.

Funding: This work was supported by the Grants-in-Aid for Scientific Research (Grant Numbers 21K18839 and 22H02593) from the Japan Society for the Promotion of Science (JSPS) and JP21am0101078. Funders had no role in the study design, data collection and analysis, decision to publish, and manuscript preparation.

Abstract

Immune checkpoint inhibitors (ICIs) have been developed for canine tumour treatment, and pilot clinical studies have demonstrated their antitumour efficacy in dogs with oral malignant melanoma (OMM). Although ICIs have been approved for various human malignancies, their clinical benefits in other tumour types remain to be elucidated in dogs. Here, we conducted a clinical study of c4G12, a canine chimeric anti-PD-L1 antibody, to assess its safety and efficacy in dogs with various advanced malignant tumours ($n = 12$) at the Veterinary Teaching Hospital of Hokkaido University from 2018 to 2023. Dogs with digit or foot pad malignant melanoma ($n = 4$), osteosarcoma ($n = 2$), hemangiosarcoma ($n = 1$), transitional cell carcinoma ($n = 1$), nasal adenocarcinoma ($n = 1$), B-cell lymphoma ($n = 1$), or undifferentiated sarcoma ($n = 2$) were treated with 2 or 5 mg/kg c4G12 every 2 weeks. Treatment-related adverse events of any grade were observed in eight dogs (66.7%), including elevated aspartate aminotransferase (grade 3) in one dog (8.3%) and thrombocytopenia (grade 4) in another dog (8.3%). Among dogs with target disease at baseline ($n = 8$), as defined by the response evaluation criteria for solid tumours in dogs (cRECIST), one dog with nasal adenocarcinoma and another with osteosarcoma experienced a partial response effects in dogs with malignant tumours other than OMM. Further clinical studies are warranted to identify the tumour types that are most likely to benefit from c4G12 treatment.

出典 doi: 10.1371/journal.pone.0291727.

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0291727

最終責任者 Satoru Konnai (Corresponding Author)

PLOS ONE

RESEARCH ARTICLE

Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors

Naoya Maekawa[1], Satoru Konnai[1,2,3]*, Yumie Asano[2], Takumi Otsuka[2], Eri Aoki[2], Hiroto Takeuchi[2], Yukinari Kato[4,5], Mika K. Kaneko[4], Shinji Yamada[4], Yumiko Kagawa[5], Maki Nishimura[6], Satoshi Takagi[7,8], Tatsuya Deguchi[7,9], Hiroshi Ohta[7,9], Takayuki Nakagawa[10], Yasuhiko Suzuki[1,3,11,12], Tomohiro Okagawa[1], Shiro Murata[1,2], Kazuhiko Ohashi[1,2,13]

1 Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **2** Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **3** Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Sapporo, Japan, **4** Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan, **5** Department of Molecular Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan, **6** North Lab, Sapporo, Japan, **7** Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **8** Department of Veterinary Surgery 1, School of Veterinary Medicine, Azabu University, Sagamihara, Japan, **9** Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, **10** Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Japan, **11** International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, **12** Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo, Japan, **13** International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

* konnai@vetmed.hokudai.ac.jp

OPEN ACCESS

Citation: Maekawa N, Konnai S, Asano Y, Otsuka T, Aoki E, Takeuchi H, et al. (2023) Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors. PLoS ONE 18(1): e0281143. https://doi.org/10.1371/journal.pone.0281143

Editor: Avaniyapuram Kannan Murugan, King Faisal Specialist Hospital and Research Center, SAUDI ARABIA

Received: November 4, 2022

Accepted: January 11, 2023

Published: January 26, 2023

Data Availability Statement: All data are fully available without restriction. All relevant data are within the manuscript and its Supporting

21K14983(H) and 22H02503) from the Japan Society for the Promotion of Science (JSPS) and by Japan Agency for Medical Research and Development (AMED) under Grant Numbers: JP22fk0210076, JP22am1210008, and JP21am0101078. Funders had no role in the study design, data collection and analysis, decision to publish, and manuscript preparation.

Abstract

Spontaneous tumors are a major cause of death in cats. Treatment of human tumors has progressed dramatically in the past decade, partly due to the success of immunotherapies using immune checkpoint inhibitors, such as anti-programmed death 1 (PD-1) and anti-PD-ligand 1 (PD-L1) antibodies. However, little is known about the PD-1 pathway and its association with tumor disease in cats. This study investigated the applicability of anti-PD-1/PD-L1 therapy in feline tumors. We first determined the complete coding sequence of feline *PD-L1* and *PD-L2*, and found that the deduced amino acid sequences of feline PD-L1/PD-L2 share high sequence identities (66–83%) with orthologs in other mammalian species. We prepared recombinant feline PD-1, PD-L1, and PD-L2 proteins and confirmed receptor–ligand binding between PD-1 and PD-L1/PD-L2 using flow cytometry. Next, we established an anti-feline PD-L1 monoclonal antibody (clone CL1Mab-7) to analyze the expression of PD-

80%), fibrosarcoma (5/5, 100%), and renal cell carcinoma (2/2, 100%) tissues. Our results strongly encourage further investigations of the PD-1/PD-L1 pathway as a potential therapeutic target for feline tumors.

PD-L1 in a KNp, FMCm, ted by inter- wed PD-L1 carcinoma (4/5,

出典 doi: 10.1371/journal.pone.0281143.

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0281143

最終責任者 Satoru Konnai (Corresponding Author)

獣医麻酔学（Veterinary Anesthesiology）

Tadashi Sano

Associate Professor

准教授　佐野　忠士

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

Ⅱ．その他　＜Others＞

1）Beraprost and overall survival in cats with chronic kidney disease.
Ito H, Matsuura T, **Sano T**.
Vet Sci. 10:459. 2023. doi: 10.3390/vetsci10070459

2）Kinematic characteristics of canine hindlimb movement during sit-to-stand and stand-to-sit motions.
Yoshikawa K, Kitazawa T, **Sano T**, Ino T, Miyasaka T.
Res Vet Sci. 162:104944. 2023. doi: 10.1016/j.rvsc.2023.104944.

3）The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits.
Wei Y, Chen IY, Tamogi H, Sugita C, Daimaruya N, Hirokawa T, Kato K, Itami T, **Sano T**, Yamashita K.
J Vet Med Sci. 85: 471–478. 2023. doi: 10.1292/jvms.22-0484.

4）Sedative and cardiorespiratory effects of intranasal atomized alfaxalone in Japanese White rabbits.
Wei Y, Nakagawa M, Chen IY, Itami T, **Sano T**, Pasloske K, Yamashita K.
Vet Anaesth Analg. 50:255–262. 2023. doi: 10.1016/j.vaa.2023.02.001.

5）ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs.
Chen IY, Sugita C, Wei Y, Daimaruya N, Itami T, **Sano T**, Yamashita K. *Vet Anaesth Analg.* 50:204–210. 2023. doi: 10.1016/j.vaa.2023.02.008.

6）Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs.
Chen IY, Sugita C, Wei Y, Daimaruya N, Itami T, **Sano T**, Yamashita K.
Vet Anaesth Analg. 50:485–491. 2023. doi: 10.1016/j.vaa.2023.08.002.

veterinary sciences

MDPI

Article

Beraprost and Overall Survival in Cats with Chronic Kidney Disease

Hiroyuki Ito [1,*,†], Takumi Matsuura [2,†] and Tadashi Sano [3]

[1] Ichikawa General Hospital, Kariya Animal Hospital Group, Chiba 272-0034, Japan
[2] Toray Industries, Inc., Tokyo 103-8666, Japan
[3] School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
* Correspondence: h.ito@kariya-ah.co.jp
† These authors contributed equally to this work.

Simple Summary: Chronic kidney disease (CKD) is a highly prevalent disorder in senior cats. CKD is commonly diagnosed together with other disorders; therefore, overall survival, based on all-cause death, including complications, is the most important outcome for treatment response in feline CKD. Although researchers have been seeking a pharmaceutical to improve overall survival, unfortunately, they have not discovered it yet. Beraprost (also called beraprost sodium or BPS) is a prostacyclin analogue that has a vasoprotective effect, which is unique and completely different from renin–angiotensin inhibitors. This study demonstrated that beraprost therapy was associated with better overall survival, and the findings shed light on cats suffering with CKD, their owners, and veterinarians in clinical practice.

Abstract: Background: Overall survival is the most important outcome for treatment response in feline chronic kidney disease (CKD). Beraprost has been shown to reduce the kidney function decline in cats with International Renal Interest Society (IRIS) stage 2 and 3 CKD. However, the association with prolonged survival has not yet been examined. Objective: To assess the relationship between beraprost and overall survival in cats with CKD in real clinical practice. Animals: Client-owned cats with IRIS stage 3 CKD (n = 134) were evaluated between 2017 and 2020. Methods: A retrospective cohort study based on data from electronic medical records of one hospital. Results: The cohort was divided into "beraprost therapy" and "no beraprost therapy" groups, and survival analyses revealed that overall survival was significantly longer in the beraprost therapy group, using Kaplan–Meier curves (p = 0.004). However, baseline phosphate is known to be an important prognostic indicator and was not well balanced between the two groups. Therefore, a subcohort of 97 cats was selected (those having baseline phosphate <6.0 mg/dL) that allowed for this parameter to be balanced between groups. The survival data in this subcohort were consistent with those of the overall study cohort. Conclusions: In feline patients with CKD, beraprost therapy is associated with better overall survival.

Keywords: beraprost; feline; chronic kidney disease; progression-free survival; overall survival

Citation: Ito, H.; Matsuura, T.; Sano, T. Beraprost and Overall Survival in Cats with Chronic Kidney Disease. *Vet. Sci.* **2023**, *10*, 459. https://doi.org/10.3390/vetsci10070459

Academic Editor: Jose Alberto Montoya-Alonso

Received: 11 May 2023
Revised: 7 July 2023
Accepted: 11 July 2023
Published: 13 July 2023

1. Introduction

Chronic kidney disease (CKD) is a common disease worldwide and a leading cause of mortality and morbidity in older cats. Advanced disease is characterized by incurable kidney function decline and poor prognosis [1,2]. Cardiac function can be impaired by the presence of decreased kidney function in cats, which is known as cardiovascular–renal disorder (CvRD) [3]; a subset of cats with CKD can die from heart failure. In addition, the complications of feline CKD include hypertension, proteinuria, hypokalemia, hyperphosphatemia, urinary tract infections, anemia, and CKD-related mineral bone disorders [1,2]. Hyperthyroidism [4] and diabetes mellitus [5] also occur as co-morbidities. All such disorders can also contribute to early mortality. Therefore, overall survival, based on all-cause

出典 doi: 10.3390/vetsci10070459

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10384921/pdf/vetsci-10-00459.pdf

最終責任者 Hiroyuki Itoh (Corresponding Author)

Research in Veterinary Science 162 (2023) 104944

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Kinematic characteristics of canine hindlimb movement during sit-to-stand and stand-to-sit motions

Kazuyuki Yoshikawa [a,b,*], Takio Kitazawa [c], Tadashi Sano [c], Takumi Ino [d], Tomoya Miyasaka [d]

[a] Japan Small Animal Medical Center, 1-10-4 Higashi-tokorozawa, Tokorozawa, 8, Saitama 359-0025, Japan
[b] Graduate School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[c] Department of Veterinary Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[d] Department of Physical Therapy, Faculty of Health Sciences, Hokkaido University of Science, Hokkaido 006-8585, Japan

ARTICLE INFO

Keywords:
Canine physical therapy
Canine rehabilitation
Kinematics
Sit-to-stand
Stand-to-sit
Therapeutic exercise

ABSTRACT

Sit-to-stand and stand-to-sit motions are basic motions for daily animal life, and these motions are used as therapeutic exercises for dogs with functional impairments. The sit-to-stand motion is divided into several phases for kinesiological assessment in human rehabilitation and physical therapy. However, these motions in dogs have not been characterized in detail. We examined canine hindlimb kinematic characteristics during sit-to-stand/stand-to-sit motions and compared the characteristics with those during walking. In addition, we tried to classify phases of the movements based on kinematic characteristics of the transition of the range of motion of the hindlimb. We used a three-dimensional motion analysis system to evaluate the motions of eight clinically healthy beagles. During the sit-to-stand motion, the total range of motion (ROM) in the hip joint flexion/extension was half of that of during walking, but the total ROM of the hindlimb external/internal rotation relative to the pelvis and flexion/extension of the stifle and the tarsal joints were significantly larger than those of walking, suggesting that sit-to-stand exercise causes movements of hindlimb joints without marked changes in hip joint flexion/extension movement. Both sit-to-stand and stand-to-sit motions could not be divided into multiple phases only by the transition of the range of motion of the hindlimb.

Recently, canine rehabilitation has been focused on as an important field of veterinary medicine (Millis and Ciuperca, 2015; Millis and Levine, 2014). Evaluation of a patient's motion and kinesiological knowledge in therapeutic exercises are necessary to select precise rehabilitation plans for an individual patient (Marcellin-Little et al., 2021).

Sit-to-stand motion is essential for the daily life of animals such as dogs, and these movements have been used as a therapeutic exercise following musculoskeletal injury or surgery because this exercise can be performed without using any special equipment. However, there have been only two reports concerning kinematic analysis of sit-to-stand motion (Feeney et al., 2007; McEachern and Headrick, 2006) and one report examining the motion of prone-to-stand (Ellis et al., 2018). Different species of dogs were used in two studies of sit-to-stand and the range of motions of the hip and tarsal flexion were slightly different. In the case of human medicine, the sit-to-stand motion has been divided into three to five phases to conduct precise analysis, and this phase classification contributes to human rehabilitation (Li et al., 2021; Mao et al., 2018; Millington et al., 1992). However, phase classification of sit-to-stand motion in dogs has not been examined yet. Precise phase classifications of sit-to-stand motion in normal dogs and accurate characterization of the range of motion transitions can potentially contribute to the development of personalized rehabilitation plans for individual canine patients. Additionally, this approach can complement medical examinations in identifying abnormal movements in patients.

In this study, canine hindlimb kinematic characteristics of the sit-to-stand motion were investigated and these characteristics were compared with those of walking, which is another general exercise of rehabilitation. We also tried to establish phase classification of sit-to-stand and stand-to-sit motions considering the transition of range of motion of hindlimb joints.

All of the procedures were approved by the Experimental Animal Research Committee of Rakuno Gakuen University (VH17B7). Eight clinically healthy beagles (3 females and 5 males) owned by Rakuno Gakuen University with an average ± SEM weight of 12.3 ± 0.6 kg and age of 4–8 years (5.6 ± 0.6 years) were used in this study. All of the dogs

* Corresponding author at: Japan Small Animal Medical Center, 1-10-4 Higashi-tokorozawa, Tokorozawa, 8, Saitama 359-0025, Japan.
E-mail address: kyoshikawa@jsamc.jp (K. Yoshikawa).

https://doi.org/10.1016/j.rvsc.2023.104944
Received 8 April 2023; Received in revised form 25 June 2023; Accepted 29 June 2023
Available online 30 June 2023

出典 doi: 10.1016/j.rvsc.2023.104944.
https://www.sciencedirect.com/science/article/pii/S0034528823001893/pdff
最終責任者 Kazuyuki Yoshikawa (Corresponding Author)

The Journal of Veterinary Medical Science

FULL PAPER
Surgery

The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits

Yixian WEI[1)], I-Ying CHEN[1)], Haruka TAMOGI[1)], Chihiro SUGITA[1)], Nozomi DAIMARUYA[1)], Taku HIROKAWA[1)], Keiko KATO[1)], Takaharu ITAMI[1)], Tadashi SANO[1)], Kazuto YAMASHITA[1)]*

[1)]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. To prevent aspiration in Japanese White (JW) rabbits, the maximum single volume of medetomidine administered intranasally is 0.3 mL per nostril using a mucosal atomization device (MAD). This study aimed to examine the sedative effect of intranasal administration of medetomidine using MAD in eight healthy female JW rabbits. Each rabbit received intranasal atomization (INA) of saline (Control treatment) along with three doses of 1 mg/mL medetomidine (0.3 mL to one nostril [MED0.3 treatment]; 0.3 mL each to both nostrils [MED0.6 treatment]; 0.3 mL twice to both nostrils [MED1.2 treatment]), with a washout period of at least 7 days between treatments. The actual doses of medetomidine were 82 (75–84) µg/kg (median [25th–75th percentile]), 163 (156–168) µg/kg, and 323 (295–343) µg/kg for the MED0.3, MED0.6, and MED1.2 treatments, respectively. A medetomidine-dose dependent sedative effect was detected, and the loss of righting reflex (LRR) was achieved in one rabbit at 18 min, seven rabbits at 11 (9–18) min, and eight rabbits at 7 (4–18) min after the MED0.3, MED0.6, and MED1.2 treatments, respectively. The LRR was maintained for 63 (29–71) min and 83 (68–101) min after the MED0.6 and MED1.2 treatments, respectively. Additionally, the INA of medetomidine produced a significant dose-dependent cardiorespiratory depression including a decrease in pulse rate, respiratory rate, percutaneous oxygen saturation, and arterial partial pressure of oxygen, and an increase in arterial partial pressure of carbon dioxide in the rabbits.

KEYWORDS: intranasal atomization, medetomidine, mucosal atomization device, rabbit

J. Vet. Med. Sci.
85(4): 471–478, 2023
doi: 10.1292/jvms.22-0484

Received: 25 October 2022
Accepted: 22 February 2023
Advanced Epub:
6 March 2023

*Correspondence to: Yamashita K: yamasita@rakuno.ac.jp, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

出典　doi: 10.1292/jvms.22-0484.
https://www.jstage.jst.go.jp/article/jvms/85/4/85_22-0484/_pdf/-char/en

最終責任者　Kazuto Yamashita (Corresponding Author)

Veterinary Anaesthesia and Analgesia 2023, **50**, 255–262 https://doi.org/10.1016/j.vaa.2023.02.001

RESEARCH PAPER

Sedative and cardiorespiratory effects of intranasal atomized alfaxalone in Japanese White rabbits

Yixian Wei[a], **Mari Nakagawa**[a], **I-Ying Chen**[a], **Takaharu Itami**[a], **Tadashi Sano**[a], **Kirby Pasloske**[b] & **Kazuto Yamashita**[a]

[a]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan

[b]Jurox Pty Ltd, Rutherford, NSW, Australia

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasita@rakuno.ac.jp

Abstract

Objective To investigate the sedative and cardiorespiratory effects of intranasal atomization (INA) of alfaxalone using a mucosal atomization device in Japanese White rabbits.

Study design Randomized, prospective, crossover study.

Animals A total of eight healthy female rabbits, weighing 3.6–4.3 kg and aged 12–24 months.

Methods Each rabbit was randomly assigned to four INA treatments administered 7 days apart: Control treatment, 0.15 mL 0.9% saline in both nostrils; treatment INA0.3, 0.15 mL 4% alfaxalone in both nostrils; treatment INA0.6, 0.3 mL 4% alfaxalone in both nostrils; treatment INA0.9, 0.3 mL 4% alfaxalone in left, then right, then left nostril. Sedation was scored 0–13 using a composite measure scoring system for rabbits. Simultaneously, pulse rate (PR), respiratory rate (f_R), noninvasive mean arterial pressure (MAP), peripheral hemoglobin oxygen saturation (SpO_2) and arterial blood gases were measured until 120 minutes. The rabbits breathed room air during the experiment and were administered flow-by oxygen when hypoxemia (SpO_2 <90% or PaO_2 <60 mmHg; 8.0 kPa) developed. Data were analyzed using the Fisher's exact test and the Friedman test ($p < 0.05$).

Results No rabbit was sedated in treatments Control and INA0.3. All rabbits in treatment INA0.9 developed loss of righting reflex for 15 (10–20) minutes [median (25th–75th percentile)]. Sedation score significantly increased from 5 to 30 minutes in treatments INA0.6 and INA0.9 with maximum scores of 2 (1–4) and 9 (9–9), respectively. f_R decreased in an alfaxalone dose-dependent manner and one rabbit developed hypoxemia in treatment INA0.9. No significant changes were observed in PR and MAP.

Conclusions and clinical relevance INA alfaxalone resulted in dose-dependent sedation and respiratory depression in Japanese White rabbits to values considered not clinically relevant. Further investigation of INA alfaxalone in combination with other drugs is warranted.

Keywords alfaxalone, intranasal mucosal atomization, rabbits, sedative effects.

Introduction

Over the past decade, interest in intranasal drug delivery has been garnering increased attention in both human and veterinary medicine. The theory behind intranasal drug absorption is that the intranasal cavity is lined by highly vascularized mucosa facilitating rapid drug absorption, and the olfactory epithelium provides direct access to the central nervous system (CNS) by bypassing the blood–brain barrier (Grassin-Delyle et al. 2012; Keller et al. 2021; Mignani et al. 2021). Drugs absorbed through the trigeminal and olfactory nerve pathways from the nasal cavity are directly delivered to the brain. Thus intranasal administration is a promising alternative route to oral, intravenous and intramuscular administration of drugs that bypasses the blood–brain barrier (Erdő et al. 2018).

In rabbits, a total volume of 0.3–0.64 mL kg^{-1} of solution containing sedative and anesthetic drugs has been administered intranasally through the nostrils using a catheter-tipped syringe to produce sedation (Santangelo et al. 2016; Weiland et al. 2017; Yanmaz et al. 2022). An intranasal combination of dexmedetomidine, midazolam and butorphanol resulted in deep sedation with moderate hypoxemia and hypercarbia in New Zealand White (NZW) rabbits (Santangelo et al. 2016). In a different study, intranasal combination of dexmedetomidine and midazolam resulted in rapid sedation in NZW rabbits;

出典 doi: 10.1016/j.vaa.2023.02.008.

https://www.sciencedirect.com/science/article/pii/S1467298723000326?via%3Dihub

最終責任者 Kazuto Yamashita (Corresponding Author)

RESEARCH PAPER

ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia in dogs

I-Ying Chen, Chihiro Sugita, Yixian Wei, Nozomi Daimaruya, Takaharu Itami, Tadashi Sano & Kazuto Yamashita

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

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasita@rakuno.ac.jp

Abstract

Objective To determine the median effective dose (ED_{50}) and effective dose required to depress the twitch value by 95% (ED_{95}) of rocuronium during alfaxalone anesthesia in dogs.

Study design A randomized, prospective, crossover experimental study.

Animals A total of eight adult Beagle dogs (four female, four male), weighing 10.3–14.6 kg and aged 6–8 years.

Methods The dogs were anesthetized three times with 1.25-fold the individual minimum infusion rate of alfaxalone at intervals of ≥ 14 days. Neuromuscular function was monitored with train-of-four (TOF) stimulation of the peroneal nerve by acceleromyography. After recording the control TOF ratio (TOFRC) and first twitch of TOF (T1C), a single bolus dose of rocuronium 100, 175 or 250 μg kg^{-1} (treatments R100, R175 or R250) was administered intravenously. The maximum suppression of the first twitch of TOF (T1) was recorded and calibrated with T1C to construct the dose–response curve, from which ED_{50} and ED_{95} were calculated. Time from rocuronium administration to TOF ratio/TOFRC > 0.9 (duration TOFR0.9) was recorded.

Results ED_{50} and ED_{95} of rocuronium during alfaxalone anesthesia were 175 and 232 μg kg^{-1}, respectively. The median (range) duration TOFR0.9 was longer in treatment R250 [10.1 (9.2–10.9) minutes] than in treatments R100 [3.1 (2.9–4.4) minutes; $p < 0.0001$] and R175 [7.7 (6.9–8.1) minutes; $p < 0.0001$]; and longer in treatment R175 than in treatment R100 ($p < 0.0001$).

Conclusions and clinical relevance The duration of TOFR0.9 correlated positively with the dosage of rocuronium, indicating that recovery time of rocuronium was also dose-dependent in dogs anesthetized with alfaxalone. The duration TOFR0.9 of rocuronium 250 μg kg^{-1} was 10 minutes during alfaxalone anesthesia in dogs.

Keywords administration and dosage, alfaxalone, dose–response relationship, drug, prospective studies, veterinary.

Introduction

Rocuronium bromide is an intermediate-acting non-depolarizing neuromuscular blocking agent (NMBA) in dogs (Marshall et al. 1994; Dugdale et al. 2002). As rocuronium does not produce active metabolites in human plasma (Appiah-Ankam & Hunter 2004), it is now widely used as an intravenous (IV) bolus, for incremental IV doses, and for constant rate infusion in anesthetized dogs (Dugdale et al. 2002; Alderson et al. 2007; Auer et al. 2007; Briganti et al. 2015; Haga et al. 2019). Nevertheless, NMBAs do not have an anesthetic effect and, therefore, should be administered during general anesthesia (Kastrup et al. 2005).

Alfaxalone is a synthetic neuroactive steroid anesthetic agent that is used for induction and maintenance of anesthesia in veterinary practice (Quirós-Carmona et al. 2017; Bennell et al. 2019; Dehuisser et al. 2019; Bustamante et al. 2020). The single-dose rocuronium-induced neuromuscular blockade was longer in alfaxalone than propofol anesthesia in dogs (Chen et al. 2022). However, information to guide veterinary physicians in clinical practice regarding the effective doses of rocuronium with alfaxalone is still insufficient.

The potency of NMBAs can be evaluated by constructing dose–response curves from a linear least squares regression analysis model using the single-dose method (Fuchs-Buder et al. 2007). In this method, the degree of neuromuscular block is measured following administration of different doses of NMBA to determine the median effective dose 50 (ED_{50}) and

出典 doi: 10.1016/j.vaa.2023.02.008.

https://www.sciencedirect.com/science/article/pii/S1467298723000326

最終責任者 Kazuto Yamashita (Corresponding Author)

Veterinary Anaesthesia and Analgesia 2023, **50**, 485–491 https://doi.org/10.1016/j.vaa.2023.08.002

RESEARCH PAPER

Sugammadex for reversal of rocuronium-induced neuromuscular blockade during alfaxalone anesthesia in dogs

I-Ying Chen, Chihiro Sugita, Yixian Wei, Nozomi Daimaruya, Takaharu Itami, Tadashi Sano & Kazuto Yamashita

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

Correspondence: Kazuto Yamashita, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan. E-mail: yamasita@rakuno.ac.jp

Abstract

Objective To investigate the reversal effect of sugammadex on neuromuscular blockade induced by a single bolus of rocuronium in dogs under alfaxalone anesthesia.

Study design Randomized, prospective, crossover experimental study.

Animals A group of six adult Beagle dogs (three females and three males), weighing 11.3–15.8 kg and aged 6–8 years, were used.

Methods Dogs were anesthetized twice with a 1.25 times minimum infusion rate of alfaxalone, with a washout period of at least 14 days between experiments. Neuromuscular function was monitored using acceleromyography with train-of-four (TOF) stimulation of the peroneal nerve. After recording the control TOF ratio (TOFRC), rocuronium (0.5 mg kg^{-1}) was administered intravenously. Subsequently, sugammadex (4 mg kg^{-1}) or an equal volume of saline (control treatment) was administered intravenously when the TOF count returned from 0 to 1 after neuromuscular blockade. Time from rocuronium injection to TOF count = 0 (onset time), time from TOF count = 0 to TOF count = 1 (maximum blockade period), time of first twitch amplitude recovery from 0.25 to 0.75 (recovery index), and time from sugammadex or saline administration to TOF ratio/TOFRC ≥ 0.9 (recovery time) were recorded.

Results The onset time and maximum blockade duration did not differ between sugammadex treatment [1.2 (0.7–1.5) minutes and 9.9 (6.3–10.5) minutes, respectively] and control treatment [median (range); 1.0 (0.7–1.1) minutes and 9.9 (8.8–11.5) minutes, respectively] ($p = 0.219$ and 0.844, respectively). Recovery index was 0.5 (0.3–0.7) minutes in sugammadex treatment, which was shorter than that in control treatment [4.5 (3.7–4.9) minutes] ($p = 0.031$). Recovery time was 0.8 (0.5–2.8) minutes in sugammadex treatment, which was shorter than that in control treatment [10.5 (6.8–14.3) minutes] ($p = 0.031$).

Conclusions and clinical relevance Rocuronium-induced neuromuscular blockade was effectively reversed by sugammadex in dogs anesthetized with alfaxalone.

Keywords alfaxalone, dogs, neuromuscular blockade, rocuronium, sugammadex.

Introduction

Sugammadex, a gamma-cyclodextrin, selectively reverses rocuronium-induced neuromuscular blockade via chemical encapsulation (Bom et al. 2002; de Boer et al. 2006a,b). The structure of sugammadex comprises a lipophilic internal cavity that encapsulates aminosteroid neuromuscular-blocking molecules to form an inactive complex, which is then excreted unchanged via the kidneys, thus reversing the neuromuscular blockade (Bom et al. 2002; Peeters et al. 2011). The reversal effect of sugammadex on rocuronium-induced neuromuscular blockade has been evaluated in dogs under isoflurane anesthesia (Mosing et al. 2012). Compared with acetylcholinesterase inhibitors, sugammadex does not have cardiovascular side effects and can reverse profound neuromuscular block (Sacan et al. 2007). However, encapsulation of other steroidal drugs or endogenous steroids may occur with sugammadex administration (Hunter & Flockton 2006).

Alfaxalone, a synthetic neuroactive steroid anesthetic agent, is used to induce and maintain anesthesia in veterinary practice (Bennett et al. 2019). An alfaxalone formulation licensed for clinical use in dogs and cats uses 2-hydroxypropyl-beta cyclodextrin as a solubilizing excipient (Ferré et al. 2006). There is a possibility that alfaxalone, as a synthetic neuroactive

出典 doi: 10.1016/j.vaa.2023.08.002.
https://www.sciencedirect.com/science/article/pii/S1467298723001186

最終責任者 Kazuto Yamashita (Corresponding Author)

獣医臨床病理学（Veterinary Clinicopathology）

Nao Tsuzuki

Associate Professor

准教授　都築　直

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

1）Evaluation of oxidative stress in foals with Rhodococcus equi infection-induced pneumonia for the judgment of therapeutic effect.
Tsuzuki N, Maruko T, Takeyama A, Ikeda H, Mizuguchi Y.
J Vet Med Sci. 85:1277–1280. doi: 10.1292/jvms.23-0260.

Ⅱ．その他＜Others＞

1）A retrospective analysis for criteria of surgical intervention in Japanese Black calves with respiratory signs caused by perinatal rib fracture.
Kim S, Naik MV, Kirino Y, Satoh H, **Tsuzuki N**, Inoue Y, Kuroda K, Hidaka Y.
J Vet Med Sci. 85:40–80. 2023. doi: 10.1292/jvms.22-0332.

The Journal of Veterinary Medical Science

NOTE
Internal Medicine

Evaluation of oxidative stress in foals with *Rhodococcus equi* infection–induced pneumonia for the judgment of therapeutic effect

Nao TSUZUKI[1], Tosei MARUKO[2], Akiko TAKEYAMA[2], Hiroki IKEDA[3], Yuya MIZUGUCHI[4]*

[1]Rakuno Gakuen University, Hokkaido, Japan
[2]Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
[3]Hidaka Horse Breeders Association, Hokkaido, Japan
[4]Mitsuishi Animal Medical Center, Hokkaido, Japan

J. Vet. Med. Sci.
85(12): 1277–1280, 2023
doi: 10.1292/jvms.23-0260

Received: 16 June 2023
Accepted: 4 October 2023
Advanced Epub:
17 October 2023

ABSTRACT. Forty-five foals with *Rhodococcus equi* infection and pneumonia symptoms were classified into a surviving group and a dead group. Using serum samples, the oxidative stress index (OSI) was determined at the first visit and the follow-up visit. The OSI of the surviving group was significantly lower at the follow-up than that at the first visit. No significant difference was observed between the OSI of the dead group at the first and follow-up visits. In the surviving group, treatment at the first visit mitigated inflammation and reduced OSI. However, in the dead group, poor response to the treatment provided at the first visit led to continued inflammation, and no change was observed the OSI.

KEYWORDS: horse, oxidative stress, pneumonia, *Rhodococcus equi*

*Correspondence to: Mizuguchi Y: yuuya_mizuguchi_g1@nosai-do.or.jp, Mitsuishi Animal Medical Center, NOSAI Minami, 200 Mitsuishi Higashihorai Shinhidaka-cho, Hokkaido 059-3105, Japan

出典　doi: 10.1292/jvms.23-0260.
https://www.jstage.jst.go.jp/article/jvms/85/12/85_23-0260/_article

最終責任者　Yuuya Mizuguchi (Corresponding Author)

The Journal of Veterinary Medical Science

NOTE

Surgery

A retrospective analysis for criteria of surgical intervention in Japanese Black calves with respiratory signs caused by perinatal rib fracture

Sueun KIM[1)], Mrunmayi Vishwanath NAIK[1)], Yumi KIRINO[1)], Hiroyuki SATOH[2)], Nao TSUZUKI[1)], Yoshiyuki INOUE[1)], Kohei KURODA[1)], Yuichi HIDAKA[1)]*

[1)]Laboratory of Veterinary Surgery, Department of Veterinary Science, University of Miyazaki, Miyazaki, Japan
[2)]Laboratory of Veterinary Clinical Radiology, Department of Veterinary Science, University of Miyazaki, Miyazaki, Japan

J. Vet. Med. Sci.
85(1): 40–43, 2023
doi: 10.1292/jvms.22-0332

Received: 23 July 2022
Accepted: 29 October 2022
Advanced Epub:
17 November 2022

ABSTRACT. Medical records of 16 Japanese Black calves with respiratory signs associated with perinatal rib fracture were analyzed, retrospectively, to speculate criteria of surgical intervention for the disease. For this analysis, the severity of respiratory condition was classified into three grades: Grade 1, with no wheezing, Grade 2, with wheezing after excitement but not at rest and Grade 3, with wheezing at rest. Grade 1 (n=3) received only conservative management. Seven of Grade 2 (n=8) and all of Grade 3 (n=5) had surgical management. Clinical outcome was good in all of Grade 1, and the surgical success rate was 83% in other groups. Our result suggests that surgical intervention should be considered for cases with more the severity of Grade 2.

KEYWORDS: calf, partial costectomy, rib fracture, surgical criteria, wheezing

*Correspondence to: Hidaka Y: yhidaka@cc.miyazaki-u.ac.jp, Laboratory of Veterinary Surgery, University of Miyazaki, 1-1 Gakuen Kibana-dai Nishi, Miyazaki 889-2192, Japan

出典　doi: 10.1292/jvms.22-0332.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9887215/

最終責任者　Yuichi Hidaka (Corresponding Author)

人獣共通感染症学（Zoonotic Diseases）

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）Identification of Three Novel Genes in Phenuiviridae Detected from Aedes Mosquitoes in Hokkaido, Japan.
Uchida L, Sakurai Y, Shimooka M, Morales-Vargas RE, Hagiwara K, **Muramatsu Y.**
*Jpn J Infect Dis.*76:55-63. 2023. doi: 10.7883/yoken.JJID.2022.179.

Ⅱ．その他　＜Others＞

Jpn. J. Infect. Dis., 76, 55-63, 2023

Original Article

Identification of Three Novel Genes in *Phenuiviridae* Detected from *Aedes* Mosquitoes in Hokkaido, Japan

Leo Uchida[1]*, Yoshimi Sakurai[1], Makoto Shimooka[1], Ronald Enrique Morales-Vargas[2], Katsuro Hagiwara[1], and Yasukazu Muramatsu[1]

[1]*School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan; and*
[2]*Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

ABSTRACT: Mosquitoes are important arthropod vectors of arboviruses. The family *Phenuiviridae* includes several medically important arboviruses, such as the Rift Valley fever phlebovirus and Toscana phlebovirus. Recent comprehensive genetic analyses have identified many novel mosquito-specific viruses that are phylogenetically related to *Phenuiviridae*. We collected mosquitoes from Hokkaido in northern Japan, and conducted reverse transcription polymerase chain reactions (RT-PCRs) targeting the RNA-dependent RNA polymerase (*RdRp*) gene of *Phenuiviridae*. A total of 285 pools, comprising 3,082 mosquitoes from 2 genera and 8 species, were collected. Partial *RdRp* sequences were detected in 97 pools, which allowed us to classify the viruses into 3 clusters provisionally designated as Etutanne virus (ETTV) 1, 2, and 3. The virus most closely related to ETTVs is Narangue virus (family *Phenuiviridae*, genus *Mobuvirus*), which was detected in *Mansonia* mosquitoes; the nucleotide and amino acid sequences of the Narangue virus are 58.4–66.2% and 64.7–86.7% similar, respectively, to those of ETTVs. PCR and RT-PCR using DNA and RNase digestion methods showed that the ETTVs are RNA viruses that do not form non-retroviral integrated RNA virus sequences in the mosquito genome.

INTRODUCTION

Arthropod-borne viruses (also known as arboviruses) are a group of viruses that "replicate in both arthropod vectors and vertebrate hosts and can be transmitted between vertebrate hosts by the arthropod vector" (1). Mosquito-borne viruses belong to a large variety of viral families, such as *Flaviviridae, Phenuiviridae* (order Bunyavirales), *Togaviridae*, and *Reoviridae*, which include several medically and veterinary important viruses; these viruses have accounted for 22.8% of emerging infectious disease events in the latter half of the 20th century (2). A recent study showed that the dengue virus (family *Flaviviridae*) has spread worldwide, with an estimated incidence of 390 million infections, with 96 million individuals showing clinical manifestations annually (3). In the veterinary field, the Rift Valley fever phlebovirus (family *Phenuiviridae*) causes not just human disease but also economic losses in the livestock industry at an estimated USD 5–471 million per outbreak (4). Vaccines and antiviral drugs for most mosquito-borne viral diseases are still under development; therefore, effective vector control strategies are required.

Mosquito-specific viruses are found in mosquitoes and mosquito-derived cell lines and generally do not infect or replicate in vertebrates (5,6). Recent studies have identified several new mosquito-specific viruses that belong to the families *Flaviviridae*, *Phenuiviridae*, *Rhabdoviridae*, and *Mesoniviridae*, as well as unassigned Negeviruses (5). These mosquito-specific viruses show no significant effects in vertebrates, but their potential as control strategies against vector-borne agents as a result of their cooperative or competitive effects on arboviruses has been a focus of interest (7,8). Next-generation sequencing technologies have led to the discovery of several partial viral genes that have been integrated into the arthropod genomes (9,10). These integrated elements are called non-retroviral integrated RNA virus sequences, and partial elements related to *Flaviviridae*, *Phenuiviridae*, *Phasmaviridae*, *Hantaviridae* (order Bunyavirales), and *Rhabdoviridae* have been identified in the mosquito genomes (9,10).

In Japan, approximately 18 genera, including 114 species of mosquitoes, are distributed from Hokkaido in the northeast to Okinawa in the southwest, encompassing an area of approximately 20° latitude

Received March 18, 2022. Accepted September 12, 2022.
J-STAGE Advance Publication September 30, 2022.
DOI: 10.7883/yoken.JJID.2022.179
*Corresponding author: Mailing address: School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu-shi, Hokkaido 069-8501, Japan. Tel: +81-11-388-4828, Fax: +81-11-388-4828, E-mail: uchidaleo@rakuno.ac.jp

出典 doi: 10.7883/yoken.JJID.2022.179.
https://www.jstage.jst.go.jp/article/yoken/76/1/76_JJID.2022.179/_pdf/-char/en
最終責任者 Leo Uchida (Corresponding Author)

獣医解剖学（Veterinary Anatomy）

Hirimi Ueda

Associate Professor

准教授　植田　弘美

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

Ⅱ．その他　＜Others＞

1）Oviduct histopathology of internal laying and egg-bound syndrome in laying hens. Hosotani M, Hamano S, Iwasaki T, Hasegawa Y, **Ueda H**, Watanabe T. *Vet Sci.* 10: 260. 2023. doi: 10.3390/vetsci10040260.

veterinary sciences

MDPI

Article

Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens

Marina Hosotani [1,*], Sohei Hamano [1], Tomohito Iwasaki [2], Yasuhiro Hasegawa [2], Hiromi Ueda [1] and Takafumi Watanabe [1]

[1] Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
[2] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Ebetsu 069-8501, Hokakido, Japan
* Correspondence: m-hosotani@rakuno.ac.jp; Tel.: +81-11-388-4763

Simple Summary: Internal laying and egg-bound syndromes are avian reproductive disorders that reduce egg productivity in laying hens. To date, the understanding of the importance of peristalsis abnormality in the smooth muscle of oviducts has been focused on the pathogeneses of internal laying and egg-bound syndrome, and the histopathology of the oviductal epithelium has not been explored. In this study, we histologically examined the oviductal ciliated epithelium of aged laying hens. We observe that the epithelial region lacking cilia is larger in the oviducts of hens with internal laying and egg-bound syndrome than in those of healthy hens. In addition, the lamina propria of the oviducts of hens with internal laying and egg-bound syndrome is affected by the infiltration of CD3-positive T-cells. The histological alternation of the ciliated epithelial cells in the oviducts owing to the oviductal inflammation is suggested as the underlying cause of the pathogeneses of internal laying and egg-bound syndrome.

Abstract: In the egg industry, common reproductive disorders, such as internal laying and egg-bound syndrome, not only reduce egg productivity but also cause deaths in severe cases. In this study, we focused on the oviduct histology of the pathogenesis of internal laying and egg-bound syndrome. We divided the aged laying hens into four groups according to the observation of the abdominal cavity and oviductal lumen: healthy, internal laying, egg-bound, and intercurrent. The percentages of healthy, internal laying, egg-bound, and intercurrent groups were 55%, 17.5%, 15%, and 12.5%, respectively. In all parts of the oviduct (i.e., infundibulum, magnum, isthmus, and uterus), the oviductal epithelium was composed of ciliated epithelial cells and secretory cells. The epithelial region lacking cilia was larger in the entire oviduct of the internal laying, and intercurrent groups than in the healthy group. In the internal laying, egg-bound, and intercurrent groups, significant T-cell infiltration was observed in the lamina propria of the entire oviduct. The morphological alteration of ciliated epithelial cells in the oviducts caused by inflammation may be the underlying cause of the pathogenesis of internal laying and egg-bound syndrome.

Keywords: cilia; chicken; egg-bound syndrome; histology; internal laying; oviduct; reproductive disorder

Citation: Hosotani, M.; Hamano, S.; Iwasaki, T.; Hasegawa, Y.; Ueda, H.; Watanabe, T. Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens. *Vet. Sci.* **2023**, *10*, 260. https://doi.org/10.3390/vetsci10040260

Academic Editor: Manuel Aviles

Received: 14 February 2023
Revised: 25 March 2023
Accepted: 27 March 2023
Published: 29 March 2023

1. Introduction

Selective breeding of laying hens has achieved an approximately two-fold higher egg productivity over the decades [1,2]; however, this has led to the emergence of reproductive disorders. Caged layer fatigue owing to the excess demand for calcium for frequent egg laying includes not only osteopenia but also oviductal inertia because muscle contraction requires calcium [3]. Laying hens spontaneously increase the risk of ovarian cancer with aging, along with decreased egg productivity [4,5]. In addition to genetic factors, rearing

出典 doi: 10.3390/vetsci10040260

https://www.ncbi.nlm.nih.gov/pubmed/37104415

最終責任者 Marina Hosotani (Corresponding Author)

獣医解剖学（Veterinary Anatomy）

Takafumi Watanabe

Associate Professor

准教授　渡邉　敬文

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

Ⅱ．その他＜Others＞

1） Physicochemical properties of wooden breast-extracted myosin and rheological properties of its heat-induced gel.
Hasegawa Y, Kawasaki T, Yamada M, Hosotani M, Maeda N, **Watanabe T**, Iwasaki T.
J Science Food Agric. 103:5609–5615. 2023. doi: 10.1002/jsfa.12636.

2） Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens.
Hosotani M, Hamano S, Iwasaki T, Hasegawa Y, Ueda H, **Watanabe T**.
Vet Sci. 10:260–260. 2023. doi: 10.3390/vetsci10040260.

3） Oocyte cumulus complex quality and oviduct transportation velocity in systemic autoimmune disease model mice.
Hosotani M, Ichii O, **Watanabe Y**, Kon Y.
Exp Biol Med (Maywood) 248:1359–1363. 2023.
doi: 10.1177/15353702231160875.

4） Collagen Network Formation in Vitro Models of Musculocontractural Ehlers-Danlos Syndrome.
Hashimoto A, Hirose T, Hashimoto K, Mizumoto S, Nitahara-Kasahara Y, Saka S, Yoshizawa T, Okada T, Yamada S, Kosho T, **Watanabe T**, Miyata S, Nomura Y.
Genes (Basel) 14:308. 2023. doi: 10.3390/genes14020308.

Journal of the Science of Food and Agriculture

Research Article

Physicochemical properties of wooden breast-extracted myosin and rheological properties of its heat-induced gel

Yasuhiro Hasegawa, Takeshi Kawasaki, Michi Yamada, Marina Hosotani, Naoyuki Maeda, Takafumi Watanabe, Tomohito Iwasaki

First published: 17 April 2023 | https://doi.org/10.1002/jsfa.12636

Abstract

BACKGROUND

It is reported that broilers with 'wooden breast' have poor processing properties, such as low binding and water-holding capacities. However, the reason for the poor functional characteristics has not been clarified. In this study, myosin was extracted from a wooden breast. Its physicochemical properties were investigated to clarify the relationship between the structure and physicochemical properties of the heating gel of myosin obtained from the wooden breast.

RESULTS

The turbidity of myosin solution extracted from wooden breast increased with increase in the heat treatment to a higher value than that from the normal breast meat myosin. The solubility of myosin collected from a wooden breast after heating decreased like normal breast muscle myosin. The surface hydrophobicity of myosin removed from wooden breast increased continually above 60 °C, unlike the change in surface hydrophobicity of normal breast myosin. The free thiol group of myosin extracted from the wooden breast was higher than normal breast myosin before and after heating. The apparent elasticity of heat-induced gels and chicken meat sausages was significantly lower in sausages and gel with wooden breast than normal ones ($P < 0.05$). The microstructure of the heated gel of normal myosin showed a fine network structure. In contrast, the heat-induced gel of wooden breast-extracted myosin showed a structure with loosely connected aggregates and many gaps.

CONCLUSION

The coarseness of the internal gel structure of myosin extracted from wooden breast was shown to affect the apparent elasticity of the gel and sausages made from the chicken meat.

出典　doi: 10.1002/jsfa.12636.

https://onlinelibrary.wiley.com/doi/10.1002/jsfa.12636

最終責任者　Tomohito Iwasaki (Corresponding Author)

veterinary sciences

MDPI

Article

Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens

Marina Hosotani [1,*], Sohei Hamano [1], Tomohito Iwasaki [2], Yasuhiro Hasegawa [2], Hiromi Ueda [1] and Takafumi Watanabe [1]

[1] Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
[2] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Ebetsu 069-8501, Hokakido, Japan
* Correspondence: m-hosotani@rakuno.ac.jp; Tel.: +81-11-388-4763

Simple Summary: Internal laying and egg-bound syndromes are avian reproductive disorders that reduce egg productivity in laying hens. To date, the understanding of the importance of peristalsis abnormality in the smooth muscle of oviducts has been focused on the pathogeneses of internal laying and egg-bound syndrome, and the histopathology of the oviductal epithelium has not been explored. In this study, we histologically examined the oviductal ciliated epithelium of aged laying hens. We observe that the epithelial region lacking cilia is larger in the oviducts of hens with internal laying and egg-bound syndrome than in those of healthy hens. In addition, the lamina propria of the oviducts of hens with internal laying and egg-bound syndrome is affected by the infiltration of CD3-positive T-cells. The histological alternation of the ciliated epithelial cells in the oviducts owing to the oviductal inflammation is suggested as the underlying cause of the pathogeneses of internal laying and egg-bound syndrome.

Abstract: In the egg industry, common reproductive disorders, such as internal laying and egg-bound syndrome, not only reduce egg productivity but also cause deaths in severe cases. In this study, we focused on the oviduct histology of the pathogenesis of internal laying and egg-bound syndrome. We divided the aged laying hens into four groups according to the observation of the abdominal cavity and oviductal lumen: healthy, internal laying, egg-bound, and intercurrent. The percentages of healthy, internal laying, egg-bound, and intercurrent groups were 55%, 17.5%, 15%, and 12.5%, respectively. In all parts of the oviduct (i.e., infundibulum, magnum, isthmus, and uterus), the oviductal epithelium was composed of ciliated epithelial cells and secretory cells. The epithelial region lacking cilia was larger in the entire oviduct of the internal laying, and intercurrent groups than in the healthy group. In the internal laying, egg-bound, and intercurrent groups, significant T-cell infiltration was observed in the lamina propria of the entire oviduct. The morphological alteration of ciliated epithelial cells in the oviducts caused by inflammation may be the underlying cause of the pathogenesis of internal laying and egg-bound syndrome.

Keywords: cilia; chicken; egg-bound syndrome; histology; internal laying; oviduct; reproductive disorder

Citation: Hosotani, M.; Hamano, S.; Iwasaki, T.; Hasegawa, Y.; Ueda, H.; Watanabe, T. Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens. *Vet. Sci.* **2023**, *10*, 260. https://doi.org/10.3390/vetsci10040260

Academic Editor: Manuel Aviles

Received: 14 February 2023
Revised: 25 March 2023
Accepted: 27 March 2023
Published: 29 March 2023

出典　doi: 10.3390/vetsci10040260.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10142957/

最終責任者　Marina Hosotani (Corresponding Author)

Brief Communication

Oocyte cumulus complex quality and oviduct transportation velocity in systemic autoimmune disease model mice

Marina Hosotani[1], Osamu Ichii[2,3], Takafumi Watanabe[1] and Yasuhiro Kon[2]

[1]Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; [2]Laboratory of Anatomy, Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan; [3]Laboratory of Agrobiomedical Science, Faculty of Agriculture, Hokkaido University, Sapporo 060-0818, Japan
Corresponding author: Marina Hosotani. Email: m-hosotani@rakuno.ac.jp

Impact Statement

Oocyte transportation consists of two steps: adhesion of cumulus cells to the ciliary tip of ciliated epithelial cells and transportation of cumulus–oocyte complexes (COCs) by ciliary beating. In the former step, the pathological factors that alter the interaction between cumulus cells and cilia are not currently understood. In this study, by using autoimmune disease-prone MRL/MpJ-*Fas*$^{lpr/lpr}$ mice that exhibited the oocyte transportation disorder by abnormal morphofunction of oviductal ciliated epithelium, we revealed that COC transportation property was determined by both the ciliary function in the infundibulum and the properties of COCs. Furthermore, we showed that the transportation velocity of COCs (TVCs) was recovered by the properties of cumulus cells and the healthy morphofunction of oviductal ciliated epithelium. These findings contribute to further investigations on novel immunological factors in COCs that can achieve efficient oocyte transportation and related processes, which provide the potential for understanding the pathogenesis of tubal infertility.

Abstract

Oocyte transportation by the oviduct involves the interaction between ciliated epithelial cells and cumulus cells. To determine whether the quality of cumulus–oocyte complexes (COCs) changes the transportation property of COCs, we compared the transportation velocity of COCs (TVC) by the infundibulum *ex vivo* with various combinations of infundibula and COCs collected from different mice. We used young and aged C57BL/6N and MRL/MpJ, and MRL/MpJ-*Fas*$^{lpr/lpr}$ mice as the strains with intact female reproductive function and the systemic autoimmune disease model exhibiting oocyte pick-up dysfunction owing to the morphofunctional abnormality of ciliated epithelium, respectively. The TVC of aged MRL strains was less than that of aged C57BL/6N mice, suggesting that aging affects the transportation of COCs in MRL strains. The TVC of aged MRL/MpJ-*Fas*$^{lpr/lpr}$ mice was the least among all examined combinations, whereas the TVC accelerated when the infundibulum or COCs were collected from other strains. These results indicate that the transportation property of COCs is determined not only by the ciliary function in the infundibulum but also by the properties of COCs.

Keywords: Autoimmune disease, cumulus cell, cilia, *ex vivo* experiment, oocyte transportation, oviduct

Experimental Biology and Medicine **2023; 248: 1359–1363. DOI: 10.1177/15353702231160875**

出典　doi: 10.1177/15353702231160875.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10657593/
最終責任者　Marina Hosotani (Corresponding Author)

genes

MDPI

Article

Collagen Network Formation in In Vitro Models of Musculocontractural Ehlers–Danlos Syndrome

Ayana Hashimoto [1], Takuya Hirose [2], Kohei Hashimoto [1], Shuji Mizumoto [3], Yuko Nitahara-Kasahara [4], Shota Saka [1], Takahiro Yoshizawa [5], Takashi Okada [4], Shuhei Yamada [3], Tomoki Kosho [6,7,8,9], Takafumi Watanabe [2], Shinji Miyata [1,*] and Yoshihiro Nomura [1]

[1] Graduate School of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan
[2] Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
[3] Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University, Nagoya 468-8503, Aichi, Japan
[4] Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639, Japan
[5] Division of Animal Research, Research Center for Advanced Science and Technology, Shinshu University, Matsumoto 390-8621, Nagano, Japan
[6] Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto 390-8621, Nagano, Japan
[7] Center for Medical Genetics, Shinshu University Hospital, Matsumoto 390-8621, Nagano, Japan
[8] Division of Clinical Sequencing, Shinshu University School of Medicine, Matsumoto 390-8621, Nagano, Japan
[9] Research Center for Supports to Advanced Science, Matsumoto 390-8621, Nagano, Japan
* Correspondence: smiyata@go.tuat.ac.jp

Abstract: Loss-of-function mutations in *carbohydrate sulfotransferase 14* (*CHST14*) cause musculocontractural Ehlers–Danlos syndrome-*CHST14* (mcEDS-*CHST14*), characterized by multiple congenital malformations and progressive connective tissue fragility-related manifestations in the cutaneous, skeletal, cardiovascular, visceral and ocular system. The replacement of dermatan sulfate chains on decorin proteoglycan with chondroitin sulfate chains is proposed to lead to the disorganization of collagen networks in the skin. However, the pathogenic mechanisms of mcEDS-*CHST14* are not fully understood, partly due to the lack of in vitro models of this disease. In the present study, we established in vitro models of fibroblast-mediated collagen network formation that recapacitate mcEDS-*CHST14* pathology. Electron microscopy analysis of mcEDS-*CHST14*-mimicking collagen gels revealed an impaired fibrillar organization that resulted in weaker mechanical strength of the gels. The addition of decorin isolated from patients with mcEDS-*CHST14* and *Chst14*$^{-/-}$ mice disturbed the assembly of collagen fibrils in vitro compared to control decorin. Our study may provide useful in vitro models of mcEDS-*CHST14* to elucidate the pathomechanism of this disease.

Keywords: Ehlers–Danlos syndrome; decorin; collagen; dermatan sulfate proteoglycan; fibrillogenesis; carbohydrate sulfotransferase 14; mcEDS-*CHST14*

Citation: Hashimoto, A.; Hirose, T.; Hashimoto, K.; Mizumoto, S.; Nitahara-Kasahara, Y.; Saka, S.; Yoshizawa, T.; Okada, T.; Yamada, S.; Kosho, T.; et al. Collagen Network Formation in In Vitro Models of Musculocontractural Ehlers–Danlos Syndrome. *Genes* **2023**, *14*, 308. https://doi.org/10.3390/genes14020308

Academic Editor: Marina Colombi

Received: 30 November 2022
Revised: 5 January 2023

出典　doi: 10.3390/genes14020308.
https://www.mdpi.com/2073-4425/14/2/308

最終責任者　Shinji Miyata (Corresponding Author)

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

Hiroki Yamazaki

Associate Professor

准教授　山﨑　裕毅

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

1) Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism.
Yamazaki H, Onoyama S, Gotani S, Deguchi T, Tamura M, Ohta H, Hidetomo I, Nishida H, Dickinson PJ, Akiyoshi H.
Cancers 15:5537. 2023. doi: 10.3390/cancers15235537
2) Assessment of hypoxia-targeting therapy for gastrointestinal lymphoma in dogs: Preclinical test using murine models.
Yamazaki H, Tanaka T, Nishida H, Hatoya S, Akiyoshi H.
Res Vet Sci. 154:22-28. 2023. doi: 10.1016/j.rvsc.2022.11.003.
3) Hypoxia-targeting therapy for intestinal T-cell lymphoma in dogs: Preclinical study using 3D in vitro models.
Yamazaki H, Tanaka T, Nishida H, Hatoya S, Akiyoshi H.
Vet Comp Oncol. 21:12-19. 2023. doi: 10.1111/vco.12855.

Ⅱ．その他　＜Others＞

cancers

MDPI

Article

Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism

Hiroki Yamazaki [1,*], Seio Onoyama [1], Shunichi Gotani [1], Tatsuya Deguchi [1], Masahiro Tamura [1], Hiroshi Ohta [1], Hidetomo Iwano [2], Hidetaka Nishida [3], Peter J. Dickinson [4] and Hideo Akiyoshi [5]

[1] Laboratory of Veterinary Internal Medicine, Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu 069-0836, Japan; t-deguchi@rakuno.ac.jp (T.D.); m-tamura@rakuno.ac.jp (M.T.); h-ohta@rakuno.ac.jp (H.O.)
[2] Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu 069-0836, Japan; h-iwano@rakuno.ac.jp
[3] Laboratory of Small Animal Clinics, Veterinary Teaching Hospital, Graduate School of Veterinary Science, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara 52-5201, Japan; h-nishida@azabu-u.ac.jp
[4] Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA; pjdickinson@ucdavis.edu
[5] Laboratory of Veterinary Surgery, Graduate School of Life and Environmental Sciences, Osaka Metropolitan University, 1-58 Rinku-Oraikita, Izumisano 598-8531, Japan
* Correspondence: author. hyamazaki@rakuno.ac.jp; Tel.: +81-011-388-4767

Simple Summary: This study investigated the anti-glycolytic effects of evofosfamide (EVO) on three canine glioma (GL)-derived cell lines with activated hypoxia-inducible factor 1α (HIF-1α). Our clinical data showed that glycolytic activity was correlated with poorer outcomes in dogs with spontaneous GL. Our in vitro studies showed that EVO inhibited glycolytic metabolism by targeting HIF-1α-positive cells under hypoxic culture conditions, resulting in the suppression of cellular ATP production. Our in vivo studies showed that EVO significantly decreased tumor development compared to controls or temozolo-mid in orthotopic murine GL models. A metabolic analysis demonstrated that EVO suppressed glycolytic activity by eliminating HIF-1α-positive cells. Our findings suggest that EVO may improve cancer metabolism and restore the microenvironment for both canine and human GL.

Abstract: The transcription factor hypoxia-inducible factor 1α (HIF-1α) drives metabolic reprogramming in gliomas (GLs) under hypoxic conditions, promoting glycolysis for tumor development. Evofosfamide (EVO) releases a DNA-alkylating agent within hypoxic regions, indicating that it may serve as a hypoxia-targeted therapy. The aim of this study was to investigate the glycolytic metabolism and antitumor effects of EVO in a canine GL model. Our clinical data showed that overall survival was significantly decreased in GL dog patients with higher HIF-1α expression compared to that of those with lower HIF-1α expression, and there was a positive correlation between HIF-1α and pyruvate dehydrogenase kinase 1 (PDK1) expression, suggesting that glycolytic activity under hypoxia conditions may contribute to poor outcomes in canine GL. Our glycolysis assay tests showed that the glycolytic ATP level was higher than the mitochondrial ATP level in three types of canine GL cell lines by activating the HIF-1 signal pathway under hypoxia conditions, resulting in an overall increase in total cellular ATP production. However, treatment with EVO inhibited the glycolytic ATP level in the GL cell lines under hypoxia conditions by targeting HIF-1α-positive cells, leading to decrease in total cellular ATP production. Our in vivo tests showed that EVO significantly reduced tumor development compared to controls and temozolomide in murine GL models. A metabolic analysis demonstrated that EVO effectively suppressed glycolytic metabolism by eliminating HIF-1α-positive cells, suggesting that it may restore metabolism in canine GLs. The evidence presented here supports the favorable preclinical evaluation of EVO as a potential improvement in cancer metabolism.

check for updates

Citation: Yamazaki, H.; Onoyama, S.; Gotani, S.; Deguchi, T.; Tamura, M.; Ohta, H.; Iwano, H.; Nishida, H.; Dickinson, P.J.; Akiyoshi, H. Influence of the Hypoxia-Activated Prodrug Evofosfamide (TH-302) on Glycolytic Metabolism of Canine Glioma: A Potential Improvement in Cancer Metabolism. *Cancers* **2023**, *15*, 5537. https://doi.org/10.3390/cancers15235537

Academic Editors: Panagiotis Liakos and Ilias Mylonis

Received: 18 September 2023
Revised: 8 November 2023
Accepted: 16 November 2023
Published: 22 November 2023

出典 doi:10.3390/cancers15235537

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10705110/pdf/cancers-15-05537.pdf

最終責任者 Hiroki Yamazaki (First Author and Corresponding Author)

Research in Veterinary Science
Volume 154, January 2023, Pages 22-28

Assessment of hypoxia-targeting therapy for gastrointestinal lymphoma in dogs: Preclinical test using murine models

Hiroki Yamazaki [a, b] ✉, Toshiyuki Tanaka [c], Hidetaka Nishida [b], Shingo Hatoya [d], Hideo Akiyoshi [b]

[a] Laboratory of Veterinary Internal Medicine, Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582-1 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

[b] Laboratory of Veterinary Surgery, Graduate School of Life and Environmental Sciences, Osaka Metropolitan University, 1-58 Rinku-orai-kita, Izumisano, Osaka 598-8531, Japan

[c] Veterinary Medical Center, Graduate School of Life and Environmental Sciences, Osaka Metropolitan University, Osaka, Japan

[d] Laboratory of Cell Pathobiology, Graduate School of Life and Environmental Sciences, Osaka Metropolitan University, 1-58 Rinku-orai-kita, Izumisano, Osaka 598-8531, Japan

Received 21 October 2022, Revised 31 October 2022, Accepted 2 November 2022, Available online 12 November 2022, Version of Record 17 November 2022.

Abstract

The transcription factor hypoxia-inducible factor 1α (HIF-1α) is expressed in several cancers under intratumoral hypoxic stress that arises during pathogenic processes, resulting in malignant progression. We previously reported that hypoxic stimulation enhances the growth potential of canine lymphoma cells by activating the HIF-1α signaling pathway. In contrast, evofosfamide (Evo) releases a DNA-alkylating moiety within hypoxic tumor regions, suggesting that Evo could serve as a hypoxia-targeting drug in canine lymphoma. This study aimed to use Evo to evaluate hypoxia-targeted therapy in dogs with gastrointestinal lymphoma (GIL) and investigate how Evo affects antitumor efficacy and adverse events in three type of murine xenograft models using T-cell GIL cells. In vitro tests, the sensitivity to Evo of three T-cell GIL cell lines under hypoxic culture was significantly higher than that under normoxic culture. Our metabolic analysis suggested that the three murine models might have high reproducibility as clinical cases in canine GIL. Our data showed that Evo showed significantly higher tumor growth potential and fewer adverse events in three type of murine models compared to lomustine; CeeNu (CCNU). Additionally, Evo suppressed the expression of HIF-1α protein in tumor tissues, suggesting that it may preferentially target and inhibit tumor cells in a hypoxic region. The evidence presented here supports the favorable preclinical evaluation that Evo may be effective for GIL in dogs.

出典 doi: 10.1016/j.rvsc.2022.11.003.

https://www.sciencedirect.com/science/article/abs/pii/S0034528822003605?via%3Dihub

最終責任者 Hiroki Yamazaki (First Author and Corresponding Author)

Veterinary and Comparative Oncology

ORIGINAL ARTICLE

Hypoxia-targeting therapy for intestinal T-cell lymphoma in dogs: Preclinical study using 3D in vitro models

Hiroki Yamazaki, Toshiyuki Tanaka, Hidetaka Nishida, Shingo Hatoya, Hideo Akiyoshi

First published: 11 August 2022 | https://doi.org/10.1111/vco.12855

Funding information: Japan Society for the Promotion of Science; Japan Society for the Promotion of Science (JSPS) KAKENHI, Grant/Award Numbers: 20K15682, 18K14591

Abstract

The transcription factor hypoxia-inducible factor 1α (HIF-1α) is activated in response to oxygen deficiency, and is expressed in several cancers under intratumoral hypoxic stress that arises during pathogenic processes. Hypoxic stimulation enhanced the growth potential of canine lymphoma cells by activating the HIF-1α signalling pathway in a previously reported study. The aim of this study was to establish a molecular design strategy for a novel hypoxia-targeting therapy for intestinal T-cell lymphoma (ITL) in dogs. We assessed the relationship between immunohistochemistry-based HIF-1α expression and clinical information, including signalment, tumour area, clinical signs, systemic diseases, treatment protocol, follow-up information, chemotherapy response and overall survivals (OS), using 48 tissue samples from dogs with ITL. We investigated the effects of hypoxic stimulation on the biological behaviour of cell lines from three different types of canine ITL. We assessed the effects of evofosfamide (Evo; hypoxia-activated prodrug) on cell lines cultured under hypoxic conditions. Our data showed that treatment response and overall survival might be significantly decreased in dogs with higher HIF-1α expression than in those with lower HIF-1α expression. Hypoxic culture (1% O_2, 72 h) enhanced the invasiveness of cell lines and decreased their sensitivity to CCNU, resulting in hypoxia-dependent aggressive behaviour. Sensitivity to Evo significantly increased in cell lines cultured under hypoxia compared with those cultured under normoxia, which exhibited hypoxia-dependent apoptosis. Additionally, Evo downregulated HIF-1α expression in cell lines cultured under hypoxia, suggesting that Evo might inhibit cell growth by inactivating HIF-1α-dependent cell signalling. Our results revealed the preclinical antitumor activity of Evo and provide a rationale for treatment strategies for dogs with ITL.

出典　doi: 10.1111/vco.12855.

https://onlinelibrary.wiley.com/doi/10.1111/vco.12855

最終責任者　Hiroki Yamazaki (First Author and Corresponding Author)

獣医生化学（Veterinary Biochemistry）

Jumpei Fujiki

Lecturer

講師　藤木　純平

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

1) Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy.
Fujiki J, Nakamura K, Nakamura T, Iwano H.
Int J Mol Sci. 24:15628. 2023. doi: 10.3390/ijms242115628.

2) Phage therapy: Targeting intestinal bacterial microbiota for the treatment of liver diseases.
Fujiki J, Schnabl B.
JHEP Rep. 5:100909. 2023. doi: 10.1016/j.jhepr.2023.100909.

Ⅱ．その他＜Others＞

1) Mechanism of skeletal muscle atrophy by muscle fiber types in male rats under long-term fasting stress.
Ieko T, **Fujiki J**, Hasegawa Y, Iwasaki T, Iwano H, Maeda N.
Steroids. 200:109328. 2023. doi: 10.1016/j.steroids.2023.109328.

2) Isolation of Streptococcus mutans temperate bacteriophage with broad killing activity to S. mutans clinical isolates.
Sugai K, Kawada-Matsuo M, Nguyen-Tra Le M, Sugawara Y, Hisatsune J, **Fujiki J**, Iwano H, Tanimoto K, Sugai M, Komatsuzawa H.
iScience. 26:108465. 2023. doi: 10.1016/j.isci.2023.108465.

International Journal of
Molecular Sciences

MDPI

Review

Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy

Jumpei Fujiki [1,2], Keisuke Nakamura [1], Tomohiro Nakamura [1,3,4,5] and Hidetomo Iwano [1,3,*]

[1] Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; j-fujiki@rakuno.ac.jp (J.F.); s21661158@g.rakuno.ac.jp (K.N.); t-nakamura@azabu-u.ac.jp (T.N.)
[2] Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA
[3] Phage Therapy Institute, Waseda University, Tokyo 169-8050, Japan
[4] Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases, Tokyo 208-0011, Japan
[5] Department of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan
* Correspondence: h-iwano@rakuno.ac.jp; Tel./Fax: +81-11-388-4885

Abstract: In recent decades, phage therapy has been overshadowed by the widespread use of antibiotics in Western countries. However, it has been revitalized as a powerful approach due to the increasing prevalence of antimicrobial-resistant bacteria. Although bacterial resistance to phages has been reported in clinical cases, recent studies on the fitness trade-offs between phage and antibiotic resistance have revealed new avenues in the field of phage therapy. This strategy aims to restore the antibiotic susceptibility of antimicrobial-resistant bacteria, even if phage-resistant variants develop. Here, we summarize the basic virological properties of phages and their applications within the context of antimicrobial resistance. In addition, we review the occurrence of phage resistance in clinical cases, and examine fitness trade-offs between phage and antibiotic sensitivity, exploring the potential of an evolutionary fitness cost as a countermeasure against phage resistance in therapy. Finally, we discuss future strategies and directions for phage-based therapy from the aspect of fitness trade-offs. This approach is expected to provide robust options when combined with antibiotics in this era of phage 're'-discovery.

Keywords: bacteriophage; antimicrobial resistance (AMR); fitness cost; phage cocktail; engineered phage; infectious disease; infection control; *P. aeruginosa*; *K. pneumoniae*; *A. baumannii*; *S. aureus*; evolution; arms race

Citation: Fujiki, J.; Nakamura, K.; Nakamura, T.; Iwano, H. Fitness Trade-Offs between Phage and Antibiotic Sensitivity in Phage-Resistant Variants: Molecular Action and Insights into Clinical Applications for Phage Therapy. *Int. J. Mol. Sci.* **2023**, *24*, 15628. https://doi.org/10.3390/ijms242115628

Academic Editor: Kosuke Fujimoto

Received: 6 October 2023
Revised: 23 October 2023
Accepted: 23 October 2023
Published: 26 October 2023

1. Introduction

Bacteriophages, also known simply as phages, are prokaryotic viruses that exclusively infect and kill bacteria. While phages were already being explored as antimicrobial agents in the early 1900s [1,2], the rise of antimicrobial chemical agents that became increasingly popular after the discovery of penicillin, overshadowed their use in Western countries [3]. However, after decades of being overlooked, the emergence of bacteria that are resistant to these antibacterial agents has refocused efforts on the use of phages for treating infectious diseases [4–6]. On the other hand, bacterial resistance to phages can also arise through a variety of molecular mechanisms. Several clinical studies on phage therapy have reported the occurrence of phage-resistant variants, which represents a significant concern for the successful development of phage-based therapies [7]. It is therefore important to carefully address phage resistance within the context of developing anti-bacterial treatments and therapies.

Phages have played a significant role in shaping the evolution of bacterial communities and populations through a co-evolutionary mechanism known as an arms race [8]. Recent

出典 doi: 10.3390/ijms242115628.

https://www.mdpi.com/1422-0067/24/21/15628

最終責任者 Hidetomo Iwano (Corresponding Author)

Review JHEP|Reports

Phage therapy: Targeting intestinal bacterial microbiota for the treatment of liver diseases

Jumpei Fujiki,[1,2] Bernd Schnabl[1,3,*]

Summary

Phage therapy has been overshadowed by antibiotics for decades. However, it is being revisited as a powerful approach against antimicrobial-resistant bacteria. As bacterial microbiota have been mechanistically linked to gastrointestinal and liver diseases, precise editing of the gut microbiota via the selective bactericidal action of phages has prompted renewed interest in phage therapy. In this review, we summarise the basic virological properties of phages and the latest findings on the composition of the intestinal phageome and the changes associated with liver diseases. We also review preclinical and clinical studies assessing phage therapy for the treatment of gastrointestinal and liver diseases, as well as future prospects and challenges.

Keywords: Microbiome; Virome; Phageome; Gut-liver-axis; NAFLD; NASH; PSC; Alcohol use disorder; Alcohol-associated hepatitis

Received 11 July 2023; received in revised form 16 August 2023; accepted 22 August 2023; available online 23 September 2023

Introduction

Bacteriophages, or simply phages, are prokaryotic viruses that infect and kill bacteria. Although phages were already known antimicrobial agents by the early 1900s,[1,2] the development and spread of antimicrobial chemicals, beginning with the discovery of penicillin,[3] made phage therapy less important; however, the emergence of antimicrobial-resistant bacteria has brought phages back into focus for the treatment of infectious diseases.[4,5] Notably, since several specific bacteria in the gut are involved in the pathogenesis of gastrointestinal and liver diseases, restoring eubiosis by selectively eliminating these so-called pathobionts is an attractive approach.[6,7] Treatment with broad-spectrum antibacterial agents may promote further dysbiosis.[8,9] In this context, the host specificity of phages has the potential to precisely edit the intestinal bacterial microbiota. Taken together, "old and new" phages can target antimicrobial-resistant bacteria for the treatment of infectious diseases and precisely edit the bacterial microbiota for the treatment of diseases linked to pathobionts. From these perspectives, this review outlines the basic virological properties of phages and their current applications. We will summarise the role and composition, including disease-associated changes, of phages in the virome, and the applicability of phage therapy for the treatment of gastrointestinal and liver diseases. Finally, we will review challenges and future prospects for phage-based therapy.

Phage biology

Phages are ubiquitous, with an estimated population of more than 10^{31} particles, making them the most enriched biological entities in the biosphere.[10,11] Phages exist universally wherever living bacteria are present, including soil, lakes, oceans and the human body, and act as predators to regulate the bacterial microbiota.[12–15]

All phages possess a capsid enclosing their genome. The genomic nucleic acid of phages can be categorised as linear double-stranded DNA, linear single-stranded or double-stranded RNA, or circular single-stranded DNA. In addition, almost all phage capsids are connected to the tail, which is essential for attachment to host cells and injection of the genome from the capsid.[3,5,7] Previously, *Caudovirales* was a class of viruses known as tailed phages with double-stranded DNA that were divided into three groups based on their morphologies, namely *Myoviridae* (contractile tail), *Siphoviridae* (long non-contractile tail), and *Podoviridae* (short tail). Although phages have previously been classified by their morphology, the International Committee on Taxonomy of Viruses recently updated viral taxa based on the sequence information alone and abolished morphology-based taxa.[16] The renewed viral taxa encompass the class *Caudoviricetes*, comprised of the *Crassvirales*, *Kirjokansivirales*, *Thumleimavirales*, *Methanobavirales* and unspecified orders, giving rise to 22 newly identified bacterial virus families within this class.

The phage life cycle can be classified into lysogenic and lytic (Fig. 1). Phages that go through these two cycles are defined as temperate phages, whereas those that only go through lytic cycles are called virulent phages. During the lytic infection cycle, phages are primarily adsorbed to specific receptors on the bacterial surface, which allows them to inject their genome into the bacterium. After phage gene expression and genome replication,

[1]*Department of Medicine, University of California San Diego, La Jolla, CA, USA;* [2]*Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan;* [3]*Department of Medicine, VA San Diego Healthcare System, San Diego, CA, USA*

* Corresponding author. Address: Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA; Tel.: +1-858-822-5311, fax: +1-858-246-1788.
E-mail address: beschnabl@health.ucsd.edu (B. Schnabl).

出典 doi: 10.1016/j.jhepr.2023.100909.

https://www.sciencedirect.com/science/article/pii/S2589555923002409?via%3Dihub

最終責任者 Bernd Schnabl (Corresponding Author)

Steroids 200 (2023) 109328

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Mechanism of skeletal muscle atrophy by muscle fiber types in male rats under long-term fasting stress

Takahiro Ieko [a], Jumpei Fujiki [a], Yasuhiro Hasegawa [b], Tomohito Iwasaki [b], Hidetomo Iwano [a], Naoyuki Maeda [a,b,*]

[a] Laboratory of Veterinary Biochemistry, Department of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[b] Laboratory of Meat Science, Department of Food Science and Human Wellness, Rakuno Gakuen University, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Corticosterone
Mammalian target of rapamycin
Interleukin-6
Branched-chain amino acids
And fasting stress

ABSTRACT

Fasting induces metabolic changes in muscles, which are differentiated by muscle fiber type. In this study, the mechanism of fasting-induced muscle atrophy in rats was examined to determine the differences between muscle fiber types in energy production. Fasting for 96 h did not alter the weight of the soleus (SOL), a fiber type I muscle, but did significantly reduce the weight of gastrocnemius (GM), a fiber type II muscle. GM, SOL and blood pregnenolone and testosterone levels decreased under fasting, which induced energy deprivation, whereas corticosterone (CORT) levels significantly increased. However, the expression of 3β-HSD and P45011β in GM was unaffected by fasting. The decrease in GM weight may be due to decreased levels of testosterone and reduced synthesis of mammalian target of rapamycin (mTOR). Significant increases in CORT both GM and SOL were associated with increases in the amount of branched-chain amino acids available for energy production. However, decreased levels of mTOR and IGF1 and increased levels of CORT and IL-6 in SOL suggest that GM proteolysis was followed by SOL proteolysis for additional energy production. In conclusion, IGF1 levels decreased significantly in SOL, whereas those of IL-6 significantly increased in SOL and blood but decreased in GM. Blood branched-chain amino acids (BCAA) levels were unaffected due to fasting, whereas an increase was noted in the levels of BCAA in GM and SOL. These results show that fasting for 96 h restricts energy supply, producing fast-twitch muscle atrophy followed by slow-twitch muscle atrophy.

出典　doi: 10.1016/j.steroids.2023.109328.
https://www.sciencedirect.com/science/article/pii/S0039128X23001563?via%3Dihub

最終責任者　Naoyuki Maeda (Corresponding Author)

iScience

CellPress
OPEN ACCESS

Article

Isolation of *Streptococcus mutans* temperate bacteriophage with broad killing activity to *S. mutans* clinical isolates

Katsuhito Sugai,[1] Miki Kawada-Matsuo,[2,3,*] Mi Nguyen-Tra Le,[2,3] Yo Sugawara,[4] Junzo Hisatsune,[4] Jumpei Fujiki,[5] Hidetomo Iwano,[5] Kotaro Tanimoto,[1] Motoyuki Sugai,[4] and Hitoshi Komatsuzawa[2,3,6,*]

SUMMARY

Bacteriophages are expected to be therapeutic agents against infectious diseases. *Streptococcus mutans* are involved in dental plaque formation related to dental caries and periodontitis. In *S. mutans*, lytic bacteriophages have been isolated previously, but the isolation of temperate bacteriophage has not been reported although their presence in the genome has been confirmed. Here, we report the isolation of temperate bacteriophage, φKSM96, from *S. mutans*. φKSM96 has a circular DNA 39,820 bp long and reveals Siphoviridae morphology. φKSM96 shows a broad range of susceptibility against *S. mutans* strains with different serotypes. By the addition of φKSM96, *S. mutans* growth and biofilm formation were significantly inhibited. In cocultures of *S. mutans* with other bacterial species, the proportion of *S. mutans* significantly decreased in the presence of φKSM96. In summary, φKSM96 shows selective anti-*S. mutans* activity. The isolation of temperate bacteriophage is important for future genetic manipulation to create more efficient bacteriophages.

出典　doi: 10.1016/j.isci.2023.108465.

https://www.sciencedirect.com/science/article/pii/S2589004223025427?via%3Dihub

最終責任者　Hitoshi Komatsuzawa (Corresponding Author)

食品衛生学（Food Microbiology and Food Safety）

Akira Fukuda

Lecturer

講師　福田　昭

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

1）Antimicrobial resistant bacteria monitoring in raw seafood retailed: A pilot study focused on *Vibrio* and *Aeromonas*.
Fukuda A, Tsunashima R, Usui M.
Food Saf. 11:65-77. 2023. doi: 10.14252/foodsafetyfscj.D-23-00006

Ⅱ．その他＜Others＞

1）Antimicrobial resistance and molecular epidemiological analysis of *Escherichia fergusonii* harboring the *mcr* gene in pigs and broiler chickens in Okinawa, Japan.
Kakita T, Shigemura H, **Fukuda A**, Katamune C, Nidaira M, Kudeken T, Kyan H.
J Vet Med Sci. 85:149-156. 2023. doi: 10.1292/jvms.22-0288

2）16S rRNA nanopore sequencing for rapid diagnosis of causative bacteria in bovine mastitis
Usui M, Akiyoshi M, **Fukuda A**, Iwano H, Kato K.
Res Vet Sci. 162:45:49. 2023. doi: 10.1016/j.rvsc.2023.06.006

FOOD SAFETY

doi: 10.14252/foodsafetyfscj.D-23-00006
Food Safety 2023; Vol. 11, No. 4, 65–77

Original Article

Antimicrobial Resistant Bacteria Monitoring in Raw Seafood Retailed: a Pilot Study Focused on *Vibrio* and *Aeromonas*

Akira Fukuda, Ryu Tsunashima, Masaru Usui

Food Microbiology and Food Safety Unit, Division of Preventive Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

In aquaculture, bacterial infections in sea animals are treated using antimicrobials. As seafood is frequently consumed in its raw form, seafood contaminated with water-borne antimicrobial-resistant bacteria presents a potential transmission route to humans and can influence food safety. In this study, we aimed to determine the abundance of water-borne bacteria in retail raw seafood and to characterize their antimicrobial resistance profiles. In total, 85 retail raw seafood samples (32 fish, 26 shellfish, 25 mollusks, and two crustaceans) were purchased from supermarkets in Japan, and water-borne bacteria were isolated. The isolated bacterial species predominantly included *Vibrio* spp. (54.1%) and *Aeromonas* spp. (34.1%). *Vibrio* or *Aeromonas* spp. were isolated from more than 70% of the seafood samples. Tetracycline-, sulfamethoxazole-, and/or trimethoprim/sulfamethoxazole-resistant *Vibrio* or *Aeromonas* spp. isolates were detected in seven (21.9%) fish samples (two wild-caught and five farm-raised) harboring *tet*, *sul*, and/or *dfr* genes. Sulfamethoxazole- and trimethoprim/sulfamethoxazole-resistant isolates were only detected in farm-raised fish. Tetracycline and sulfamethoxazole are commonly used in aquaculture. These results suggest that water-borne bacteria like *Vibrio* and *Aeromonas* spp. should be the primary focus of antimicrobial-resistant bacteria monitoring to effectively elucidate their spread of bacteria via seafood.

Key word: *Vibrio*, *Aeromonas*, seafood, antimicrobial resistant bacteria, antimicrobial resistance genes

Received: 7 May 2023; Accepted: 8 September 2023; Published online: 14 October 2023
Corresponding author: Masaru Usui, Food Microbiology and Food Safety Unit, Division of Preventive Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan (usuima@rakuno.ac.jp)
The contents of this article reflect solely the view of the author(s).
Abbreviations: ARB: antimicrobial-resistant bacteria, ARGs: antimicrobial resistance genes
Suggested citation: Akira Fukuda, Ryu Tsunashima, Masaru Usui. Antimicrobial Resistant Bacteria Monitoring in Raw Seafood Retailed: a Pilot Study Focused on *Vibrio* and *Aeromonas*. *Food Safety*. 2023; 11 (4) 65–77. doi: 10.14252/foodsafetyfscj.D-23-00006

出典　doi: 10.14252/foodsafetyfscj.D-23-00006

https://www.jstage.jst.go.jp/browse/foodsafetyfscj/advpub/0/_contents/-char/ja

最終責任者　Masaru Usui (Corresponding Author)

The Journal of Veterinary Medical Science

FULL PAPER
Bacteriology

Antimicrobial resistance and molecular epidemiological analysis of *Escherichia fergusonii* harboring the *mcr* gene in pigs and broiler chickens in Okinawa, Japan

Tetsuya KAKITA[1)]*, Hiroaki SHIGEMURA[2)], Akira FUKUDA[3)], Chiharu KATAMUNE[2)], Minoru NIDAIRA[1)], Tsuyoshi KUDEKEN[1)], Hisako KYAN[1)]

[1)]Department of Biological Sciences, Okinawa Prefectural Institute of Health and Environment, Okinawa, Japan
[2)]Division of Pathology and Microbiology, Department of Health Science, Fukuoka Institute of Health and Environmental Sciences, Fukuoka, Japan
[3)]Laboratory of Food Microbiology and Food Safety, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. The dissemination of *mcr*-harboring *Enterobacteriaceae*, e.g., *Escherichia fergusonii*, with resistance to colistin via animal products is a public health concern. In our previous study, *E. fergusonii* harboring the *mcr* gene were isolated from 11 pigs and 43 chickens. To understand the spread of *mcr*-harboring *E. fergusonii* in Okinawa, Japan, and to gain further insights into how they can be controlled, an antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), a conjugation test for the transferability of *mcr*-harboring plasmids, and PCR-based replicon typing (PBRT) were performed using the 54 strains. According to the disk-diffusion and broth microdilution methods, 9 of the 11 strains from pigs and 9 of the 43 strains from chickens had multidrug resistance (MDR). The broth microdilution method showed that all strains were resistant to colistin, and the minimum inhibitory concentration of colistin was 4–16 µg/mL. PFGE suggested identical PFGE types were being transmitted within one pig farm, within one chicken farm, and among several chicken farms. These findings showed that some *mcr*-harboring *E. fergusonii* in Okinawa exhibited MDR, and these had spread within farms and between farms. In the *mcr* gene conjugation test and PBRT, a type IncI2 plasmid replicon was detected in all *mcr-1*-harboring transconjugants. Therefore, evidence suggests that the IncI2 plasmid is probably involved in the transmission of the *mcr-1* gene. It is important to monitor the antimicrobial resistance profile and dissemination of the IncI2 plasmid in *mcr*-harboring *E. fergusonii*.

KEYWORDS: broiler chicken, *Escherichia fergusonii*, IncI2, *mcr-1*, pig

J. Vet. Med. Sci.
85(2): 149–156, 2023
doi: 10.1292/jvms.22-0288

Received: 20 June 2022
Accepted: 30 November 2022
Advanced Epub:
9 December 2022

*Correspondence to: Kakita T: kakitatt@pref.okinawa.lg.jp, Department of Biological Sciences, Okinawa Prefectural Institute of Health and Environment, 17-1 Kanekadan, Uruma-shi, Okinawa 904-2241, Japan
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

出典　doi: 10.1292/jvms.22-0288.
https://www.jstage.jst.go.jp/article/jvms/85/2/85_22-0288/_article/-char/ja/

最終責任者　Tetsuya Kakita (Corresponding Author)

Research in Veterinary Science 161 (2023) 45–49

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

16S rRNA nanopore sequencing for rapid diagnosis of causative bacteria in bovine mastitis

Masaru Usui [a,*], Misaki Akiyoshi [a,b], Akira Fukuda [a], Hidetomo Iwano [c], Toshihide Kato [b]

[a] Laboratory of Food Microbiology and Food Safety, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan
[b] Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan
[c] Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Bovine mastitis
Nanopore sequence
16S rRNA sequencing

ABSTRACT

The rapid identification of specific bacterial pathogens in bovine mastitis is crucial for appropriate antimicrobial treatment. Sequencing of 16S rRNA gene amplicons is a proven, useful strategy for diagnosing bacterial infections. In this study, the use of 16S rRNA analysis with nanopore sequencer for the rapid identification of causative bacteria in bovine mastitis, was evaluated. DNA was extracted from 122 milk samples from cattle with suspected mastitis based on clinical symptoms. 16S rRNA gene amplicon sequencing was conducted using a nanopore sequencer. The efficacy of bacterial identification was verified by comparison with conventional culture methods. Nanopore sequencing identified the causative bacteria with high accuracy within approximately 6 h from the time of sample collection. When the major causative bacteria of bovine mastitis (*Escherichia coli*, *Streptococcus uberis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*) were detected by nanopore sequencing, 98.3% of the results were consistent with identification through conventional culturing methods. 16S rRNA gene analysis using a nanopore sequencer enabled the rapid and accurate identification of bacterial species in bovine mastitis.

出典　doi: 10.1016/j.rvsc.2023.06.006.
https://doi.org/10.1016/j.rvsc.2023.06.006

最終責任者　Masaru Usui (Corresponding Author)

* Corresponding author at: Laboratory of Food Microbiology and Food Safety, Department of Health and Environmental Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Midorimachi, Bunkyodai, Ebetsu, Hokkaido 069-8501, Japan.
E-mail address: usuima@rakuno.ac.jp (M. Usui).

https://doi.org/10.1016/j.rvsc.2023.06.006
Received 3 December 2022; Received in revised form 4 June 2023; Accepted 5 June 2023
Available online 10 June 2023

生産動物病態学（Farm Animal Pathophysiology）

Shigeo Fukuda

Lecturer

講師　福田　茂夫

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

Ⅱ．その他＜Others＞

1）Histological Characteristics of Conjunctiva-Associated Lymphoid Tissue in Young and Adult Holstein Cattle.
Kosenda K, Ichii O, Yamashita Y, Ohtsuka H, **Fukuda S**, Kon Y.
Animals (Basel). 13:3481. 2023. doi: 10.3390/ani13223481.

animals

MDPI

Article

Histological Characteristics of Conjunctiva-Associated Lymphoid Tissue in Young and Adult Holstein Cattle

Keigo Kosenda [1,*], Osamu Ichii [2,3], Yusuke Yamashita [4], Hiromichi Ohtsuka [5], Shigeo Fukuda [1] and Yasuhiro Kon [2]

[1] Laboratory of Farm Animal Pathophysiology, Department of Farm Animal Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-0836, Japan; fukuda@rakuno.ac.jp
[2] Laboratory of Anatomy, Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan; ichi-o@vetmed.hokudai.ac.jp (O.I.); y-kon@vetmed.hokudai.ac.jp (Y.K.)
[3] Laboratory of Agrobiomedical Science, Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
[4] Nayoro Veterinary Clinical Center, Hokkaido Agricultural Mutual Aid Association, Nayoro 096-0072, Japan; jiji.shizuku@gmail.com
[5] Section of Large Animal Clinical Sciences, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan; ohtsuka@obihiro.ac.jp
* Correspondence: k-kosenda@rakuno.ac.jp; Tel.: +81-011-388-4856

Simple Summary: Many animals exhibit immune cell aggregation in the conjunctiva which prevents the invasion of viruses and bacteria. However, the features and functions of conjunctival immune cell aggregates in cows are not fully understood. Microscopic observations confirmed conjunctival immune cell aggregation in calves and adult cows, similar to that in other animals. However, these structures of calves are small and poor in immune cells compared to those of adult cows. These results suggest that cows have a conjunctival local immune system similar to that of other animals, and that conjunctival immune function is weak in calves. These findings may help to prevent infectious diseases in cows.

Abstract: The conjunctiva-associated lymphoid tissue (CALT) has been used as a target site for mucosal vaccinations in several animals. In this study, we compared the morphological features of CALT in the eyelid and third eyelid between Holstein calves and adult cows. In the eyelids, CALTs in the form of diffused lymphoid tissue (DLT) and lymphatic follicles (LF) were observed, where DLTs were dominant and LFs were scarce. The CALTs of cows comprised T-, B-cells, macrophages, and antigen-presenting cells (APCs). In particular, B-cells were dominant except in the eyelids of the calves. The epithelial layer covering the CALT is often discontinuous and lacks goblet cells. Cytokeratin18 is strongly expressed in the epithelial layer covering the CALT, except in the third eyelids of adult cows. IgA-positive cells were diffusely distributed in the lamina propria of the conjunctiva of the eyelids and third eyelids. The eyelid CALT area in calves was lower than that in adult cows. Furthermore, the CALT of calves had a lower cellularity of B-cells and a higher cellularity of macrophages than that of adult cows. These histological characteristics indicate that CALT plays a role in the mucosal immune-inductive and effector sites. Furthermore, lower cellularity of B-cells in the CALT of calves indicates that the function of CALT as a mucosal immune induction site is less developed in calves than in adult cows.

Keywords: cow; conjunctiva; mucosa; lymphoid tissue; histology

Citation: Kosenda, K.; Ichii, O.; Yamashita, Y.; Ohtsuka, H.; Fukuda, S.; Kon, Y. Histological Characteristics of Conjunctiva-Associated Lymphoid Tissue in Young and Adult Holstein Cattle. *Animals* **2023**, *13*, 3481. https://doi.org/10.3390/ani13223481

Academic Editors: Steven Van Winden and Nicola Blackie

Received: 4 September 2023
Revised: 7 November 2023
Accepted: 9 November 2023
Published: 11 November 2023

出典　doi: 10.3390/ani13223481.
https://www.mdpi.com/2076-2615/13/22/3481

最終責任者　Keigo Kosenda (Corresponding Author)

伴侶動物外科学（companion animal surgery）

Yuji Hamamoto

Lecturer

講師　濱本　裕仁

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

Ⅱ．その他＜Others＞

1）Investigation of the effect and availability of ketamine on electroencephalography in cats with temporal lobe epilepsy.
Mizuno S, Asada R, Yu Y, **Hamamoto Y,** Hasegawa D.
Front Vet Sci. 10:1236275. 2023. doi: 10.3389/fvets.2023.1236275.

frontiers Frontiers in Veterinary Science

TYPE Original Research
PUBLISHED 25 July 2023
DOI 10.3389/fvets.2023.1236275

OPEN ACCESS

EDITED BY
Edward (Ned) E. Patterson,
University of Minnesota Twin Cities, United States

REVIEWED BY
Alejandra Mondino,
North Carolina State University, United States
Sam Long,
Veterinary Referral Hospital, Australia

*CORRESPONDENCE
Daisuke Hasegawa
✉ disk-hsgw@nvlu.ac.jp

†PRESENT ADDRESSES:
Yoshihiko Yu,
Mitaka, Tokyo, Japan

Yuji Hamamoto,
Laboratory of Companion Animal Surgery, Rakuno Gakuen University, Ebetsu, Japan

RECEIVED 07 June 2023
ACCEPTED 10 July 2023
PUBLISHED 25 July 2023

CITATION
Mizuno S, Asada R, Yu Y, Hamamoto Y and Hasegawa D (2023) Investigation of the effect and availability of ketamine on electroencephalography in cats with temporal lobe epilepsy.
Front. Vet. Sci. 10:1236275.
doi: 10.3389/fvets.2023.1236275

Investigation of the effect and availability of ketamine on electroencephalography in cats with temporal lobe epilepsy

Satoshi Mizuno[1], Rikako Asada[1], Yoshihiko Yu[1†], Yuji Hamamoto[1,2†] and Daisuke Hasegawa[1*]

[1]Laboratory of Veterinary Clinical Neurology, Graduate School of Nippon Veterinary and Life Science University, Musashino, Japan, [2]Veterinary Medical Teaching Hospital, Nippon Veterinary and Life Science University, Musashino, Japan

In recent years, electroencephalography (EEG) in veterinary medicine has become important not only in the diagnosis of epilepsy, but also in determining the epileptogenic focus. In cats, sedation and immobilization, usually with medetomidine or dexmedetomidine, are necessary to place the electrodes and to obtain stable scalp EEG recordings. In this study, we hypothesized that, for cats with temporal lobe epilepsy (TLE), ketamine, a sedative/anesthetic and N-methyl-D-aspartate (NMDA) antagonist that activates the limbic system and is also used to treat refractory status epilepticus in dogs, would induce sufficient sedation and immobilization for EEG, as well as induce interictal epileptiform discharges (IEDs) that are more pronounced than those induced with medetomidine. We obtained EEG recordings from TLE cats and healthy cats administered either ketamine or medetomidine alone (study 1) or ketamine after medetomidine sedation (study 2). In study 1, the frequency of IEDs showed no statistically significant difference between ketamine and medetomidine in both TLE and healthy cats. Seizures were observed in 75% (9/12) cats of the TLE group with ketamine alone. When ketamine was administered after sedation with medetomidine (study 2), 3/18 cats in the TLE group developed generalized tonic-clonic seizure and 1/18 cats showed subclinical seizure activity. However, no seizures were observed in all healthy cats in both study 1 and study 2. Slow wave activity at 2–4 Hz was observed in many individuals after ketamine administration regardless studies and groups, and quantitative analysis in study 2 showed a trend toward increased delta band activities in both groups. While there was no significant difference in the count of IEDs between medetomidine and ketamine, ketamine caused seizures in cats with TLE similar to their habitual seizure type and with a higher seizure frequency. Our results suggest that ketamine may activate epileptiform discharges during EEG recordings. However, caution should be used for cats with TLE.

KEYWORDS
EEG, feline, interictal epileptiform discharge, ketamine, medetomidine, seizure, TLE

出典　doi: 10.3389/fvets.2023.1236275.
https://www.frontiersin.org/articles/10.3389/fvets.2023.1236275/full

最終責任者　Daisuke Hasegawa (Corresponding Author)

獣医疫学（Veterinary Epidemiology

Ryota Matsuyama

Lecturer

講師　松山　亮太

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

Ⅱ．その他＜Others＞

1）Impact of eHealth education to reduce anemia among school-going adolescent girls in rural Bangladesh: Study protocol of a randomized controlled trial.
Rahman MJ, Rahman MM, Kakehashi M, **Matsuyama R**, Sarker MHR, Ali M, Promitee SK, Prihanto JB, Ahmed A, Shimpuku Y.
J Family Med Prim Care 12:2569-2575. 2023.
doi: 10.4103/jfmpc.jfmpc_1010_23.

Research Protocol

Impact of eHealth education to reduce anemia among school-going adolescent girls in rural Bangladesh: Study protocol of a randomized controlled trial

Md Jiaur Rahman[1], Md Moshiur Rahman[2], Masayuki Kakehashi[2], Ryota Matsuyama[3], Mohammad Habibur Rahman Sarker[4], Mohammad Ali[5], Sumaita Kabir Promitee[6], Junaidi Budi Prihanto[7], Ashir Ahmed[8], Yoko Shimpuku[1]

[1]Global Health Nursing, Department of Health Science, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [2]Department of Health Science, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [3]Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan, [4]Training Unit, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [5]Department of Medicine, Comilla Medical College, Cumilla, Bangladesh, [6]Nutrition and Clinical Service Division, International Centre for Diarrhoeal Disease Research, Bangladesh, Chandpur, Bangladesh, [7]Physical Education, Health and Recreation, Sport Science Faculty, Universitas Negeri Surabaya, Indonesia, [8]Department of Advanced Information Technology, Graduate School of Information Science and Electrical Engineering, Kyushu University, Fukuoka, Japan

Abstract

Adolescent girls are highly vulnerable to developing anemia due to reproductive immaturity, poor personal hygiene, and lack of nutritional intake and health education in rural Bangladesh. Digital health technology is a promising tool to overcome barriers and provide appropriate health guidelines. We aim to evaluate eHealth education's impact and changes in adolescent girls' knowledge, attitude, and practice regarding anemia. A 1:1 parallel randomized control trial was conducted among school-going adolescent girls in rural Bangladesh. A total of 138 anemic (mild and moderate) participants were enrolled. We randomized schools to reduce the health education bias through a simple coin toss technique, then allocated participants to the intervention group (n = 69) and control group (n = 69) by stratified random sampling technique. The intervention group received two online counseling sessions and 8-month eHealth education through mobile phone calls and short message service regarding anemia. The control group received the usual care. The primary endpoint changes the anemic level through changing knowledge, healthy lifestyle behavior, and an iron-rich food dietary plan. Per-protocol analysis will utilize to compare the control and intervention groups using SPSS software. Descriptive statistics (frequencies, percentages, mean, SD) will be employed, and continuous variables will be compared using the t-test/Mann–Whitney test. Two-way analysis of variance will assess outcome variables at baseline, 4 months, and 8 months. The 8-month intervention is designed from May 2022 to February 2023. Participants' age range of 10-14 years was 60.9% in the intervention group and 56.5% in the control group. Among the participants, 89.9% and 88.4% were mild anemic; 11.11 (SD ± 0.80) and 11.06 (SD ± 0.96) were mean hemoglobin in the intervention and control groups, respectively. eHealth education is expected to be an effective way to increase knowledge and healthy behavioral change, which can reduce the anemia burden among adolescent girls.

Address for correspondence: Md Jiaur Rahman, Global Health Nursing, Department of Health Science, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima - 734-8553, Japan.
E-mail: jiaur9731@gmail.com

Keywords: Adolescent girl, anemia, Bangladesh, eHealth, mHealth

Received: 18-06-2023 **Revised:** 10-08-2023
Accepted: 08-09-2023 **Published:** 21-11-2023

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_1010_23

How to cite this article: Rahman MJ, Rahman MM, Kakehashi M, Matsuyama R, Sarker MH, Ali M, *et al.* Impact of eHealth education to reduce anemia among school-going adolescent girls in rural Bangladesh: Study protocol of a randomized controlled trial. J Family Med Prim Care 2023;12:2569-75.

出典 doi: 10.4103/jfmpc.jfmpc_1010_23.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10771192/pdf/JFMPC-12-2569.pdf

最終責任者 Md Jiaur Rahman (First and Corresponding Author)

獣医薬理学（Veterinary Pharmacology）

Tatsuro Nakamura

Lecturer

講師　中村　達朗

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

Ⅱ．その他　＜Others＞

1）Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane Grus japonensis in Eastern Hokkaido, Japan.
Yokokawa A, Dong W, Momose K, Iima H, Yoshino T, Izumi K, Kawai Y, Amano T, **Nakamura T**, Sawada A, Endoh D, Nakajima N, Teraoka H. *Animals (Basel)*. 13:3167. 2023. doi: 10.3390/ani13203167.

2）Production profile of lipid mediators in conjunctival lavage fluid in allergic and infectious conjunctivitis in guinea pigs.
Hayashi A, Kobayashi K, **Nakamura T**, Nagata N, Murata T.
Front Allergy 4:1218447 . 2023. doi: 10.3389/falgy.2023.1218447.

3）Mast cell-derived prostaglandin D2 limits the subcutaneous absorption of honey bee venom in mice.
Fujiwara Y, **Nakamura T**, Maehara T, Hayashi A, Aritake K, Murata T.
Proc Natl Acad Sci U S A. 120:e2300284120. 2023.
doi: 10.1073/pnas.2300284120.

4）Augmentation of Pectoral Fin Teratogenicity by Thalidomide in Human Cytochrome P450 3A-Expressing Zebrafish.
Dong W, Akasaka I, Komiyama A, **Nakamura T**, Mizoguchi N, Nawaji T, Ikushiro S, Kobayashi M, Teraoka H.
Pharmaceuticals (Basel) 16:368. 2023. doi: 10.3390/ph16030368

5）Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina.
Sato Y, Dong W, **Nakamura T**, Mizoguchi N, Nawaji T, Nishikawa M, Onaga T, Ikushiro S, Kobayashi M, Teraoka H.
Int J Mol Sci. 24:4013. 2023. ;doi: 10.3390/ijms24044013

animals

MDPI

Article

Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan

Ayaka Yokokawa [1], Wenjing Dong [1], Kunikazu Momose [2], Hiroko Iima [3], Tomoo Yoshino [3], Kenichi Izumi [4], Yusuke Kawai [5], Tomoko Amano [4], Tatsuro Nakamura [1], Akira Sawada [6], Daiji Endoh [1], Nobuyoshi Nakajima [6] and Hiroki Teraoka [1,2,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; yokokawa-ayaka@bozo.co.jp (A.Y.); dongwenjing_2015@163.com (W.D.); t-naka@rakuno.ac.jp (T.N.); dendoh@rakuno.ac.jp (D.E.)
[2] NPO Red-Crowned Crane Conservancy, 9-21 Wakatake-Cho, Kushiro 085-0036, Japan; dzi00244@nifty.com
[3] Kushiro Zoo, 11 Shimoninishibetsu, Kushiro 085-0204, Japan; hiroko.iima@city.kushiro.lg.jp (H.I.); garrulaxcanorus@yahoo.co.jp (T.Y.)
[4] College of Agriculture, Food and Environment Sciences, Rakuno Gakuen University, Ebetsu 069-8501, Japan; izmken@rakuno.ac.jp (K.I.); amano@rakuno.ac.jp (T.A.)
[5] Laboratory of Toxicology, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, 2-11 Inada-cho Nishi, Obihiro 080-8555, Japan; ykawai@obihiro.ac.jp
[6] Biodiversity Division, National Institute for Environmental Studies, Tsukuba 305-8506, Japan; akira.sawada.1312@gmail.com (A.S.); naka-320@nies.go.jp (N.N.)
* Correspondence: hteraoka08@gmail.com

Simple Summary: The red-crowned crane (*Grus japonensis*), which is an endangered and highly protected bird species, is distributed in two populations: a mainland population in far eastern Eurasia and an island population in Hokkaido, Japan. Red-crowned cranes in Japan are resident birds mainly in the eastern part of Hokkaido. As omnivores, they feed on plants, grains, insects, and fish. Most cranes spend the winter around feeding stations in southeastern Hokkaido, where people provide corn. Since most of the cranes in Hokkaido now live near areas inhabited by humans, cases of crop damage caused by cranes have recently been reported. This study showed that the cranes feed on various crops of human origin, mostly outside farmlands.

Abstract: Total DNA extracts from the intestinal contents of 60 flying red-crowned cranes (juveniles, subadults and adults) found dead in 2006–2021, and the feces of 25 chicks collected in June and July of 2016–2018, were used for PCR reactions with primers specific for 16 crops, followed by high-throughput sequencing. The most predominant crop detected was corn in adult and subadult cranes (61.7%). Other grains (barley, wheat, soybean) (5.0–8.3%) and vegetables (tomatoes, Chinese cabbage, etc.) (1.7–6.7%) were also detected in flying cranes. Surprisingly, some of the detected crops were not grown in the Kushiro and Nemuro regions. There was no significant difference in crop intake status in winter and that in other seasons for most of the crops. Corn (28.0%), soybeans (8.0%), wheat and beet (4.0%) were detected in crane chicks in summer, though the detection rates were generally lower than those in flying cranes. Alfalfa, which is not grown in eastern Hokkaido but is used in some cattle feed, was detected in some cranes. Rice, buckwheat, adzuki beans, common beans, potatoes and carrots were not detected at any life stage, indicating the preferences of red-crowned cranes. The results suggest that red-crowned cranes in Hokkaido are dependent on dairy farmers for their feed supply.

Keywords: amplicon sequencing; crop consumption; *Grus japonensis*; Japan; scatological samples

Citation: Yokokawa, A.; Dong, W.; Momose, K.; Iima, H.; Yoshino, T.; Izumi, K.; Kawai, Y.; Amano, T.; Nakamura, T.; Sawada, A.; et al. Analysis of Crop Consumption Using Scatological Samples from the Red-Crowned Crane *Grus japonensis* in Eastern Hokkaido, Japan. *Animals* **2023**, *13*, 3167. https://doi.org/10.3390/ani13203167

Academic Editor: Barry Hartup

Received: 27 July 2023
Revised: 6 October 2023
Accepted: 9 October 2023
Published: 10 October 2023

出典 doi: 10.3390/ani13203167.
https://www.mdpi.com/2076-2615/13/20/3167
責任著者 Hiroki Teraoka (Corresponding Author)

frontiers | Frontiers in Allergy

TYPE Brief Research Report
PUBLISHED 06 July 2023
DOI 10.3389/falgy.2023.1218447

OPEN ACCESS

EDITED BY
Julie Weidner,
AstraZeneca, Sweden

REVIEWED BY
DeGaulle I. Chigbu,
Salus University, United States
Ying Jie,
Capital Medical University, China

*CORRESPONDENCE
Takahisa Murata
amurata@mail.ecc.u-tokyo.ac.jp

RECEIVED 07 May 2023
ACCEPTED 26 June 2023
PUBLISHED 06 July 2023

CITATION
Hayashi A, Kobayashi K, Nakamura T, Nagata N and Murata T (2023) Production profile of lipid mediators in conjunctival lavage fluid in allergic and infectious conjunctivitis in guinea pigs. Front. Allergy 4:1218447. doi: 10.3389/falgy.2023.1218447

Production profile of lipid mediators in conjunctival lavage fluid in allergic and infectious conjunctivitis in guinea pigs

Akane Hayashi[1], Koji Kobayashi[2], Tatsuro Nakamura[3], Nanae Nagata[1] and Takahisa Murata[1,2,3]*

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

Introduction: Conjunctivitis is a major ocular disease classified into allergic or infectious. The pathological features of conjunctivitis are not fully understood despite its high morbidity rate; thus, its differentiation can be difficult.
Materials and methods: We used ovalbumin-induced allergic conjunctivitis and lipopolysaccharide-induced infectious conjunctivitis models of guinea pigs. Both models showed conjunctival swelling. Histological studies revealed that numerous eosinophils infiltrated the conjunctiva in the allergic model, whereas neutrophils infiltrated the conjunctiva in the infectious model. We collected conjunctival lavage fluid (COLF) and comprehensively analyzed lipid production using liquid chromatography-tandem mass spectrometry.
Results: COLF showed increase of 20 and 12 lipid species levels in the allergic and infectious models, respectively. Specifically, the levels of a major allergic mediator, prostaglandin D_2 and its three metabolites and several cytochrome P450-catalyzed lipids increased in the allergic model. In the infectious model, the levels of prostaglandin E_2 and 8-iso-prostaglandin E_2 increased, indicating tissue inflammation. Moreover, the level of 12-oxo-eicosatetraenoic acid, a lipoxygenase metabolite, increased in the infectious model.
Conclusion: These differences in lipid production in the COLF reflected the pathological features of allergic and infectious conjunctivitis.

KEYWORDS
conjunctivitis, lipid mediators, tear, biomarker, eosinophils, neutrophils

出典　doi: 10.3389/falgy.2023.1218447.
https://www.frontiersin.org/articles/10.3389/falgy.2023.1218447/full
責任著者　Takahisa Murata (Corresponding Author)

PNAS BRIEF REPORT | IMMUNOLOGY AND INFLAMMATION OPEN ACCESS

Mast cell-derived prostaglandin D_2 limits the subcutaneous absorption of honey bee venom in mice

Yuki Fujiwara[a], Tatsuro Nakamura[a], Toko Maehara[a], Akane Hayashi[a], Kosuke Aritake[b], and Takahisa Murata[a,1]

Edited by Philippa Marrack, National Jewish Health, Denver, CO; received January 10, 2023; accepted April 14, 2023

Mast cells play pivotal roles in innate host defenses against venom. Activated mast cells release large amounts of prostaglandin D_2 (PGD_2). However, the role of PGD_2 in such host defense remains unclear. We found that c-kit-dependent and c-kit-independent mast cell–specific hematopoietic prostaglandin D synthase (*H-pgds*) deficiency significantly exacerbated honey bee venom (BV)–induced hypothermia and increased mortality rates in mice. BV absorption via postcapillary venules in the skin was accelerated upon endothelial barrier disruption resulting in increased plasma venom concentrations. These results suggest that mast cell–derived PGD_2 may enhance host defense against BV and save lives by inhibiting BV absorption into circulation.

lipid mediator | bee venom | biological defense | vasculature | barrier

出典 doi: 10.1073/pnas.2300284120.
https://www.pnas.org/doi/epdf/10.1073/pnas.2300284120

責任著者 Takahisa Murata (Corresponding Author)

pharmaceuticals

MDPI

Article

Augmentation of Pectoral Fin Teratogenicity by Thalidomide in Human Cytochrome P450 3A-Expressing Zebrafish

Wenjing Dong [1], Ippo Akasaka [1], Akifumi Komiyama [1], Tatsuro Nakamura [1], Naohiro Mizoguchi [2], Tasuku Nawaji [2], Shinichi Ikushiro [3], Makoto Kobayashi [4] and Hiroki Teraoka [1,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[2] Chemicals Evaluation and Research Institute, Japan (CERI), 3-2-7, Miyanojin, Kurume 839-0801, Fukuoka, Japan
[3] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180, Kurokawa, Imizu 939-0398, Toyama, Japan
[4] Department of Molecular and Developmental Biology, Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan
* Correspondence: hteraoka@rakuno.ac.jp or hteraoka08@gmail.com

Abstract: The pharmacological and toxicological effects of active metabolites of enzymes including cytochrome P450 (CYP) are important. While it has been believed for a long time that thalidomide causes characteristic limb malformation only in rabbits and primates including humans, the involvement of their CYP3A subtypes (CYP3As) has been suggested. Recently, however, it was reported that zebrafish were sensitive to thalidomide, showing defects of pectoral fins, homologous organs of forelimbs in mammals, as well as other deformities. In this study, we prepared human CYP3A7 (hCYP3A7)-expressing zebrafish (F0) using a transposon system. Thalidomide caused pectoral fin defects and other malformations including pericardial edema in hCYP3A7-expressing embryos/larvae but not in wild-type and hCYP1A1-expressing embryos/larvae. Thalidomide also reduced the expression of fibroblast growth factor 8 in pectoral fin buds in only hCYP3A7-expressing embryos/larvae. The results suggest the involvement of human-type CYP3A in thalidomide teratogenicity.

Keywords: CYP1A1; CYP3A4; CYP3A7; human; teratogenicity; thalidomide; zebrafish

Citation: Dong, W.; Akasaka, I.; Komiyama, A.; Nakamura, T.; Mizoguchi, N.; Nawaji, T.; Ikushiro, S.; Kobayashi, M.; Teraoka, H. Augmentation of Pectoral Fin Teratogenicity by Thalidomide in

出典 doi: 10.3390/ph16030368

https://www.mdpi.com/1424-8247/16/3/368

最終責任者 Hiroki Teraoka (Corresponding Author)

International Journal of
Molecular Sciences

MDPI

Article

Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina

Yoshinori Sato [1,†], Wenjing Dong [1,†], Tatsuro Nakamura [1], Naohiro Mizoguchi [2], Tasuku Nawaji [2], Miyu Nishikawa [3], Takenori Onaga [1], Shinichi Ikushiro [3], Makoto Kobayashi [4] and Hiroki Teraoka [1,*]

[1] School of Veterinary Medicine, Rakuno Gakuen University, 582, Bunkyodai-Midorimachi, Ebetsu 069-8501, Hokkaido, Japan
[2] Chemicals Evaluation and Research Institute, Japan (CERI), 3-2-7, Miyanojin, Kurume 839-0801, Fukuoka, Japan
[3] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180, Kurokawa, Imizu 939-0398, Toyama, Japan
[4] Department of Molecular and Developmental Biology, Institute of Medicine, University of Tsukuba, Tsukuba 305-8575, Ibaraki, Japan
* Correspondence: hteraoka@rakuno.ac.jp
† These authors contributed equally to this work.

Abstract: Metabolic activation is the primary cause of chemical toxicity including hepatotoxicity. Cytochrome P450 2E (CYP2E) is involved in this process for many hepatotoxicants, including acetaminophen (APAP), one of the most common analgesics and antipyretics. Although the zebrafish is now used as a model for toxicology and toxicity tests, the CYP2E homologue in zebrafish has not been identified yet. In this study, we prepared transgenic zebrafish embryos/larvae expressing rat CYP2E1 and enhanced green fluorescent protein (EGFP) using a β-actin promoter. Rat CYP2E1 activity was confirmed by the fluorescence of 7-hydroxycoumarin (7-HC), a metabolite of 7-methoxycoumarin that was specific for CYP2 in transgenic larvae with EGFP fluorescence (EGFP [+]) but not in transgenic larvae without EGFP fluorescence (EGFP [−]). APAP (2.5 mM) caused reduction in the size of the retina in EGFP [+] larvae but not in EGFP [−] larvae, while APAP similarly reduced pigmentation in both larvae. APAP at even 1 mM reduced the liver size in EGFP [+] larvae but not in EGFP [−] larvae. APAP-induced reduction of liver size was inhibited by *N*-acetylcysteine. These results suggest that rat CYP2E1 is involved in some APAP-induced toxicological endpoints in the retina and liver but not in melanogenesis of the developing zebrafish.

Keywords: acetaminophen; bioactivation; CYP2E1; rat; zebrafish

Citation: Sato, Y.; Dong, W.; Nakamura, T.; Mizoguchi, N.; Nawaji, T.; Nishikawa, M.; Onaga, T.; Ikushiro, S.; Kobayashi, M.; Teraoka, H. Transgenic Zebrafish Expressing Rat Cytochrome P450 2E1 (CYP2E1): Augmentation of Acetaminophen-Induced Toxicity in the Liver and Retina. *Int. J. Mol. Sci.* **2023**, *24*, 4013. https://doi.org/10.3390/ijms24044013

出典 doi: 10.3390/ijms24044013
https://www.mdpi.com/1422-0067/24/4/4013

最終責任者 Hiroki Teraoka (Corresponding Author)

生産動物外科学（Farm Animal Surgery）

Ayano Sato

Lecturer

講師　佐藤　綾乃

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

1）Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows.
Sato A, Sugiura T, Kosenda K, Murakami T
J Am Vet Med Assoc. 261:1374-1379. 2023. doi.org/10.2460/javma.23.02.0062

Ⅱ．その他　＜Others＞

1）mRNA expression of immune factors by milk somaticcells from healthy Holstein lactating cows.
Murakami K, Fukuhara T, Kure S, Shimosakai T, **Sato A**, Murata R, Kosenda K, Ohtsuka H
Can J Vet Res. 87:231-236. 2023.

JAVMA AVMA

Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows

Ayano Sato, DVM, PhD*; Tomochika Sugiura, DVM, PhD; Keigo Kosenda, DVM; Takashi Murakami, DVM, PhD

Division of Farm Animal Clinical Sciences, Rakuno Gakuen University, Ebetsu, Japan

*Corresponding author: Dr. Sato (ayanok@rakuno.ac.jp)

Received February 3, 2023
Accepted April 24, 2023

doi.org/10.2460/javma.23.02.0062

OBJECTIVE
To evaluate analgesic efficacy of 3 different preoperative protocols in cows undergoing right flank laparotomy for displaced abomasum.

ANIMALS
40 cows diagnosed with displaced abomasum.

PROCEDURES
The cows were assigned by block randomization to 1 of 3 preoperative protocols: inverted L-block using 50 mL of 2% lidocaine (ILB; n = 13), ILB plus preoperative flunixin meglumine (2 mg/kg, IV; ILB-F; 13), and dorsolumbar epidural anesthesia using 2% xylazine (0.8 mL) and 2% lidocaine (4 mL; EPI; 14). Venous blood samples were collected for CBC, serum biochemistry, and cortisol preoperatively and at 0 (immediately after), 3, 17, and 48 hours postoperatively.

RESULTS
The mean (95% CI) of the serum cortisol in ILB, ILB-F, and EPI were 108.7 (66.7 to 150.7), 150.7 (116.4 to 185.0), and 139.8 (93.4 to 186.3), respectively. The serum cortisol concentrations decreased over time in all groups (ILB, $P = .001$; ILB-F and EPI, $P < .001$). In the ILB group, the cortisol concentration at 17 and 48 hours postoperatively decreased ($P = .026$ and $P = .009$, respectively), compared with that preoperatively. In the ILB-F and EPI groups, the preoperative cortisol concentration was the highest and then decreased at 0, 3, 17, and 48 hours postoperatively (ILB-F, 0 hours [$P = .001$] and 3, 17, and 48 hours [$P < .001$]; EPI, all [$P < .001$]).

CLINICAL RELEVANCE
ILB-F and EPI improved intraoperative and immediate postoperative indicators of pain-related stress when compared to standard ILB. EPI requires less anesthetic, which may be beneficial when in short supply.

出典　doi: 10.2460/javma.23.02.0062

https://avmajournals.avma.org/view/journals/javma/261/9/javma.23.02.0062.xml

最終責任者　Ayano Sato (Corresponding Author)

mRNA expression of immune factors by milk somatic cells from healthy Holstein lactating cows

Kenji Murakami, Tomochika Fukuhara, Shunsuke Kure, Takaaki Shimosakai, Ayano Sato, Ryo Murata, Keigo Kosenda, Hiromichi Ohtsuka

Abstract

This study investigated the mRNA of immune factors expressed by milk somatic cells from 72 healthy lactating Holstein cows on 1 farm. Milk samples were collected aseptically from the right front mammary gland before milking. The milk samples that had a negative reaction to the California mastitis test were used to analyze the mRNA of immune factors. Cows were divided into 2 groups based on the detection of bacteria in milk samples: positive group (n = 22 cows), which showed bacteria in cultures, and negative group (n = 50 cows), which did not show bacteria in cultures. There were significant positive correlations among the relative mRNA levels of interleukin (IL)-6, IL-8, arginase 1, chemokine (C-C motif) ligand (CCL) 1, and chemokine (C-X-C motif) ligand (CXCL) 13, as well as among the relative mRNA levels of IL-10, pentraxin 3, CCL5, and CCL14. Significantly high levels of IL-1β, IL-6, IL-8, arginase 1, Batf, CCL1, CXCL14, and toll-like receptor 4 in the positive group were discovered compared to the negative group. These results suggest that the presence of bacteria in lactating healthy dairy cows may affect mRNA levels of inflammatory mediators expressed by somatic cells.

出典 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10291702/

最終責任者 Hiromichi Ohtsuka (Corresponding Author)

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

Masahiro Tamura

Lecturer

講師　田村　昌大

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

1）Detecting antegrade urine flow using detective flow imaging in a cat with chronic kidney disease.
Tamura M, Ohta H.
J Small Anim Pract. 2023 Oct 31(Online ahead of print.).
doi: 10.1111/jsap.13684.

2）Effectiveness of 2-dimensional shear wave elastography for noninvasive and reliable estimation of right atrial pressure in dogs with induced volume overload.
Tamura M, Ohta H, Osuga T, Takiguchi M.
J Vet Intern Med. 37:866–874. 2023. doi: 10.1111/jvim.16705.

Ⅱ．その他　＜Others＞

BSAVA
L OF SMALL ANIMAL PRACTICE

IMAGES IN SMALL ANIMAL PRACTICE

Detecting antegrade urine flow using detective flow imaging in a cat with chronic kidney disease

Detective flow imaging represents an innovative advancement in ultrasound Doppler technology, enabling the visualisation of low-velocity flow without contrast. A 14-year-old neutered cat was referred for a screening examination for chronic kidney disease (CKD), which had been diagnosed over 2 months prior. Blood tests revealed elevated levels of blood urea nitrogen (19.64 mmol/L; reference interval, 6.28 to 11.71 mmol/L) and creatinine (398.7 μmol/L; reference interval, 79.6 to 185.6 μmol/L), as well as hypokalaemia (3.1 mmol/L; reference interval, 3.4 to 4.6 mmol/L). Packed cell volume was within normal limits. Urine analysis indicated a decreased urine specific gravity of 1.009, but no other significant abnormalities were noted. The cat was diagnosed with IRIS CKD stage 3. Radiography showed that the left kidney was small, measuring approximately 1.2 times the length of the second lumber vertebra, but the right kidney was normal limits (2.4 times the length of the left kidney). Abdominal ultrasonographic examination (ARIETTA 850; FUJIFILM Medical Co., Ltd., Tokyo, Japan) revealed a small left kidney length measuring 2.1 cm with a mild irregular shape (Fig 1A) and a normal-sized right kidney measuring 4.2 cm. Both kidneys exhibited poor renal architecture. The ultrasound findings were consistent with clinically diagnosed chronic degenerative nephropathy. Detective flow imaging was performed at the level of the ureterovesicular junctions to evaluate the urine production in the small left kidney using an 18 to 5-MHz liner probe (L64; FUJIFILM Medical). The ureteral jet phenomenon was observed as antegrade urine flow from the ureterovesicular junction of the left ureter (Fig 1B) as well as that of the right ureter (Fig 1C). This phenomenon occurred at intervals of once every 10 to 30 s. While not a measure of renal function, detective flow imaging may be used for detection of ureteral jets to confirm urine production and ureteral patency in kidneys with ultrasound findings indicative of chronic degenerative nephropathy.

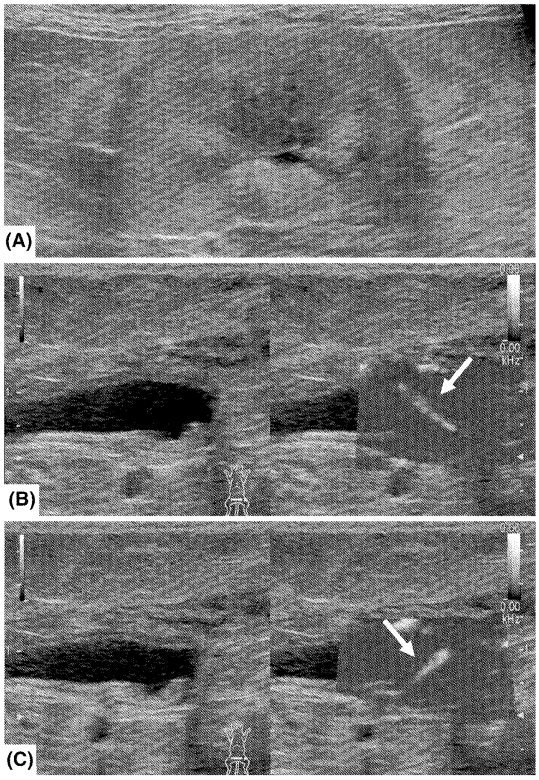


FIG 1. Ultrasonography was used to detect the small size, mild irregular shape and poor renal architecture of the left kidney (A). Detective flow imaging revealed the ureteral jet phenomenon from the ureterovesicular junction of the left ureter (B: white arrow) as well as that of the right ureter (C: white arrow)

M. Tamura and H. Ohta[1]

Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University
Ebetsu, Hokkaido 069-8501, Japan

[1]Corresponding author email: h-ohta@rakuno.ac.jp

出典 doi: 10.1111/jsap.13684
https://onlinelibrary.wiley.com/doi/10.1111/jsap.13684
最終責任者 Ohta Hiroshi (Corresponding Author)

Received: 12 May 2022 | Accepted: 24 March 2023
DOI: 10.1111/jvim.16705

STANDARD ARTICLE

Journal of Veterinary Internal Medicine ACVIM
Open Access
American College of Veterinary Internal Medicine

Effectiveness of 2-dimensional shear wave elastography for noninvasive and reliable estimation of right atrial pressure in dogs with induced volume overload

Masahiro Tamura[1,2] | Hiroshi Ohta[1] | Tatsuyuki Osuga[3] | Mitsuyoshi Takiguchi[2]

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

[2]Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

[3]Laboratory of Veterinary Internal Medicine, Department of Veterinary Sciences, University of Miyazaki, Miyazaki, Miyazaki, Japan

Correspondence
Mitsuyoshi Takiguchi, Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9, Sapporo, Hokkaido 060-0818, Japan.
Email: mtaki@vetmed.hokudai.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: JP22K15014; Promotion and Mutual Aid Corporation for Private Schools of Japan

Abstract

Background: Two-dimensional shear wave elastography (2D-SWE) provides information on hepatic elastic modulus as shear wave velocity (SWV).

Hypothesis/Objectives: To assess SWV using 2D-SWE in dogs with induced volume overload, investigate the relationship between this information and right atrial pressure (RAP) measured by invasive right heart catheterization, and also evaluate the difference in SWV before and after diuretic administration.

Animals: Six healthy beagles.

Methods: Prospective experimental study. Right heart catheterization and 2D-SWE were performed in 6 anesthetized beagles at baseline and after the induction of volume overload. Volume overload was induced by IV hydroxyethyl starch 70/0.5 infusion (100 mL/kg/h). Furosemide (4-6 mg/kg, IV) was administered, and the SWVs were measured.

Results: Shear wave velocity showed a significant gradual increase during acute volume overload compared to baseline. SWV was significantly positively correlated with RAP ($P < .0001$, $\rho = 0.9729$). The area under the curve of SWV to predict RAP at >10, >15, and >20 mm Hg was 0.9896 (95% confidence interval [95% CI], 0.9690-1.000), 0.9907 (95% CI, 0.9701-1.000), and 0.9722 (95% CI, 0.9280-1.000), respectively. The SWV after diuretic use decreased significantly.

Conclusions and Clinical Importance: Two-dimensional shear wave elastography might be useful for noninvasive and reliable estimation of RAP in dogs with acute volume overload and has potential as a quantitative biomarker for evaluating therapeutic response in dogs with right sided congestive heart failure.

KEYWORDS
2D-SWE, canine, right atrial pressure, SWV

Abbreviations: 2D-SWE, 2-dimensional shear wave elastography; AUC, area under the curve; CI, confidence interval; IVC, interior vena cava; RAP, right atrial pressure; R-CHF, right-sided congestive heart failure; ROC, receiver-operating characteristic; ROI, region of interest; SWV, shear wave velocity; TE, transient elastography.

出典 doi: 10.1111/jvim.16705.
https://onlinelibrary.wiley.com/doi/10.1111/jvim.16705
最終責任者 Takiguchi Mitsuyoshi (Corresponding Author)

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

Tatsuya Deguchi

Assistant professor

助教　出口　辰弥

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

1）Enhanced Systemic Antitumour Immunity by Hypofractionated Radiotherapy and Anti-PD-L1 Therapy in Dogs with Pulmonary Metastatic Oral Malignant Melanoma.
Deguchi T, Maekawa N, Konnai S, Owaki R, Hosoya K, Morishita K, Nakamura M, Okagawa T, Takeuchi H, Kim S, Kinoshita R, Tachibana Y, Yokokawa M, Takagi S, Kato Y, Suzuki Y, Murata S, Ohashi K.
Cancers 15:3013. 2023. doi:10.3390/cancers15113013.

2）Regulation of programmed death ligand 1 expression by interferon-γ and tumour necrosis factor-α in canine tumour cell lines.
Owaki R, **Deguchi T**, Konnai S, Maekawa N, Okagawa T, Hosoya K, Kim S, Sunaga T, Okumura M.
Vet Comp Oncol. 21:279-290. 2023. doi: 10.1111/vco.12886.

Ⅱ．その他　＜Others＞

1）Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors.
Maekawa N, Konnai S, Asano Y, Otsuka T, Aoki E, Takeuchi H, Kato Y, Kaneko M, Yamada S, Kagawa Y, Nishimura M, Takagi S, **Deguchi T**, Ohta H, Nakagawa T, Suzuki Y, Okagawa T, Murata S, Ohashi K.
PLoS One. 18:e0281143. 2023. doi:10.1371/journal.pone.0281143.

2）Safety and clinical efficacy of an anti-PD-L1 antibody (c4G12) in dogs with advanced malignant tumours.
Maekawa N, Konnai S, Hosoya K, Kim S, Kinoshita R, **Deguchi T**, Owaki R, Tachibana Y, Yokokawa M, Takeuchi H, Kagawa Y, Takagi S, Ohta H, Kato Y, Yamamoto S, Yamamoto K, Suzuki Y, Okagawa T, Murata S, Ohashi K.
PLoS One. 18:e0291727. 2023. doi: 10.1371/journal.pone.0291727.

cancers

MDPI

Article

Enhanced Systemic Antitumour Immunity by Hypofractionated Radiotherapy and Anti-PD-L1 Therapy in Dogs with Pulmonary Metastatic Oral Malignant Melanoma

Tatsuya Deguchi [1,†], Naoya Maekawa [2,†], Satoru Konnai [2,3,*], Ryo Owaki [1], Kenji Hosoya [1], Keitaro Morishita [1], Motoji Nakamura [1], Tomohiro Okagawa [2], Hiroto Takeuchi [3], Sangho Kim [1], Ryohei Kinoshita [1], Yurika Tachibana [1], Madoka Yokokawa [1], Satoshi Takagi [4], Yukinari Kato [5,6], Yasuhiko Suzuki [7,8], Shiro Murata [2,3] and Kazuhiko Ohashi [2,3]

[1] Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0819, Japan
[2] Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[3] Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[4] Department of Veterinary Surgery 1, School of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan
[5] Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan
[6] Department of Molecular Pharmacology, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan
[7] International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan
[8] Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo 060-0808, Japan
* Correspondence: konnai@vetmed.hokudai.ac.jp
† These authors contributed to this work equally.

Simple Summary: Immune checkpoint inhibitors (ICIs) are promising treatment options for spontaneous cancers in dogs. To optimize the clinical benefit of ICIs, combination therapy with radiation has been proposed for human cancers; however, the safety and efficacy of the combination approach have not yet been reported in dogs. The retrospective analysis of dogs bearing pulmonary metastatic oral malignant melanoma suggested that the use of previous (≤8 weeks) hypofractionated radiation therapy is associated with a better clinical response to anti-PD-L1 immunotherapy. Radiotherapy before the initiation of anti-PD-L1 therapy can be an effective approach to enhance antitumor immunity in dogs.

Abstract: Although immune checkpoint inhibitors (ICIs), such as the anti-programmed death-ligand 1 (PD-L1) antibody, have been developed for the treatment of canine malignant melanoma, desirable clinical efficacies have not been achieved. Recent studies in humans have suggested that radiation therapy (RT) combined with ICIs induces robust systemic antitumour immunity in patients with cancer. This study retrospectively examined the therapeutic efficacy of combination therapy (hypofractionated RT and anti-PD-L1 antibody [c4G12]) in dogs with pulmonary metastatic oral malignant melanoma. The intrathoracic clinical benefit rate (CBR)/median overall survival (OS) in the no RT (n = 20, free from the effect of RT), previous RT (n = 9, received RT ≤8 weeks prior to the first c4G12 dose), and concurrent RT (n = 10, c4G12 therapy within ±1 week of the first RT fraction) groups were 10%/185 days, 55.6%/283.5 days ($p < 0.05$ vs. no RT group), and 20%/129 days ($p > 0.05$ vs. no RT group), respectively. The adverse events were considered to be tolerable in the combination therapy. Thus, hypofractionated RT before the initiation of c4G12 therapy can be an effective approach for enhancing the therapeutic efficacy of immunotherapy, with acceptable safety profiles. Further prospective clinical studies are required to confirm the findings of this study.

check for updates

Citation: Deguchi, T.; Maekawa, N.; Konnai, S.; Owaki, R.; Hosoya, K.; Morishita, K.; Nakamura, M.; Okagawa, T.; Takeuchi, H.; Kim, S.; et al. Enhanced Systemic Antitumour Immunity by Hypofractionated Radiotherapy and Anti-PD-L1 Therapy in Dogs with Pulmonary Metastatic Oral Malignant Melanoma. *Cancers* **2023**, *15*, 3013. https://doi.org/10.3390/cancers15113013

Academic Editor: David Wong

Received: 7 April 2023
Revised: 9 May 2023
Accepted: 24 May 2023
Published: 1 June 2023

出典 doi: 10.3390/cancers15113013.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10252299/pdf/cancers-15-03013.pdf

最終責任者 Satoru Konnai (Corresponding Author)

Received: 18 October 2022 | Revised: 4 February 2023 | Accepted: 6 February 2023
DOI: 10.1111/vco.12886

ORIGINAL ARTICLE

Veterinary and Comparative Oncology WILEY

Regulation of programmed death ligand 1 expression by interferon-γ and tumour necrosis factor-α in canine tumour cell lines

Ryo Owaki[1] | Tatsuya Deguchi[2] | Satoru Konnai[3,4] | Naoya Maekawa[3] | Tomohiro Okagawa[3] | Kenji Hosoya[2] | Sangho Kim[1] | Takafumi Sunaga[1] | 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]Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Correspondence
Tatsuya Deguchi, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan.
Email: t-deguchi@rakuno.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Numbers: JP19K23702, JP20K15655; JST SPRING, Grant/Award Number: JPMJSP2119

Abstract

Expression of programmed death ligand 1 (PD-L1) on tumour cells provides an immune evasion mechanism by inducing suppression of cytotoxic T cells. Various regulatory mechanisms of PD-L1 expression have been described in human tumours, however, little is known in canine tumours. To investigate whether inflammatory signalling is involved in PD-L1 regulation in canine tumours, the effects of interferon (IFN)-γ and tumour necrosis factor (TNF)-α treatment were examined in canine malignant melanoma cell lines (CMeC and LMeC) and an osteosarcoma cell line (HMPOS). The protein level of PD-L1 expression was upregulated by IFN-γ and TNF-α stimulation. Upon IFN-γ stimulation, all cell lines showed an increase in expression of *PD-L1*, *signal transducer and activator of transcription (STAT)1*, *STAT3* and genes regulated by STAT activation. Upregulated expression of these genes was suppressed by the addition of a JAK inhibitor, oclacitinib. Contrastingly, upon TNF-α stimulation, all cell lines exhibited higher gene expression of the nuclear factor kappa B (NF-κB) gene *RELA* and genes regulated by NF-κB activation, whereas expression of *PD-L1* was upregulated in LMeC only. Upregulated expression of these genes was suppressed by the addition of an NF-κB inhibitor, BAY 11-7082. The expression level of cell surface PD-L1 induced by IFN-γ and TNF-α treatment was reduced by oclacitinib and BAY 11-7082, respectively, indicating that upregulation of PD-L1 expression by IFN-γ and TNF-α stimulation is regulated via the JAK-STAT and NF-κB signalling pathways, respectively. These results provide insights into the role of inflammatory signalling in PD-L1 regulation in canine tumours.

KEYWORDS
cell signalling, immunology, in vitro models, oncology, small animal

出典　doi: 10.1111/vco.12886.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/vco.12886
最終責任者　Tatsuya Deguchi (Corresponding Author)

PLOS ONE

RESEARCH ARTICLE

Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors

Naoya Maekawa[1], Satoru Konnai[1,2,3]*, Yumie Asano[2], Takumi Otsuka[2], Eri Aoki[2], Hiroto Takeuchi[2], Yukinari Kato[4,5], Mika K. Kaneko[4], Shinji Yamada[4], Yumiko Kagawa[6], Maki Nishimura[6], Satoshi Takagi[7,8], Tatsuya Deguchi[7,9], Hiroshi Ohta[7,9], Takayuki Nakagawa[10], Yasuhiko Suzuki[1,3,11,12], Tomohiro Okagawa[1], Shiro Murata[1,2], Kazuhiko Ohashi[1,2,13]

1 Department of Advanced Pharmaceutics, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **2** Department of Disease Control, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **3** Institute for Vaccine Research and Development (HU-IVReD), Hokkaido University, Sapporo, Japan, **4** Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan, **5** Department of Molecular Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan, **6** North Lab, Sapporo, Japan, **7** Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan, **8** Department of Veterinary Surgery 1, School of Veterinary Medicine, Azabu University, Sagamihara, Japan, **9** Companion Animal Internal Medicine, Department of Companion Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan, **10** Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Japan, **11** International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan, **12** Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Sapporo, Japan, **13** International Affairs Office, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

* konnai@vetmed.hokudai.ac.jp

Citation: Maekawa N, Konnai S, Asano Y, Otsuka T, Aoki E, Takeuchi H, et al. (2023) Molecular characterization of feline immune checkpoint molecules and establishment of PD-L1 immunohistochemistry for feline tumors. PLoS ONE 18(1): e0281143. https://doi.org/10.1371/journal.pone.0281143

Editor: Avaniyapuram Kannan Murugan, King Faisal Specialist Hospital and Research Center, SAUDI ARABIA

Received: November 4, 2022

Accepted: January 11, 2023

Published: January 26, 2023

Data Availability Statement: All data are fully available without restriction. All relevant data are within the manuscript and its Supporting information file. Nucleotide sequences of feline PD-L1 and PD-L2 were submitted to the DDBJ/EMBLBank/GenBank database under accession numbers LC735019, LC735020, and LC735021.

Funding: This work was supported by the Grant-in-Aid for Scientific Research (Grant Numbers

Abstract

Spontaneous tumors are a major cause of death in cats. Treatment of human tumors has progressed dramatically in the past decade, partly due to the success of immunotherapies using immune checkpoint inhibitors, such as anti-programmed death 1 (PD-1) and anti-PD-ligand 1 (PD-L1) antibodies. However, little is known about the PD-1 pathway and its association with tumor disease in cats. This study investigated the applicability of anti-PD-1/PD-L1 therapy in feline tumors. We first determined the complete coding sequence of feline *PD-L1* and *PD-L2*, and found that the deduced amino acid sequences of feline PD-L1/PD-L2 share high sequence identities (66–83%) with orthologs in other mammalian species. We prepared recombinant feline PD-1, PD-L1, and PD-L2 proteins and confirmed receptor–ligand binding between PD-1 and PD-L1/PD-L2 using flow cytometry. Next, we established an anti-feline PD-L1 monoclonal antibody (clone CL1Mab-7) to analyze the expression of PD-L1. Flow cytometry using CL1Mab-7 revealed the cell surface expression of PD-L1 in a feline macrophage (Fcwf-4) and five mammary adenocarcinoma cell lines (FKNp, FMCm, FYMp, FONp, and FONm), and showed that PD-L1 expression was upregulated by interferon-γ stimulation. Finally, immunohistochemistry using CL1Mab-7 also showed PD-L1 expression in feline squamous cell carcinoma (5/5, 100%), mammary adenocarcinoma (4/5,

出典　doi: 10.1371/journal.pone.0281143

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9879432/pdf/pone.0281143.pdf

最終責任者　Satoru Konnai (Corresponding Author)

PLOS ONE

RESEARCH ARTICLE

Safety and clinical efficacy of an anti-PD-L1 antibody (c4G12) in dogs with advanced malignant tumours

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

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

* konnai@vetmed.hokudai.ac.jp

OPEN ACCESS

Citation: Maekawa N, Konnai S, Hosoya K, Kim S, Kinoshita R, Deguchi T, et al. (2023) Safety and clinical efficacy of an anti-PD-L1 antibody (c4G12) in dogs with advanced malignant tumours. PLoS ONE 18(10): e0291727. https://doi.org/10.1371/journal.pone.0291727

Editor: Alessandro Rizzo, IRCCS Giovanni Paolo II Cancer Hospital, ITALY

Received: July 20, 2023

Accepted: September 2, 2023

Published: October 4, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by the Grant-in-Aid for Scientific Research (Grant Numbers 21K1498301 and 22H02503) from the Japan Society for the Promotion of Science (JSPS) and by Japan Agency for Medical Research and Development (AMED) under Grant Numbers: JP223fa627005, JP22ama121008, and JP21am0101078. Funders had no role in the study

Abstract

Immune checkpoint inhibitors (ICIs) have been developed for canine tumour treatment, and pilot clinical studies have demonstrated their antitumour efficacy in dogs with oral malignant melanoma (OMM). Although ICIs have been approved for various human malignancies, their clinical benefits in other tumour types remain to be elucidated in dogs. Here, we conducted a clinical study of c4G12, a canine chimeric anti-PD-L1 antibody, to assess its safety and efficacy in dogs with various advanced malignant tumours ($n = 12$) at the Veterinary Teaching Hospital of Hokkaido University from 2018 to 2023. Dogs with digit or foot pad malignant melanoma ($n = 4$), osteosarcoma ($n = 2$), hemangiosarcoma ($n = 1$), transitional cell carcinoma ($n = 1$), nasal adenocarcinoma ($n = 1$), B-cell lymphoma ($n = 1$), or undifferentiated sarcoma ($n = 2$) were treated with 2 or 5 mg/kg c4G12 every 2 weeks. Treatment-related adverse events of any grade were observed in eight dogs (66.7%), including elevated aspartate aminotransferase (grade 3) in one dog (8.3%) and thrombocytopenia (grade 4) in another dog (8.3%). Among dogs with target disease at baseline ($n = 8$), as defined by the response evaluation criteria for solid tumours in dogs (cRECIST), one dog with nasal adenocarcinoma and another with osteosarcoma experienced a partial response (PR), with an objective response rate of 25.0% (2 PR out of 8 dogs; 95% confidence interval: 3.2–65.1%). These results suggest that c4G12 is safe and tolerable and shows antitumor

出典　doi: 10.1371/journal.pone.0291727

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10550157/pdf/pone.0291727.pdf

最終責任者　Satoru Konnai (Corresponding Author)

獣医解剖学（Veterinary Anatomy）

Marina Hosotani

Assistant Professor

助教　細谷　実里奈

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

1）Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens.
Hosotani M, Hamano S, Iwasaki T, Hasegawa Y, Ueda H, Watanabe T.
Vet Sci. 10:260–260. 2023. doi: 10.3390/vetsci10040260.

2）Oocyte cumulus complex quality and oviduct transportation velocity in systemic autoimmune disease model mice.
Hosotani M, Ichii O, Watanabe Y, Kon Y.
Exp Biol Med (Maywood) 248:1359–1363. 2023.
doi: 10.1177/15353702231160875.

Ⅱ．その他＜Others＞

1）Physicochemical properties of wooden breast-extracted myosin and rheological properties of its heat-induced gel.
Hasegawa Y, Kawasaki T, Yamada M, **Hosotani M,** Maeda N, Watanabe T, Iwasaki T.
J Science Food Agric. 103:5609–5615. 2023. doi: 10.1002/jsfa.12636.

veterinary sciences

MDPI

Article

Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens

Marina Hosotani [1,*], Sohei Hamano [1], Tomohito Iwasaki [2], Yasuhiro Hasegawa [2], Hiromi Ueda [1] and Takafumi Watanabe [1]

[1] Laboratory of Veterinary Anatomy, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
[2] Department of Food Science and Human Wellness, College of Agriculture, Food and Environment Science, Rakuno Gakuen University, Ebetsu 069-8501, Hokakido, Japan
* Correspondence: m-hosotani@rakuno.ac.jp; Tel.: +81-11-388-4763

Simple Summary: Internal laying and egg-bound syndromes are avian reproductive disorders that reduce egg productivity in laying hens. To date, the understanding of the importance of peristalsis abnormality in the smooth muscle of oviducts has been focused on the pathogeneses of internal laying and egg-bound syndrome, and the histopathology of the oviductal epithelium has not been explored. In this study, we histologically examined the oviductal ciliated epithelium of aged laying hens. We observe that the epithelial region lacking cilia is larger in the oviducts of hens with internal laying and egg-bound syndrome than in those of healthy hens. In addition, the lamina propria of the oviducts of hens with internal laying and egg-bound syndrome is affected by the infiltration of CD3-positive T-cells. The histological alternation of the ciliated epithelial cells in the oviducts owing to the oviductal inflammation is suggested as the underlying cause of the pathogeneses of internal laying and egg-bound syndrome.

Abstract: In the egg industry, common reproductive disorders, such as internal laying and egg-bound syndrome, not only reduce egg productivity but also cause deaths in severe cases. In this study, we focused on the oviduct histology of the pathogenesis of internal laying and egg-bound syndrome. We divided the aged laying hens into four groups according to the observation of the abdominal cavity and oviductal lumen: healthy, internal laying, egg-bound, and intercurrent. The percentages of healthy, internal laying, egg-bound, and intercurrent groups were 55%, 17.5%, 15%, and 12.5%, respectively. In all parts of the oviduct (i.e., infundibulum, magnum, isthmus, and uterus), the oviductal epithelium was composed of ciliated epithelial cells and secretory cells. The epithelial region lacking cilia was larger in the entire oviduct of the internal laying, and intercurrent groups than in the healthy group. In the internal laying, egg-bound, and intercurrent groups, significant T-cell infiltration was observed in the lamina propria of the entire oviduct. The morphological alteration of ciliated epithelial cells in the oviducts caused by inflammation may be the underlying cause of the pathogenesis of internal laying and egg-bound syndrome.

Keywords: cilia; chicken; egg-bound syndrome; histology; internal laying; oviduct; reproductive disorder

Citation: Hosotani, M.; Hamano, S.; Iwasaki, T.; Hasegawa, Y.; Ueda, H.; Watanabe, T. Oviduct Histopathology of Internal Laying and Egg-Bound Syndrome in Laying Hens. *Vet. Sci.* **2023**, *10*, 260. https://doi.org/10.3390/vetsci10040260

Academic Editor: Manuel Aviles

Received: 14 February 2023
Revised: 25 March 2023
Accepted: 27 March 2023
Published: 29 March 2023

1. Introduction

Selective breeding of laying hens has achieved an approximately two-fold higher egg productivity over the decades [1,2]; however, this has led to the emergence of reproductive disorders. Caged layer fatigue owing to the excess demand for calcium for frequent egg laying includes not only osteopenia but also oviductal inertia because muscle contraction requires calcium [3]. Laying hens spontaneously increase the risk of ovarian cancer with aging, along with decreased egg productivity [4,5]. In addition to genetic factors, rearing

出典 doi: 10.3390/vetsci10040260.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10142957/
最終責任者 Marina Hosotani (Corresponding Author)

Brief Communication

Oocyte cumulus complex quality and oviduct transportation velocity in systemic autoimmune disease model mice

Marina Hosotani[1], Osamu Ichii[2,3], Takafumi Watanabe[1] and Yasuhiro Kon[2]

[1]Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; [2]Laboratory of Anatomy, Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan; [3]Laboratory of Agrobiomedical Science, Faculty of Agriculture, Hokkaido University, Sapporo 060-0818, Japan
Corresponding author: Marina Hosotani. Email: m-hosotani@rakuno.ac.jp

Impact Statement

Oocyte transportation consists of two steps: adhesion of cumulus cells to the ciliary tip of ciliated epithelial cells and transportation of cumulus–oocyte complexes (COCs) by ciliary beating. In the former step, the pathological factors that alter the interaction between cumulus cells and cilia are not currently understood. In this study, by using autoimmune disease-prone MRL/MpJ-*Fas*$^{lpr/lpr}$ mice that exhibited the oocyte transportation disorder by abnormal morphofunction of oviductal ciliated epithelium, we revealed that COC transportation property was determined by both the ciliary function in the infundibulum and the properties of COCs. Furthermore, we showed that the transportation velocity of COCs (TVCs) was recovered by the properties of cumulus cells and the healthy morphofunction of oviductal ciliated epithelium. These findings contribute to further investigations on novel immunological factors in COCs that can achieve efficient oocyte transportation and related processes, which provide the potential for understanding the pathogenesis of tubal infertility.

Abstract

Oocyte transportation by the oviduct involves the interaction between ciliated epithelial cells and cumulus cells. To determine whether the quality of cumulus–oocyte complexes (COCs) changes the transportation property of COCs, we compared the transportation velocity of COCs (TVC) by the infundibulum *ex vivo* with various combinations of infundibula and COCs collected from different mice. We used young and aged C57BL/6N and MRL/MpJ, and MRL/MpJ-*Fas*$^{lpr/lpr}$ mice as the strains with intact female reproductive function and the systemic autoimmune disease model exhibiting oocyte pick-up dysfunction owing to the morphofunctional abnormality of ciliated epithelium, respectively. The TVC of aged MRL strains was less than that of aged C57BL/6N mice, suggesting that aging affects the transportation of COCs in MRL strains. The TVC of aged MRL/MpJ-*Fas*$^{lpr/lpr}$ mice was the least among all examined combinations, whereas the TVC accelerated when the infundibulum or COCs were collected from other strains. These results indicate that the transportation property of COCs is determined not only by the ciliary function in the infundibulum but also by the properties of COCs.

Keywords: Autoimmune disease, cumulus cell, cilia, *ex vivo* experiment, oocyte transportation, oviduct

Experimental Biology and Medicine **2023; 248: 1359–1363. DOI: 10.1177/15353702231160875**

出典　doi: 10.1177/15353702231160875.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10657593/

最終責任者　Marina Hosotani (Corresponding Author)

Journal of the Science of Food and Agriculture

Research Article

Physicochemical properties of wooden breast-extracted myosin and rheological properties of its heat-induced gel

Yasuhiro Hasegawa, Takeshi Kawasaki, Michi Yamada, Marina Hosotani, Naoyuki Maeda, Takafumi Watanabe, Tomohito Iwasaki

First published: 17 April 2023 | https://doi.org/10.1002/jsfa.12636

Abstract

BACKGROUND

It is reported that broilers with 'wooden breast' have poor processing properties, such as low binding and water-holding capacities. However, the reason for the poor functional characteristics has not been clarified. In this study, myosin was extracted from a wooden breast. Its physicochemical properties were investigated to clarify the relationship between the structure and physicochemical properties of the heating gel of myosin obtained from the wooden breast.

RESULTS

The turbidity of myosin solution extracted from wooden breast increased with increase in the heat treatment to a higher value than that from the normal breast meat myosin. The solubility of myosin collected from a wooden breast after heating decreased like normal breast muscle myosin. The surface hydrophobicity of myosin removed from wooden breast increased continually above 60 °C, unlike the change in surface hydrophobicity of normal breast myosin. The free thiol group of myosin extracted from the wooden breast was higher than normal breast myosin before and after heating. The apparent elasticity of heat-induced gels and chicken meat sausages was significantly lower in sausages and gel with wooden breast than normal ones ($P < 0.05$). The microstructure of the heated gel of normal myosin showed a fine network structure. In contrast, the heat-induced gel of wooden breast-extracted myosin showed a structure with loosely connected aggregates and many gaps.

CONCLUSION

The coarseness of the internal gel structure of myosin extracted from wooden breast was shown to affect the apparent elasticity of the gel and sausages made from the chicken meat.

出典 doi: 10.1002/jsfa.12636.

https://onlinelibrary.wiley.com/doi/10.1002/jsfa.12636

最終責任者 Tomohito Iwasaki (Corresponding Author)

獣医麻酔学（Veterinary Anesthesiology）

Keiko Kato

Professor

助教　加藤　桂子

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

1）Use of a gum elastic bougie in a cat with severe upper airway stenosis.
Kato K, Itami T, Torisu S, Sakai T, Yamashita K.
Open Vet J. 13:114–118. doi: 10.5455/OVJ.2023.v13.i1.12.

Ⅱ．その他　＜Others＞

1）The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits.
Wei Y, Chen IY, Tamogi H, Sugita C, Daimaruya N, Hirokawa T, **Kato K**, Itami T, Sano T, Yamashita K.
J Vet Med Sci. 85: 471–478. 2023. doi: 10.1292/jvms.22-0484.

Open Veterinary Journal, (2023), Vol. 13(1): 114–118
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Case Report
DOI: *10.5455/OVJ.2023.v13.i1.12*

Submitted: 11/07/2022 Accepted: 05/12/2022 Published: 23/01/2023

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*

出典 doi: 10.5455/OVJ.2023.v13.i1.12.

https://www.openveterinaryjournal.com/?mno=56656

最終責任者 Takaharu Itami (Corresponding Author)

The Journal of Veterinary Medical Science

FULL PAPER
Surgery

The sedative effect of intranasal administration of medetomidine using a mucosal atomization device in Japanese White rabbits

Yixian WEI[1)], I-Ying CHEN[1)], Haruka TAMOGI[1)], Chihiro SUGITA[1)], Nozomi DAIMARUYA[1)], Taku HIROKAWA[1)], Keiko KATO[1)], Takaharu ITAMI[1)], Tadashi SANO[1)], Kazuto YAMASHITA[1)]*

[1)]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

ABSTRACT. To prevent aspiration in Japanese White (JW) rabbits, the maximum single volume of medetomidine administered intranasally is 0.3 mL per nostril using a mucosal atomization device (MAD). This study aimed to examine the sedative effect of intranasal administration of medetomidine using MAD in eight healthy female JW rabbits. Each rabbit received intranasal atomization (INA) of saline (Control treatment) along with three doses of 1 mg/mL medetomidine (0.3 mL to one nostril [MED0.3 treatment]; 0.3 mL each to both nostrils [MED0.6 treatment]; 0.3 mL twice to both nostrils [MED1.2 treatment]), with a washout period of at least 7 days between treatments. The actual doses of medetomidine were 82 (75–84) μg/kg (median [25th–75th percentile]), 163 (156–168) μg/kg, and 323 (295–343) μg/kg for the MED0.3, MED0.6, and MED1.2 treatments, respectively. A medetomidine-dose dependent sedative effect was detected, and the loss of righting reflex (LRR) was achieved in one rabbit at 18 min, seven rabbits at 11 (9–18) min, and eight rabbits at 7 (4–18) min after the MED0.3, MED0.6, and MED1.2 treatments, respectively. The LRR was maintained for 63 (29–71) min and 83 (68–101) min after the MED0.6 and MED1.2 treatments, respectively. Additionally, the INA of medetomidine produced a significant dose-dependent cardiorespiratory depression including a decrease in pulse rate, respiratory rate, percutaneous oxygen saturation, and arterial partial pressure of oxygen, and an increase in arterial partial pressure of carbon dioxide in the rabbits.

KEYWORDS: intranasal atomization, medetomidine, mucosal atomization device, rabbit

J. Vet. Med. Sci.
85(4): 471–478, 2023
doi: 10.1292/jvms.22-0484

Received: 25 October 2022
Accepted: 22 February 2023
Advanced Epub:
6 March 2023

*Correspondence to: Yamashita K: yamasita@rakuno.ac.jp, Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

出典　doi: 10.1292/jvms.22-0484.

https://www.jstage.jst.go.jp/article/jvms/85/4/85_22-0484/_pdf/-char/en

最終責任者　Kazuto Yamashita (Corresponding Author)

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＞

1）Effect of live yeast Saccharomyces cerevisiae supplementation on immune factors in Japanese Black calves during the growth periods.
Kosenda K, Yabashi E, Takeda S, Ohtsuka H.
J Vet Med Sci. 85: 290–295. 2023. doi: 10.1292/jvms.22-0437.

2）Histological Characteristics of Conjunctiva-Associated Lymphoid Tissue in Young and Adult Holstein Cattle.
Kosenda K, Ichii O, Yamashita Y, Ohtsuka H, Fukuda S, Kon Y.
Animals (Basel). 13:3481. 2023. doi: 10.3390/ani13223481.

Ⅱ．その他＜Others＞

1）mRNA expression of immune factors by milk somatic cells from healthy Holstein lactating cows.
Murakami K, Fukuhara T, Kure S, Shimosakai T, Sato A, Murata R, **Kosenda K**, Ohtsuka H.*Can J Vet Res.* 87:231-236. 2023.

2）Effect of supplementation with Saccharomyces boulardii CNCM I-1079 on vaccine response to an inactivated bacterial vaccine in young Japanese Black calves: A field trial.
Mori K, Uchiumi A, Yamamoto K, Shimizu Y, Ueda R, **Kosenda K**, Fukuhara T, Kure S, Fukazawa H, Ohtsuka H.
Can J Vet Res. 87:237-242. 2023.

3）Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows.
Sato A, Sugiura T, **Kosenda K**, Murakami T.
J Am Vet Med Assoc. 261:1374-1379. 2023. doi: 10.2460/javma.23.02.0062.

4）Antibody response to 1.0 and 0.5 mL doses of an inactivated bacterial vaccine against bovine respiratory disease in young Holstein calves: a field trial.
Mori K, Kato T, **Kosenda K**, Yokota O, Ohtsuka H.
J Vet Res. 67:315-321. 2023. doi: 10.2478/jvetres-2023-0037.

The Journal of Veterinary Medical Science

FULL PAPER
Internal Medicine

Effect of live yeast *Saccharomyces cerevisiae* supplementation on immune factors in Japanese Black calves during the growth periods

Keigo KOSENDA[1)], Eri YABASHI[1)], Shogo TAKEDA[2)], Hiromichi OHTSUKA[1)]*

[1)]Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[2)]Hidaka Central Livestock Clinic, Hokkaido, Japan

ABSTRACT. The aim of this study was to investigate changes in expression levels of immune factors of peripheral blood mononuclear cells (PBMC) after oral supplementation of live yeast *Saccharomyces cerevisiae* to healthy Japanese Black (JB) calves. This study examined JB calves (N=28): 14 calves (SC Group) received 10 g/calf/day of *Saccharomyces cerevisiae* (SC) (Acti-Saf Sc 47), and the other calves did not receive supplement (Control Group). Blood samples were collected 9 times during experimental period (1, 2, 3, 4, 5, 6, 7, 8 and 9 months of age), and analyzed for cytokines and chemokines mRNA expression of PBMC using Real-time PCR. The level of beta Hydroxybutanoic acid (BHB) in the SC Group was significantly high at 7 and 8 months of age compared to that in the Control Group. Lymphocyte counts in the SC Group were significantly higher at 2 and 5 to 6 months of age compared to the Control Group. Significant differences were found for IL-12p40 level at 4, 7 and 9 months of age, and for IFN-γ level at 6, 7 and 8 months of age. The level of CXCR3 was significantly higher at 6 to 7 months of age by dietary SC supplementation. These results indicated that SC supplementation improved the cellular immune responses of JB calves.

KEYWORDS: calf, immune factors, Japanese Black, peripheral blood mononuclear cells, yeast supplementation

J. Vet. Med. Sci.
85(3): 290–295, 2023
doi: 10.1292/jvms.22-0437

Received: 15 September 2022
Accepted: 5 January 2023
Advanced Epub:
23 January 2023

*Correspondence to: Ohtsuka H: ohtsuka@rakuno.ac.jp, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsushi Bunnkyoudai-midorimachi 582, Hokkaido 069-8501, Japan

出典 doi: 10.1292/jvms.22-0437.
https://www.jstage.jst.go.jp/article/jvms/85/3/85_22-0437/_pdf/-char/en

最終責任者 Hiromichi Ohtsuka (Corresponding Author)

animals

MDPI

Article

Histological Characteristics of Conjunctiva-Associated Lymphoid Tissue in Young and Adult Holstein Cattle

Keigo Kosenda [1,*], Osamu Ichii [2,3], Yusuke Yamashita [4], Hiromichi Ohtsuka [5], Shigeo Fukuda [1] and Yasuhiro Kon [2]

[1] Laboratory of Farm Animal Pathophysiology, Department of Farm Animal Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-0836, Japan; fukuda@rakuno.ac.jp
[2] Laboratory of Anatomy, Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan; ichi-o@vetmed.hokudai.ac.jp (O.I.); y-kon@vetmed.hokudai.ac.jp (Y.K.)
[3] Laboratory of Agrobiomedical Science, Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
[4] Nayoro Veterinary Clinical Center, Hokkaido Agricultural Mutual Aid Association, Nayoro 096-0072, Japan; jiji.shizuku@gmail.com
[5] Section of Large Animal Clinical Sciences, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan; ohtsuka@obihiro.ac.jp
* Correspondence: k-kosenda@rakuno.ac.jp; Tel.: +81-011-388-4856

Simple Summary: Many animals exhibit immune cell aggregation in the conjunctiva which prevents the invasion of viruses and bacteria. However, the features and functions of conjunctival immune cell aggregates in cows are not fully understood. Microscopic observations confirmed conjunctival immune cell aggregation in calves and adult cows, similar to that in other animals. However, these structures of calves are small and poor in immune cells compared to those of adult cows. These results suggest that cows have a conjunctival local immune system similar to that of other animals, and that conjunctival immune function is weak in calves. These findings may help to prevent infectious diseases in cows.

Abstract: The conjunctiva-associated lymphoid tissue (CALT) has been used as a target site for mucosal vaccinations in several animals. In this study, we compared the morphological features of CALT in the eyelid and third eyelid between Holstein calves and adult cows. In the eyelids, CALTs in the form of diffused lymphoid tissue (DLT) and lymphatic follicles (LF) were observed, where DLTs were dominant and LFs were scarce. The CALTs of cows comprised T-, B-cells, macrophages, and antigen-presenting cells (APCs). In particular, B-cells were dominant except in the eyelids of the calves. The epithelial layer covering the CALT is often discontinuous and lacks goblet cells. Cytokeratin18 is strongly expressed in the epithelial layer covering the CALT, except in the third eyelids of adult cows. IgA-positive cells were diffusely distributed in the lamina propria of the conjunctiva of the eyelids and third eyelids. The eyelid CALT area in calves was lower than that in adult cows. Furthermore, the CALT of calves had a lower cellularity of B-cells and a higher cellularity of macrophages than that of adult cows. These histological characteristics indicate that CALT plays a role in the mucosal immune-inductive and effector sites. Furthermore, lower cellularity of B-cells in the CALT of calves indicates that the function of CALT as a mucosal immune induction site is less developed in calves than in adult cows.

Keywords: cow; conjunctiva; mucosa; lymphoid tissue; histology

Citation: Kosenda, K.; Ichii, O.; Yamashita, Y.; Ohtsuka, H.; Fukuda, S.; Kon, Y. Histological Characteristics of Conjunctiva-Associated Lymphoid Tissue in Young and Adult Holstein Cattle. *Animals* **2023**, *13*, 3481. https://doi.org/10.3390/ani13223481

Academic Editors: Steven Van Winden and Nicola Blackie

Received: 4 September 2023
Revised: 7 November 2023
Accepted: 9 November 2023
Published: 11 November 2023

出典　doi: 10.3390/ani13223481.
https://www.mdpi.com/2076-2615/13/22/3481

最終責任者　Keigo Kosenda (Corresponding Author)

mRNA expression of immune factors by milk somatic cells from healthy Holstein lactating cows

Kenji Murakami, Tomochika Fukuhara, Shunsuke Kure, Takaaki Shimosakai, Ayano Sato, Ryo Murata, Keigo Kosenda, Hiromichi Ohtsuka

Abstract

This study investigated the mRNA of immune factors expressed by milk somatic cells from 72 healthy lactating Holstein cows on 1 farm. Milk samples were collected aseptically from the right front mammary gland before milking. The milk samples that had a negative reaction to the California mastitis test were used to analyze the mRNA of immune factors. Cows were divided into 2 groups based on the detection of bacteria in milk samples: positive group (n = 22 cows), which showed bacteria in cultures, and negative group (n = 50 cows), which did not show bacteria in cultures. There were significant positive correlations among the relative mRNA levels of interleukin (IL)-6, IL-8, arginase 1, chemokine (C-C motif) ligand (CCL) 1, and chemokine (C-X-C motif) ligand (CXCL) 13, as well as among the relative mRNA levels of IL-10, pentraxin 3, CCL5, and CCL14. Significantly high levels of IL-1β, IL-6, IL-8, arginase 1, Batf, CCL1, CXCL14, and toll-like receptor 4 in the positive group were discovered compared to the negative group. These results suggest that the presence of bacteria in lactating healthy dairy cows may affect mRNA levels of inflammatory mediators expressed by somatic cells.

Federation of Agricultural Mutual Relief Association, Fukagawa, Hokkaido 073-0022, Japan (Murakami); School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan (Fukuhara, Kure, Shimosakai, Sato, Murata, Kosenda, Ohtsuka).

Address all correspondence to Dr. Hiromichi Ohtsuka; telephone: +81-11-388-4787; email: ohtsuka@obihiro.ac.jp

Received May 5, 2022. Accepted September 25, 2022.

出典　https://www.ingentaconnect.com/contentone/cvma/cjvr/2023/00000087/00000003/art00012

最終責任者　Hiromichi Ohtsuka (Corresponding Author)

Short Communication Communication brève

Effect of supplementation with *Saccharomyces boulardii* CNCM I-1079 on vaccine response to an inactivated bacterial vaccine in young Japanese Black calves: A field trial

Kazusa Mori, Asato Uchiumi, Kai Yamamoto, Yuki Shimizu, Risa Ueda, Keigo Kosenda, Tomochika Fukuhara, Syunsuke Kure, Hiroyuki Fukazawa, Hiromichi Ohtsuka

Abstract

Saccharomyces boulardii group (SB group) calves were fed 2.0×10^{10} CFU/day of *S. boulardii* in milk replacer after 2 wk of age. All calves received inactivated vaccine for *Histophilus somni, Pasteurella multocida,* and *Mannheimia haemolytica* at 3 wk of age and 3 wk later. After vaccination, the SB group calves showed significantly higher (mean difference: 1.56-fold) antibody titer against *H. somni* than the control group. The number of calves with the antibody titer above the cut-off value for *M. haemolytica* of the SB group was significantly higher than that of the control, and the percentage was twice as high. In addition, the mRNA transcription of *IL4* and *IL10* in peripheral blood mononuclear cells at the booster of the SB group was significantly higher than those of the control. In conclusion, *S. boulardii* may have positively affected immune responses to the inactivated multi-bacterial vaccine in young calves in the field.

School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyo-dai-midorimachi, Ebetsu, Hokkaido 069-8501, Japan (Mori, Kosenda, Fukuhara, Kure, Ohtsuka); Lallemand-Biotech Co., Ltd. JRE Shiba 2-chome Daimon Bldg. 3-3 Shiba 2-chome, Minato-ku, Tokyo 105-0014, Japan (Mori, Fukazawa); Nosan Corporation, 46F Yokohama Landmark Tower, 2-2-1, Minatomirai, Nishi-ku, Yokohama, Kanagawa 220-8146, Japan (Uchiumi, Yamamoto, Shimizu, Ueda); Nosan Farm Co., Ltd. 7-32 Osarushi, Otofukecho, Katogun, Hokkaido 080-0271, Japan (Yamamoto).

Address all correspondence to Dr. Hiromichi Ohtsuka; Tel: +81-(0)11-388-4120; email: ohtsukafarm@yahoo.co.jp

Received August 21, 2022. Accepted January 31, 2023.

出典　https://www.ingentaconnect.com/contentone/cvma/cjvr/2023/00000087/00000003/art00013

最終責任者　Hiromichi Ohtsuka (Corresponding Author)

JAVMA AVMA

Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows

Ayano Sato, DVM, PhD*; Tomochika Sugiura, DVM, PhD; Keigo Kosenda, DVM; Takashi Murakami, DVM, PhD

Division of Farm Animal Clinical Sciences, Rakuno Gakuen University, Ebetsu, Japan

*Corresponding author: Dr. Sato (ayanok@rakuno.ac.jp)

Received February 3, 2023
Accepted April 24, 2023

doi.org/10.2460/javma.23.02.0062

OBJECTIVE
To evaluate analgesic efficacy of 3 different preoperative protocols in cows undergoing right flank laparotomy for displaced abomasum.

ANIMALS
40 cows diagnosed with displaced abomasum.

PROCEDURES
The cows were assigned by block randomization to 1 of 3 preoperative protocols: inverted L-block using 50 mL of 2% lidocaine (ILB; n = 13), ILB plus preoperative flunixin meglumine (2 mg/kg, IV; ILB-F; 13), and dorsolumbar epidural anesthesia using 2% xylazine (0.8 mL) and 2% lidocaine (4 mL; EPI; 14). Venous blood samples were collected for CBC, serum biochemistry, and cortisol preoperatively and at 0 (immediately after), 3, 17, and 48 hours postoperatively.

RESULTS
The mean (95% CI) of the serum cortisol in ILB, ILB-F, and EPI were 108.7 (66.7 to 150.7), 150.7 (116.4 to 185.0), and 139.8 (93.4 to 186.3), respectively. The serum cortisol concentrations decreased over time in all groups (ILB, $P = .001$; ILB-F and EPI, $P < .001$). In the ILB group, the cortisol concentration at 17 and 48 hours postoperatively decreased ($P = .026$ and $P = .009$, respectively), compared with that preoperatively. In the ILB-F and EPI groups, the preoperative cortisol concentration was the highest and then decreased at 0, 3, 17, and 48 hours postoperatively (ILB-F, 0 hours [$P = .001$] and 3, 17, and 48 hours [$P < .001$], EPI, all [$P < .001$]).

CLINICAL RELEVANCE
ILB-F and EPI improved intraoperative and immediate postoperative indicators of pain-related stress when compared to standard ILB. EPI requires less anesthetic, which may be beneficial when in short supply.

出典　doi: 10.2460/javma.23.02.0062.

https://avmajournals.avma.org/view/journals/javma/261/9/javma.23.02.0062.xml?tab_body=pdf

最終責任者　Ayano Sato (Corresponding Author)

sciendo

J Vet Res 67, 315-321, 2023
DOI: 10.2478/jvetres-2023-0037

Antibody response to 1.0 and 0.5 mL doses of an inactivated bacterial vaccine against bovine respiratory disease in young Holstein calves: a field trial

Kazusa Mori[1], Toshihide Kato[1], Keigo Kosenda[1],
Osamu Yokota[2], Hiromichi Ohtsuka[1, 3] ✉

[1]Animal Medical Center, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
[2]F. SIDE Veterinary Service, Sapporo, Hokkaido 004-0072, Japan
[3]Schools of Agriculture and Animal Science,
Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
ohtsuka@obihiro.ac.jp

Received: January 31, 2023 *Accepted: June 19, 2023*

Abstract

Introduction: Early vaccination of cattle with an inactivated commercial bacterial vaccine against bovine respiratory disease has been reported to increase antibody production and can alleviate the disease. However, its dosage has been little investigated in young Holstein calves. This study addresses the need to establish guide values for vaccine dosage in these animals. **Material and Methods:** Healthy calves received an inactivated vaccine for *Histophilus somni*, *Pasteurella multocida* and *Mannheimia haemolytica* intramuscularly at the ages of 1 and 4 weeks. Administered vaccine doses were 1.0 mL for the primary and booster vaccinations (1.0 + 1.0 group), 0.5 mL for the primary and 1.0 mL for the booster vaccination (0.5 + 1.0 group), or 0.5 mL for both vaccinations (0.5 + 0.5 group). **Results:** Differences in the vaccine responses between the 1.0 + 1.0 group and 0.5 + 1.0 group were minor. However, the number of calves with a positive vaccine response to *H. somni* in the 0.5 + 0.5 group was less than half of that in the 1.0 + 1.0 and 0.5 + 1.0 groups. In logistic regression analysis, although the booster vaccination dose was positively correlated with seropositivity for *H. somni*, the primary vaccination dose was not correlated with vaccine response. The number of calves with positive vaccine responses to *M. haemolytica* was low even after booster vaccination regardless of the dose. **Conclusion:** The dose of 0.5 mL can be used for primary vaccinations in newborn Holstein calves, but 1.0 mL may be required for booster vaccinations.

Keywords: bovine respiratory disease, early vaccination, vaccine dose, young calves.

出典 doi: 10.2478/jvetres-2023-0037.
https://sciendo.com/article/10.2478/jvetres-2023-0037

最終責任者 Hiromichi Ohtsuka (Corresponding Author)

生産動物外科学（Farm Animal Surgery）

Takashi Murakami

Assistant Professor

助教　村上　高志

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

Ⅱ．その他＜Others＞

1）Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows

Sato A, Sugiura T, Kosenda K, **Murakami T.**

J Am Vet Med Assoc. 261:1374–1379. 2023.

doi: 10.2460/JAVMA.23.02.0062.

JAVMA

AVMA

Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows

Ayano Sato, DVM, PhD*; Tomochika Sugiura, DVM, PhD; Keigo Kosenda, DVM; Takashi Murakami, DVM, PhD

Division of Farm Animal Clinical Sciences, Rakuno Gakuen University, Ebetsu, Japan

*Corresponding author: Dr. Sato (ayanok@rakuno.ac.jp)

Received February 3, 2023
Accepted April 24, 2023

doi.org/10.2460/javma.23.02.0062

OBJECTIVE
To evaluate analgesic efficacy of 3 different preoperative protocols in cows undergoing right flank laparotomy for displaced abomasum.

ANIMALS
40 cows diagnosed with displaced abomasum.

PROCEDURES
The cows were assigned by block randomization to 1 of 3 preoperative protocols: inverted L-block using 50 mL of 2% lidocaine (ILB; n = 13), ILB plus preoperative flunixin meglumine (2 mg/kg, IV; ILB-F; 13), and dorsolumbar epidural anesthesia using 2% xylazine (0.8 mL) and 2% lidocaine (4 mL; EPI; 14). Venous blood samples were collected for CBC, serum biochemistry, and cortisol preoperatively and at 0 (immediately after), 3, 17, and 48 hours postoperatively.

RESULTS
The mean (95% CI) of the serum cortisol in ILB, ILB-F, and EPI were 108.7 (66.7 to 150.7), 150.7 (116.4 to 185.0), and 139.8 (93.4 to 186.3), respectively. The serum cortisol concentrations decreased over time in all groups (ILB, $P = .001$; ILB-F and EPI, $P < .001$). In the ILB group, the cortisol concentration at 17 and 48 hours postoperatively decreased ($P = .026$ and $P = .009$, respectively), compared with that preoperatively. In the ILB-F and EPI groups, the preoperative cortisol concentration was the highest and then decreased at 0, 3, 17, and 48 hours postoperatively (ILB-F, 0 hours [$P = .001$] and 3, 17, and 48 hours [$P < .001$]; EPI, all [$P < .001$]).

CLINICAL RELEVANCE
ILB-F and EPI improved intraoperative and immediate postoperative indicators of pain-related stress when compared to standard ILB. EPI requires less anesthetic, which may be beneficial when in short supply.

Several techniques for anesthesia of the paralumbar fossa, including proximal paravertebral nerve block, distal paravertebral nerve block, inverted L-block (ILB), and infusion of the incision or line block, can be achieved for laparotomy in adult cows.[1] These anesthetic techniques are commonly used in laparotomy, such as omentopexy, abomasopexy, rumenotomy, and cesarean section. To minimize surgical pain, sedatives, tranquilizers, narcotics, anesthetics, and the strategic use of NSAIDs are recommended.[2]

In the castration of calves, preoperative administration of NSAIDs and local anesthesia significantly decreases the peak serum cortisol concentration after the procedure.[3] In the dehorning of calves, the use of local anesthetics, NSAIDs, and sedation has mitigated acute pain following dehorning.[4,5] Cortisol concentration is an important index in pain management,[3,6] but few studies have measured it for evaluating presurgical treatment, including anesthesia and preoperative analgesia, for laparotomy in adult cows.

This study aimed to evaluate the analgesic efficacy of ILB with preoperative flunixin meglumine and dorsolumbar epidural anesthesia compared to standard ILB in cows undergoing omentopexy for displaced abomasum (DA). Blood samples were collected from cows to evaluate operative stress by measuring the serum cortisol concentrations pre- and postoperatively in the 3 groups.

Materials and Methods

The study protocol was approved by the Animal Research Committee of Rakuno Gakuen University (approval No. VH18C3). Cows diagnosed with left or right DA by farm veterinarians were transported via livestock cars from the farms to the Rakuno Gakuen University Animal Medical Center (AMC). The distance from each farm to the AMC was < 35 km, and the travel time was 5 to 40 minutes. A total of 40 female Holstein dairy cows

出典 doi: 10.2460/javma.23.02.0062.

https://www.ncbi.nlm.nih.gov/pubmed/37217178

最終責任者 Ayano Sato (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＞

II. その他　＜Others＞

1）Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture.
Isaka M, Konno W, Kokubo D, Udagawa H, Hizuka S, **Sakai T**, Yamamoto S, Torisu S, Ueno H.
Res Vet Sci. 158:41–43. 2023. doi: 10.1016/j.rvsc.2023.03.006.

2）Use of a gum elastic bougie in a cat with severe upper airway stenosis.
Kato K, Itami T, Torisu S, **Sakai T**, Yamashita K.
Open Vet J. 13:114–118. 2023. doi: 10.5455/OVJ.2023.v13.i1.12.

Research in Veterinary Science 158 (2023) 41–43

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture

Mitsuhiro Isaka *, Wataru Konno, Daiki Kokubo, Hiromu Udagawa, Sho Hizuka, Toshikazu Sakai, Shushi Yamamoto, Shidow Torisu, Hiroshi Ueno

Laboratory of Companion Animal Surgery, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Cranial cruciate ligament rupture
Dogs
Osteocrin
Osteotomy

ABSTRACT

The cranial cruciate ligament (CCL) rupture is a common orthopedic disease in dogs that is usually managed with tibial plateau leveling osteotomy (TPLO) or extracapsular lateral suture (ECLS). Osteotomy is generally associated with some complications, including nonunion. The periosteum plays an important role in bone growth and remodeling. Osteocrin (OSTN), which was recently identified and is involved in bone formation and differentiation, is produced in the periosteum and osteoblasts. The aimed to investigate whether the concentrations of serum OSTN change before and after stifle surgery in dogs and compare the OSTN concentrations in the two surgical techniques (TPLO: $n = 20$ vs. ECLS: $n = 36$). The postoperative serum OSTN concentration in the TPLO group was significantly lower than the preoperative value ($p < 0.05$), while serum OSTN concentrations differed statistically between the preoperative and suture-removal periods. In contrast, no significant differences were observed in the ECLS group. In conclusion, osteotomy affects serum OSTN concentrations during the perioperative period in dogs, which may be related to periosteal injury.

The cranial cruciate ligament (CCL) rupture is a common orthopedic condition in dogs (Knight and Danielski, 2018). The surgical procedures for CCL rupture are the tibial plateau leveling osteotomy (TPLO), which is tibial osteotomy with an orthopedic plate, and an expracapsular lateral suture (ECLS), which makes the hole of the tibia passing through the suture without osteotomy techniques (Knight and Danielski, 2018; Krotscheck et al., 2016). Generally the use of osteotomy in small- animal orthopedics may be associated with some complications, including nonunion, implant failure, infection, and revision surgery (Danielski et al., 2022).

The periosteum is a dense and abundantly vascularized connective tissue membrane with fibroblasts, including multipotent mesenchymal stem cells and osteogenic progenitor cells (Allen et al., 2004; Chang and Knothe Tate, 2012). It also covers the surface of the majority of bone, facilitating the attachment of muscles, ligaments, and tendons, and delivering blood, nutrition, and regenerative cells to participate in a healing process (Roberts et al., 2015; Seeman, 2007). A previous study reported that the absence of the periosteum induces a marked decrease in new bone formation and up to 10-fold reduced vascularization (Colnot, 2009; Zhang et al., 2005). Collectively, the periosteum may be an important factor related to the complications of osteotomy.

Osteocrin (OSTN) is a recently discovered natriuretic peptide secreted from periosteum, similar to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (Nishizawa et al., 2004). It is produced in the periosteum and osteoblasts (Thomas et al., 2003) and is thought to be involved in bone formation and differentiation (Thomas et al., 2003; Watanabe-Takano et al., 2021). However, no previous reports have described the effects of osteotomy on serum OSTN concentrations during the perioperative period in veterinary clinical settings. Thus, the aim of this study was to investigate whether the serum OSTN concentrations change before and after operative stifle surgery in dogs and compare the findings between the two surgical techniques.

Fifty-six dogs, including 20 and 36 dogs in the TPLO and ECLS groups, respectively, were included in this study. The body weight and age of the dogs in the TPLO group were 25.77 ± 12.27 kg and 96.65 ± 27.28 months, respectively; the group included two male and nine castrated dogs as well as four female and six spayed dogs. The breeds were as follows: Great Dane, $n = 1$; Labrador Retriever, $n = 1$; Golden Retriever, $n = 3$; Siberian Husky, $n = 3$; Shiba Inu, $n = 2$; Welsh Corgi, $n = 2$; Mongrel, $n = 3$; Bernese Mountain Dog, $n = 2$; Border Collie, $n = 1$; and White Shepherd, $n = 2$. Alternatively, the body weight and age of the dogs in the ECLS group were 9.9 ± 7.04 kg and 95.78 ± 36.11

* Corresponding author.
E-mail address: m-isaka@rakuno.ac.jp (M. Isaka).

https://doi.org/10.1016/j.rvsc.2023.03.006
Received 6 October 2022; Received in revised form 15 February 2023; Accepted 5 March 2023
Available online 8 March 2023

出典　doi: 10.1016/j.rvsc.2023.03.006.
https://www.sciencedirect.com/science/article/pii/S0034528823000735?via%3Dihub
最終責任者　Mitsuhiro Isaka (Corresponding Author)

Open Veterinary Journal, (2023), Vol. 13(1): 114–118
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Case Report
DOI: *10.5455/OVJ.2023.v13.i1.12*

Submitted: 11/07/2022 Accepted: 05/12/2022 Published: 23/01/2023

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*

出典 doi: 10.5455/OVJ.2023.v13.i1.12.

https://www.openveterinaryjournal.com/?mno=56656

最終責任者 Takaharu Itami (Corresponding Author)

動物生殖学（Theriogenology）

Tomochika Sugiura

Assistant Professor

助教　杉浦　智親

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

II. その他 ＜Others＞

1）Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows

Sato A, Sugiura T, Kosenda K, **Murakami T.**

J Am Vet Med Assoc. 261:1374–1379. 2023.

doi: 10.2460/JAVMA.23.02.0062.

JAVMA

Preoperative protocol for right flank laparotomy affects postoperative serum cortisol concentrations in dairy cows

Ayano Sato, DVM, PhD*; Tomochika Sugiura, DVM, PhD; Keigo Kosenda, DVM; Takashi Murakami, DVM, PhD

Division of Farm Animal Clinical Sciences, Rakuno Gakuen University, Ebetsu, Japan

*Corresponding author: Dr. Sato (ayanok@rakuno.ac.jp)

Received February 3, 2023
Accepted April 24, 2023

doi.org/10.2460/javma.23.02.0062

OBJECTIVE
To evaluate analgesic efficacy of 3 different preoperative protocols in cows undergoing right flank laparotomy for displaced abomasum.

ANIMALS
40 cows diagnosed with displaced abomasum.

PROCEDURES
The cows were assigned by block randomization to 1 of 3 preoperative protocols: inverted L-block using 50 mL of 2% lidocaine (ILB; n = 13), ILB plus preoperative flunixin meglumine (2 mg/kg, IV; ILB-F; 13), and dorsolumbar epidural anesthesia using 2% xylazine (0.8 mL) and 2% lidocaine (4 mL; EPI; 14). Venous blood samples were collected for CBC, serum biochemistry, and cortisol preoperatively and at 0 (immediately after), 3, 17, and 48 hours postoperatively.

RESULTS
The mean (95% CI) of the serum cortisol in ILB, ILB-F, and EPI were 108.7 (66.7 to 150.7), 150.7 (116.4 to 185.0), and 139.8 (93.4 to 186.3), respectively. The serum cortisol concentrations decreased over time in all groups (ILB, $P = .001$; ILB-F and EPI, $P < .001$). In the ILB group, the cortisol concentration at 17 and 48 hours postoperatively decreased ($P = .026$ and $P = .009$, respectively), compared with that preoperatively. In the ILB-F and EPI groups, the preoperative cortisol concentration was the highest and then decreased at 0, 3, 17, and 48 hours postoperatively (ILB-F, 0 hours [$P = .001$] and 3, 17, and 48 hours [$P < .001$]; EPI, all [$P < .001$]).

CLINICAL RELEVANCE
ILB-F and EPI improved intraoperative and immediate postoperative indicators of pain-related stress when compared to standard ILB. EPI requires less anesthetic, which may be beneficial when in short supply.

Several techniques for anesthesia of the paralumbar fossa, including proximal paravertebral nerve block, distal paravertebral nerve block, inverted L-block (ILB), and infusion of the incision or line block, can be achieved for laparotomy in adult cows.[1] These anesthetic techniques are commonly used in laparotomy, such as omentopexy, abomasopexy, rumenotomy, and cesarean section. To minimize surgical pain, sedatives, tranquilizers, narcotics, anesthetics, and the strategic use of NSAIDs are recommended.[2]

In the castration of calves, preoperative administration of NSAIDs and local anesthesia significantly decreases the peak serum cortisol concentration after the procedure.[3] In the dehorning of calves, the use of local anesthetics, NSAIDs, and sedation has mitigated acute pain following dehorning.[4,5] Cortisol concentration is an important index in pain management,[3,6] but few studies have measured it for evaluating presurgical treatment, including anesthesia and preoperative analgesia, for laparotomy in adult cows.

This study aimed to evaluate the analgesic efficacy of ILB with preoperative flunixin meglumine and dorsolumbar epidural anesthesia compared to standard ILB in cows undergoing omentopexy for displaced abomasum (DA). Blood samples were collected from cows to evaluate operative stress by measuring the serum cortisol concentrations pre- and postoperatively in the 3 groups.

Materials and Methods

The study protocol was approved by the Animal Research Committee of Rakuno Gakuen University (approval No. VH18C3). Cows diagnosed with left or right DA by farm veterinarians were transported via livestock cars from the farms to the Rakuno Gakuen University Animal Medical Center (AMC). The distance from each farm to the AMC was < 35 km, and the travel time was 5 to 40 minutes. A total of 40 female Holstein dairy cows

出典 doi: 10.2460/javma.23.02.0062.

https://www.ncbi.nlm.nih.gov/pubmed/37217178

最終責任者 Ayano Sato (Corresponding Author)

伴侶動物外科学（Companion Animal Surgery）

Shushi Yamamoto

Assistant Professor

助教　山本　集士

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

Ⅱ．その他＜Others＞

1）Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture.
Isaka M, Konno W, Kokubo D, Udagawa H, Hizuka S, Sakai T, **Yamamoto S**, Torisu S, Ueno H.
Res Vet Sci. 158:41–43. 2023. doi:10.1016/j.rvsc.2023.03.006

Research in Veterinary Science 158 (2023) 41–43

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Comparison of perioperative serum osteocrin concentrations between surgical techniques in dogs with cranial cruciate ligament rupture

Mitsuhiro Isaka *, Wataru Konno, Daiki Kokubo, Hiromu Udagawa, Sho Hizuka, Toshikazu Sakai, Shushi Yamamoto, Shidow Torisu, Hiroshi Ueno

Laboratory of Companion Animal Surgery, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

ARTICLE INFO

Keywords:
Cranial cruciate ligament rupture
Dogs
Osteocrin
Osteotomy

ABSTRACT

The cranial cruciate ligament (CCL) rupture is a common orthopedic disease in dogs that is usually managed with tibial plateau leveling osteotomy (TPLO) or extracapsular lateral suture (ECLS). Osteotomy is generally associated with some complications, including nonunion. The periosteum plays an important role in bone growth and remodeling. Osteocrin (OSTN), which was recently identified and is involved in bone formation and differentiation, is produced in the periosteum and osteoblasts. The aimed to investigate whether the concentrations of serum OSTN change before and after stifle surgery in dogs and compare the OSTN concentrations in the two surgical techniques (TPLO: $n = 20$ vs. ECLS: $n = 36$). The postoperative serum OSTN concentration in the TPLO group was significantly lower than the preoperative value ($p < 0.05$), while serum OSTN concentrations differed statistically between the preoperative and suture-removal periods. In contrast, no significant differences were observed in the ECLS group. In conclusion, osteotomy affects serum OSTN concentrations during the perioperative period in dogs, which may be related to periosteal injury.

* Corresponding author.
E-mail address: m-isaka@rakuno.ac.jp (M. Isaka).

https://doi.org/10.1016/j.rvsc.2023.03.006
Received 6 October 2022; Received in revised form 15 February 2023; Accepted 5 March 2023
Available online 8 March 2023

出典 doi: 10.1016/j.rvsc.2023.03.006
https://www.sciencedirect.com/science/article/pii/S0034528823000735?via%3Dihub
最終責任者 Mitsuhiro Isaka (Corresponding Author)

獣医細菌学（Veterinary bacteriology）

Tomoyuki Yamaguchi

Assistant Professor

助教　山口　智之

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

Ⅱ．その他＜Others＞

1）Surveillance, Isolation, and Genetic Characterization of Bat Herpesviruses in Zambia.
Harima H, Qiu Y, Yamagishi J, Kajihara M, Changula K, Okuya K, Isono M, **Yamaguchi T**, Ogawa H, Nao N, Sasaki M, Simulundu E, Mweene AS, Sawa H, Ishihara K, Hang'ombe BM, Takada A.
Viruses 15:1369. 2023. doi: 10.3390/v15061369.

viruses

MDPI

Article

Surveillance, Isolation, and Genetic Characterization of Bat Herpesviruses in Zambia

Hayato Harima [1], Yongjin Qiu [2,3], Junya Yamagishi [4,5], Masahiro Kajihara [6], Katendi Changula [7], Kosuke Okuya [8], Mao Isono [9], Tomoyuki Yamaguchi [10], Hirohito Ogawa [11], Naganori Nao [6,12], Michihito Sasaki [13], Edgar Simulundu [14,15], Aaron S. Mweene [14,16,†], Hirofumi Sawa [12,14,16,17], Kanako Ishihara [1], Bernard M. Hang'ombe [7,16] and Ayato Takada [5,9,12,14,16,*]

1 Laboratory of Veterinary Public Health, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan; harima@go.tuat.ac.jp (H.H.); kanako-i@cc.tuat.ac.jp (K.I.)
2 Management Department of Biosafety, Laboratory Animal and Pathogen Bank, National Institute of Infectious Diseases, Tokyo 162-8640, Japan; qiu-y@niid.go.jp
3 Department of Virology-I, National Institute of Infectious Diseases, Tokyo 162-8640, Japan
4 Division of Collaboration and Education, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan; junya@czc.hokudai.ac.jp
5 International Collaboration Unit, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan
6 Division of International Research Promotion, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan; kajihara@czc.hokudai.ac.jp (M.K.); n-nao@czc.hokudai.ac.jp (N.N.)
7 Department of Para-Clinical Studies, School of Veterinary Medicine, The University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia; katendi.changula@sacids.org (K.C.); mudenda68@yahoo.com (B.M.H.)
8 Department of Pathogenetic and Preventive Veterinary Science, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima 890-0065, Japan; kokuya@vet.kagoshima-u.ac.jp
9 Division of Global Epidemiology, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan; maoisono@gmail.com
10 Department of Oral Microbiology, Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama 700-8558, Japan; tomoyuki.yamaguchi@okayama-u.ac.jp
11 Department of Virology, Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama 700-8558, Japan; hogawa@okayama-u.ac.jp
12 One Health Research Center, Hokkaido University, Sapporo 060-0818, Japan; h-sawa@ivred.hokudai.ac.jp
13 Division of Molecular Pathobiology, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan; m-sasaki@czc.hokudai.ac.jp
14 Department of Disease Control, School of Veterinary Medicine, The University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia; esikabala@yahoo.com
15 Macha Research Trust, Choma 20100, Zambia
16 Africa Center of Excellence for Infectious Diseases of Humans and Animals, The University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia
17 Institute for Vaccine Research and Development, Hokkaido University, Sapporo 001-0021, Japan
* Correspondence: atakada@czc.hokudai.ac.jp; Tel.: +81-11-706-9502
† Deceased 27 April 2019.

Citation: Harima, H.; Qiu, Y.; Yamagishi, J.; Kajihara, M.; Changula, K.; Okuya, K.; Isono, M.; Yamaguchi, T.; Ogawa, H.; Nao, N.; et al. Surveillance, Isolation, and Genetic Characterization of Bat Herpesviruses in Zambia. *Viruses* **2023**, *15*, 1369. https://doi.org/10.3390/v15061369

Academic Editors: Patrick C.Y. Woo and Kenneth Li

Received: 23 May 2023
Revised: 12 June 2023
Accepted: 12 June 2023
Published: 13 June 2023

Abstract: Bats are of significant interest as reservoirs for various zoonotic viruses with high diversity. During the past two decades, many herpesviruses have been identified in various bats worldwide by genetic approaches, whereas there have been few reports on the isolation of infectious herpesviruses. Herein, we report the prevalence of herpesvirus infection of bats captured in Zambia and genetic characterization of novel gammaherpesviruses isolated from striped leaf-nosed bats (*Macronycteris vittatus*). By our PCR screening, herpesvirus DNA polymerase (DPOL) genes were detected in 29.2% (7/24) of Egyptian fruit bats (*Rousettus aegyptiacus*), 78.1% (82/105) of *Macronycteris vittatus*, and one Sundevall's roundleaf bat (*Hipposideros caffer*) in Zambia. Phylogenetic analyses of the detected partial DPOL genes revealed that the Zambian bat herpesviruses were divided into seven betaherpesvirus groups and five gammaherpesvirus groups. Two infectious strains of a novel gammaherpesvirus, tentatively named Macronycteris gammaherpesvirus 1 (MaGHV1), were successfully isolated from *Macronycteris vittatus* bats, and their complete genomes were sequenced. The genome of MaGHV1 encoded 79 open reading frames, and phylogenic analyses of the DNA polymerase and glycoprotein B demonstrated that MaGHV1 formed an independent lineage sharing a common origin with other bat-derived gammaherpesviruses. Our findings provide new information regarding the genetic diversity of herpesviruses maintained in African bats.

Keywords: herpesvirus; bat; surveillance; complete genome; Zambia

出典 doi: 10.3390/v15061369

https://www.mdpi.com/1999-4915/15/6/1369

最終責任者 Ayato Takada (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＞

II. その他　＜Others＞

1）Housing Systems Affect Eggshell Lightness and Free Amino Acid Contents of Egg Albumen in Tosa-Jidori Chickens: A Preliminary Research.
Kawamura N, Takaya M, **Hayashi H**, Goto T.
Animals (Basel). 13:1837. 2023. doi: 10.3390/ani13111837.

animals

MDPI

Article

Housing Systems Affect Eggshell Lightness and Free Amino Acid Contents of Egg Albumen in Tosa-Jidori Chickens: A Preliminary Research

Nonoka Kawamura [1], Masahiro Takaya [1,2], Hideaki Hayashi [3] and Tatsuhiko Goto [1,4,*]

[1] Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan
[2] Hokkaido Tokachi Area Regional Food Processing Technology Center, Tokachi Foundation, Obihiro 080-2462, Hokkaido, Japan
[3] Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
[4] Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Hokkaido, Japan
* Correspondence: tats.goto@obihiro.ac.jp; Tel.: +81-155-49-5426

Simple Summary: Although there are several housing systems (e.g., cage and litter) for egg layers, there is still no consensus about housing system effects on egg quality traits. Therefore, the purpose of this study is to determine housing effects on egg quality traits, including free amino acids of albumen and yolk. We observed significant housing effects in body weight gain, eggshell weight, yolk weight, eggshell thickness, eggshell lightness, and several albumen amino acids. Staying 7 weeks in a litter condition seemed to be enough to cause a significantly lighter eggshell color regardless of the egg production stages. These results will be important knowledge in the future layer industry.

Citation: Kawamura, N.; Takaya, M.; Hayashi, H.; Goto, T. Housing Systems Affect Eggshell Lightness and Free Amino Acid Contents of Egg Albumen in Tosa-Jidori Chickens: A Preliminary Research. *Animals* **2023**, *13*, 1837. https://doi.org/10.3390/ani13111837

Academic Editors: Guillermo Tellez-Isaias, Jayant Lohakare and Ahmed Abdel-wareth

Received: 9 April 2023
Revised: 29 May 2023
Accepted: 30 May 2023
Published: 1 June 2023

Abstract: Many countries have gradually shifted to animal welfare-friendly housing systems for egg layers. However, there is still no consensus among researchers on whether the housing system affects egg quality traits. Therefore, this study aimed to determine the effects of housing systems on egg traits and free amino acid contents of albumen and yolk using two types of housing systems, the conventional cage (cage) system and a floor rearing (litter) system. Tosa-jidori (n = 20) hens were divided into two groups. Experiments during the 7 weeks were performed twice by switching the housing systems (first and second stages). One-way analysis of variance was used to evaluate the effects of housing systems on body weight gain, egg traits, albumen and yolk amino acid contents, and fecal corticosterone. We observed significant housing effects in body weight gain, eggshell weight, yolk weight, eggshell thickness, eggshell lightness, and several albumen amino acids (A_Gln, A_His, A_Met, A_Cys, A_Lys, A_Asp, A_Glu, A_Ser, A_Thr, A_Ala, A_Pro, and A_Phe). Notably, a robust effect was seen in eggshell lightness, even after switching housing systems. These results suggest that eggshell lightness and several egg traits, including albumen amino acid contents, can be changed by using the different housing systems.

Keywords: chicken; egg; free amino acid; housing system; stress

1. Introduction

Eggs can be purchased at low prices, although they are called "complete foods" [1]. Eggs are rich in protein, essential amino acids, and vitamins, except for vitamin C [2]. Free amino acid, one of the ingredients of hens' eggs, is known to be related to the food taste and functionality after intake. For example, some free amino acids have been shown to have high antioxidant potential [3]. The production of designer eggs has been studied since 1934 [4]. Designer eggs are defined as eggs designed in advance to incorporate the nutrients

出典　doi: 10.3390/ani13111837.
https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202200924

最終責任者　Tatsuhiko Goto (Corresponding Author)

動物と人の関係学(Animal Human Relations)

Takanori Kooriyama

Professor

教授　郡山　尚紀

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

1）　Selection of Appropriate Dogs to Be Therapy Dogs Using the C-BARQ.
Sakurama M, Ito M, Nakanowataru Y, **Kooriyama T.**
Animals (Basel) 13:834. 2023. doi: 10.3390/ani13050834

II. その他　＜Others＞

animals

MDPI

Article

Selection of Appropriate Dogs to Be Therapy Dogs Using the C-BARQ

Mayu Sakurama †, Miki Ito †, Yumiko Nakanowataru and Takanori Kooriyama *

Department of Veterinary Science, Rakuno Gakuen University, Ebetsu 069-8501, Hokkaido, Japan
* Correspondence: kooriyam@rakuno.ac.jp; Tel.: +81-11-388-4755
† These authors contributed equally to this work and share co-first authorship.

Simple Summary: Animal-assisted interventions (AAI) help people relax and psychologically recover. Dogs are the most frequently used animals for this purpose and are sometimes called "therapy dogs" in Japan. Certified therapy dogs are evaluated prior to training with an aptitude test, but some owners have their dogs take this test without understanding what is required of the test. Therefore, new methods are needed to recruit candidate dogs and allow owners to easily determine whether their dogs have the potential to be therapy dogs. This study examined which items in Canine Behavior Assessment and Research Questionnaire (C-BARQ) can help to identify dogs suitable to become therapy dogs. Factor analysis identified 14 factors. Using these factors, owners can independently evaluate whether their dogs display suitable behavioural traits to become therapy dogs. The present study may help to increase the number of therapy dogs.

Abstract: In recent years, therapy dogs in medical and assisted living facilities have become popular in Japan, and the demand for such dogs has increased. However, some owners have their dogs take this test, which evaluates the dog's talent, without understanding what is required of the test. The system needs to teach owners in an understandable way whether their dog is suitable to become a therapy dog so that the owners can determine if their dog is ready to be tested. Therefore, we suggest that easy at-home testing is likely to encourage dog owners to apply for their dog to take the aptitude test. If more dogs take the test, more therapy dogs can be born. The purpose of this study was to identify the personality traits of therapy dogs that pass the aptitude test by using the Canine Behavior Assessment and Research Questionnaire (C-BARQ). The C-BARQ was administered to dogs that previously passed the aptitude test for therapy training at the Hokkaido Volunteer Dog Association, assessing their behavioural displays. A factor analysis was conducted for each questionnaire item, and a total of 98 items were analyzed. Data were collected from the results of 110 dogs encompassing 30 dog breeds, with the most common breeds including Labrador Retrievers, Golden Retrievers, and Toy Poodles. Factor analysis revealed that 14 extracted factors should be evaluated. Given these personality traits and the fact that breed and age did not influence aptitude, we believe that a variety of dogs have the potential to become therapy dogs.

Keywords: animal-assisted intervention; C-BARQ; dogs; factor analysis

Citation: Sakurama, M.; Ito, M.; Nakanowataru, Y.; Kooriyama, T. Selection of Appropriate Dogs to Be Therapy Dogs Using the C-BARQ. *Animals* **2023**, *13*, 834. https://doi.org/10.3390/ani13050834

Academic Editors: Gretchen Good and Polly Yeung

Received: 23 November 2022
Revised: 16 February 2023
Accepted: 21 February 2023
Published: 24 February 2023

1. Introduction

Dogs have long been familiar to humans and are animals that can provide psychological healing through their interactions with people [1]. In recent years, this healing effect has been scientifically demonstrated in several animal species, and awareness of animal-assisted intervention (AAI) has increased [1]. Interactions with dogs or other animal species are used in medical and assisted living facilities (i.e., hospitals and nursing homes) to improve people's quality of life (QOL) [1]. A variety of animals, from horses to dolphins, have been used in animal therapy, but dogs have become mainstream in recent years [2–5]. Specific effects of animal therapy have been reported, including decreased progression of

出典　doi: 10.3390/ani13050834
https://www.mdpi.com/2076-2615/13/5/834
責任著者　Takanori Kooriyama (Corresponding Author)

動物と人の関係学(Animal Human Relations)

Jun Noda

Professor

教授　能田　淳

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

1）　Dust and Microorganisms: Their Interactions and Health Effects.
Noda J, Morimoto K, Mitarai S, Maki T.
In: Dust and Health. Emerging Contaminants and Associated Treatment Technologies. Al-Dousari A, Hashmi M Z eds, Springer, Cham, 2023.
doi:org/10.1007/978-3-031-21209-3_8

II. その他　＜Others＞

1）Potential airborne human pathogens: A relevant inhabitant in built environments but not considered in indoor air quality standards.
Carrazana E, Ruiz-Gil T, Fujiyoshi S, Tanaka D, **Noda J**, Maruyama F, Jorquera M.
Sci Total Environ. 901:165879. 2023. doi:org/10.1016/j.scitotenv.2023.165879

2）Mass-Extinction Conversion Factor (MECF) over the Gobi Desert by a Tethered-balloon-based OPC and a Ceilometer.
Kai K, Kawai K, Ohara K, Minamoto Y, Jin Y, Maki T, **Noda J,** Shiina T, Davaanyam E.
SOLA 19:269–273. 2023. doi.org/10.2151/sola.2023-035.

3）Growth Suppression of a Robust Bacterium *Methylobacterium extorquens* by Porous Materials with Oxygen Functional Groups.
Mori T, Ogawa Y, Endo I, Matsushima K, **Noda J.**
Life (Basel) 13:2185. 2023. doi: 10.3390/life13112185

4）Airborne bacterial communities associated with particulate matter in Temuco (Chile), one of the most air−polluted cities in South America.
Ruiz-Gil T, Fujiyoshi S, Tanaka D, **Noda J**, Maruyama F, Acuña JJ, Jorquera M.
Aerobiologia 39:1–20. 2023. doi: 10.1007/s10453-023-09803-9

Chapter 8
Dust and Microorganisms: Their Interactions and Health Effects

Jun Noda, Kozo Morimoto, Satoshi Mitarai, and Teruya Maki

Abstract Desert regions emit a large amount of mineral particles to cause dust events frequently, and emitted dust from those events may circulate around the world. These desert-dust events transport inorganic and organic particulate matter for long distances and may cause health impacts. Additionally, the depositions of the particulates to marine environments supply essential nutrients to induce the growth of marine phytoplankton and bacteria. Recently, the dust events are reported to contain higher amounts of biological particles than expected. Some investigations focus on the amount of biological particles and the dust events or high concentration of dust environment, which may have strong associations with health-related effects. Also, the various types of dust may act as airborne fomites attributing to the dispersion of microorganisms in more stable conditions with prolonged viability of microorganisms to cause greater health effects such as allergy, asthma, respiratory infection, and more. The field observations collecting bacterial strains from several dust-source regions in the desert are described. Furthermore, the laboratory attempts to use a simulation chamber system to understand the attributing factor of various

J. Noda (✉)
Environment Health Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
e-mail: jnoda@rakuno.ac.jp

K. Morimoto
Division of Clinical Research, Fukujuji Hospital, Japan Anti-Tuberculosis Association, Tokyo, Japan

Department of Mycobacterium Reference and Research, Research Institute of Tuberculosis Japan Anti-Tuberculosis Association, Tokyo, Japan
e-mail: morimotok@fukujuji.org

S. Mitarai
Department of Mycobacterium Reference and Research, Research Institute of Tuberculosis Japan Anti-Tuberculosis Association, Tokyo, Japan
e-mail: mitarai@jata.or.jp

T. Maki
Department of Life Science, Faculty of Science and Engineering, Kindai University, Osaka, Japan
e-mail: makiteru@life.kindai.ac.jp

A. Al-Dousari, M. Z. Hashmi (eds.), *Dust and Health*, Emerging Contaminants and Associated Treatment Technologies,
https://doi.org/10.1007/978-3-031-21209-3_8

出典　doi: 10.1007/978-3-031-21209-3_8
https://www.researchgate.net/publication/346403506_Dust_and_Health_Challenges_and_Solutions
最終責任者 Jun Noda (Corresponding Author)

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Potential airborne human pathogens: A relevant inhabitant in built environments but not considered in indoor air quality standards

Elizabeth Carrazana [a,b], Tay Ruiz-Gil [b,c], So Fujiyoshi [d,e], Daisuke Tanaka [f], Jun Noda [g], Fumito Maruyama [d,e], Milko A. Jorquera [b,d,h,*]

[a] Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada, Universidad de La Frontera, Temuco, Chile
[b] Laboratorio de Ecología Microbiana Aplicada, Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile
[c] Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile
[d] Center for Holobiome and Built Environment (CHOBE), Hiroshima University, Japan
[e] Microbial Genomics and Ecology, PHIS, The IDEC institute, Hiroshima University, Hiroshima, Japan
[f] School of Science Academic Assembly, University of Toyama, Toyama, Japan
[g] Graduate School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan
[h] Network for Extreme Environment Research (NEXER), Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile

ARTICLE INFO

Editor: Hai Guo

Keywords:
Potential airborne human pathogens
Air microbiome
Bioaerosol
Built environments
Indoor air quality

ABSTRACT

Potential airborne human pathogens (PAHPs) may be a relevant component of the air microbiome in built environments. Despite that PAHPs can cause infections, particularly in immunosuppressed patients at medical centers, they are scarcely considered in standards of indoor air quality (IAQ) worldwide. Here, we reviewed the current information on microbial aerosols (bacteria, fungal and viruses) and PAHPs in different types of built environments (*e.g.*, medical center, industrial and non-industrial), including the main factors involved in their dispersion, the methodologies used in their study and their associated biological risks. Our analysis identified the human occupancy and ventilation systems as the primary sources of dispersal of microbial aerosols indoors. We also observed temperature and relative humidity as relevant physicochemical factors regulating the dispersion and viability of some PAHPs. Our analysis revealed that some PAHPs can survive and coexist in different environments while other PAHPs are limited or specific for an environment. In relation to the methodologies (conventional or molecular) the nature of PAHPs and sampling type are pivotal. In this context, indoors air-borne viruses are the less studies because their small size, environmental lability, and absence of efficient sampling techniques and universal molecular markers for their study. Finally, it is noteworthy that PAHPs are not commonly considered and included in IAQ standards worldwide, and when they are included, the total abundance is the single parameter considered and biological risks is excluded. Therefore, we propose a revision, design and establishment of public health policies, regulations and IAQ standards, considering the interactions of diverse factors, such as nature of PAHPs, human occupancy and type of built environments where they develop.

1. Introduction

Air pollution greatly impacts human health, and it is one of the main threats to the environmental health of our planet (+IQAir, 2021). >99 % of the world population breathes polluted air that exceeds the limits recommended by the World Health Organization (WHO; https://www.who.int/), and it is responsible for approximately 3.4 million premature deaths annually in built environments (WHO, 2021). According to the United States Environmental Protection Agency (US-EPA; https://www.epa.gov/), indoor pollution is typically 2 to 5 times higher than outdoor pollution levels, and measurements can exceed 100 times the levels of pollutants compared to the outside in some cases (United States Environmental Protection Agency, 2021a,b). However, biological pollutants, such as microbial toxins and human pathogens, have not been sufficiently considered and incorporated into indoor air quality (IAQ) worldwide.

The microbiome in the air is a key element in IAQ, and it represents approximately 34 % of the contamination present in the air (Gizaw et al.,

* Corresponding author at: Laboratorio de Ecología Microbiana Aplicada, Departmento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Ave. Francisco Salazar 01145, Temuco, Chile.
E-mail address: milko.jorquera@ufrontera.cl (M.A. Jorquera).

https://doi.org/10.1016/j.scitotenv.2023.165879
Received 29 April 2023; Received in revised form 13 July 2023; Accepted 27 July 2023
Available online 29 July 2023

出典　doi: 10.1016/j.envint.2022.107471
https://www.sciencedirect.com/science/article/pii/S0160412022003981?via%3Dihub
最終責任者　Milko A. Jorquera (Corresponding Authors)

Mass-Extinction Conversion Factor (MECF) over the Gobi Desert by a Tethered-balloon-based OPC and a Ceilometer

Kenji Kai[1], Kei Kawai[1], Kazuma Ohara[1], Yuki Minamoto[1], Yoshitaka Jin[2], Teruya Maki[3], Jun Noda[4], Tatsuo Shiina[5], and Enkhbaatar Davaanyam[6]

[1]*Graduate School of Environmental Studies, Nagoya University, Nagoya, Japan*
[2]*National Institute for Environmental Studies, Tsukuba, Japan*
[3]*Graduate School of Science and Engineering, Kindai University, Osaka, Japan*
[4]*Graduate School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan*
[5]*Graduate School of Engineering, Chiba University, Chiba, Japan*
[6]*Information and Research Institute of Meteorology, Hydrology and Environment, Ulaanbaatar, Mongolia*

(Manuscript received 9 June 2023, accepted 15 September 2023)

Abstract The mass concentration of Asian dust in the atmosphere is an essential parameter of the atmospheric environment in East Asia. In April 2016, we conducted simultaneous observations using an optical particle counter (OPC) installed on a tethered balloon and a ceilometer in the Gobi Desert. We estimated the mass-extinction conversion factor MECF (gm^{-2}) from the relationship between the dust extinction coefficient and dust mass concentration obtained by simultaneous observations. The MECF at Dalanzadgad in the Gobi Desert is 2.16 gm^{-2} at 910 nm and 1.91 gm^{-2} at 532 nm. A previous study on Asian dust showed that the values of the MECF were 1.78 gm^{-2} in Beijing, 1.40 gm^{-2} in Seoul, 1.18 gm^{-2} in Tsukuba (Japan), and 1.04 gm^{-2} at averaged AD-Net lidar stations in Japan. The MECF values decreased from the Asian dust source to the lee-side areas. This result suggests that the MECF depends on the size distribution of the dust.

Citation: Kai, K., K. Kawai, K. Ohara, Y. Minamoto, Y. Jin, T. Maki, J. Noda, T. Shiina, and E. Davaanyam, 2023: Mass-extinction conversion factor (MECF) over the Gobi Desert by a tethered-balloon-based OPC and a ceilometer. *SOLA*, **19**, 269–273, doi:10.2151/sola.2023-035.

1. Introduction

Mineral dust significantly impacts the radiation budget of the Earth's atmosphere (Knippertz and Stuut 2014; Kok et al. 2023). Asian dust plumes advance over large areas in Asia (Iwasaka et al. 1983; Kai et al. 1988; Husar et al. 2001; Yumimoto et al. 2009; Minamoto et al. 2018). Asian dust loads are high during dust outbreaks, particularly in the spring. The mass concentration of Asian dust in the atmosphere is an essential parameter of the atmospheric environment in East Asia (Sugimoto et al. 2003, 2011; Shimizu et al. 2011; Ansmann et al. 2012, 2019).

Estimating the dust mass in the atmosphere using lidar is possible if the mass-extinction conversion factor MECF (gm^{-2}) is known. The values of MECF at various places worldwide were studied; Ansmann et al. (2012) reported a MECF value of 1.93 gm^{-2} for the Saharan dust, Gasteiger et al. (2011) reported 1.45 gm^{-2} for volcanic ash, and Sugimoto et al. (2011) reported MECF values of the Asian dust 1.78 gm^{-2} at Beijing, 1.40 gm^{-2} at Seoul, 1.18 gm^{-2} at Tsukuba, and 1.04 gm^{-2} at AD-net sites in Japan. The dust blown up from the source regions is transported to wide lee-side areas by prevailing winds, keeping its own property. Thus, the MECF in the dust source is important; however, these previous studies were restricted to lee-side areas.

The present study aimed to estimate the MECF in a dust source from simultaneous optical particle counter (OPC) and ceilometer observations. Figure 1 is a map of the research area: Mongolia and the Gobi Desert.

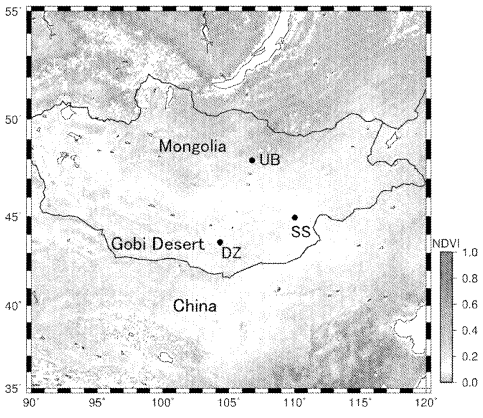


Fig. 1. Map of the research area: Mongolia and Gobi Desert. UB: Ulaanbaatar, DZ: Dalanzadgad, SS: Shainsand. The color scale indicates Terra/MODIS NDVI (April 2016).

Corresponding author: Kenji Kai, Graduate School of Environmental Studies, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan. E-mail: kenjikai789@gmail.com.

出典　doi: 10.2151/sola.2023-035.
https://www.jstage.jst.go.jp/article/sola/19/0/19_2023-035/_article/-char/ja

最終責任者　Kai K (First and Corresponding Author)

life

MDPI

Article

Growth Suppression of a Robust Bacterium *Methylobacterium extorquens* by Porous Materials with Oxygen Functional Groups

Takeshi Mori [1,2,*], Yuta Ogawa [1], Izuki Endo [2,3,4], Keiichiro Matsushima [1] and Jun Noda [2]

[1] Industrial Research Institute, Hokkaido Research Organization, Sapporo 060-0819, Japan
[2] School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; jnoda@rakuno.ac.jp (J.N.)
[3] Department of Applied Chemistry and Bioscience, Chitose Institute of Science and Technology, Chitose 066-8655, Japan
[4] School of Human Science and Environment, University of Hyogo, 1-1-12 Shinzaike-Honcho, Himeji 670-0092, Japan; izok@shse.u-hyogo.ac.jp
* Correspondence: mori-takeshi@hro.or.jp; Tel.: +81-11-726-2362

Abstract: Suppressing the growth of *Methylobacterium* species without the use of toxic chemicals has been a challenging task owing to their robustness against previous antimicrobial techniques. In this work, we prepared porous materials with various numbers and types of oxygen functional groups and investigated their ability to suppress the growth of *Methylobacterium extorquens*. It turned out that the number and type of oxygen functional groups in the porous materials greatly affected the growth of the bacterium. Three porous materials (resorcinol–formaldehyde gel (RF), hydrothermally treated RF (RFH), and Wakkanai siliceous shale (WS)) were tested, and RF exhibited the best performance in suppressing the growth of the bacterium. This performance is possibly due to abundant phenolic groups in the porous material.

Keywords: methylobacterium; porous materials; anti-bacterial materials; oxygen functional groups

Citation: Mori, T.; Ogawa, Y.; Endo, I.; Matsushima, K.; Noda, J. Growth Suppression of a Robust Bacterium *Methylobacterium extorquens* by Porous Materials with Oxygen Functional Groups. *Life* **2023**, *13*, 2185. https://doi.org/10.3390/life13112185

Academic Editor: Tao Huang

Received: 29 September 2023
Revised: 2 November 2023
Accepted: 6 November 2023
Published: 9 November 2023

1. Introduction

Methylobacterium species are Gram-negative bacteria characterized by their distinct pink-pigmented colony and unique ability to grow on C1 compounds such as methanol and methylamine [1]. This feature enables the species to inhabit plant leaf surfaces; the species can produce plant growth hormones (e.g., cytokinin and indole-3-acetic acid) from a trace amount of gaseous C1 compounds such as methanol released from plants [2,3]. Owing to this feature, the *Methylobacterium* species are promising candidates for substitutes of agrochemicals [4], whose residues often cause severe contamination in arable lands. The *Methylobacterium* species do not show high toxicity; however, the species are known to cause some opportunistic infections, which are illnesses that occur in people with weakened immune systems [5,6]. A clinical report showed that the *Methylobacterium* species were detected in biopsy specimens from patients (e.g., ulcers, phlegm, and pleural effusion) [6]. The entry of the *Methylobacterium* species into such people can lead to the occurrence of severe infections such as pneumonia, catheter-related blood stream infections, and bacteremia [6]. *Methylobacterium mesophilicum*, *Methylobacterium zatmanii*, and *Methylobacterium extorquens* are the three most commonly reported species isolated from biopsy specimens [7]. The species can be ubiquitously found in nature (e.g., soil, plants, and watersheds) [8]; therefore, techniques to suppress the growth of the *Methylobacterium* species are desired to prevent the opportunistic infections caused by the species.

While the *Methylobacterium* species possess such potential toxicity, the species are chemically robust and their growth can hardly be suppressed by typical antimicrobial techniques [9]. *Methylobacterium* species can use short-chain alcohols as a carbon source. *Methylobacterium* species contain enzymes that can assimilate one and multi-carbon substrates including short-chain alcohols [1,10]; therefore, alcohol disinfectant is not an effective

出典　doi：10.3390/life13112185
https://www.mdpi.com/2075-1729/13/11/2185
最終責任者　Takeshi Mori (First and Corresponding Author)

Aerobiologia
https://doi.org/10.1007/s10453-023-09803-9

ORIGINAL PAPER

Airborne bacterial communities associated with particulate matter in Temuco (Chile), one of the most air-polluted cities in South America

Tay Ruiz-Gil · Joaquín I. Rilling · Marco Campos · Elizabeth Carrazana · So Fujiyoshi · Fumito Maruyama · Daisuke Tanaka · Akihiro Sakatoku · Jun Noda · Jacquelinne J. Acuña · Milko A. Jorquera

Received: 4 September 2023 / Accepted: 28 November 2023

Abstract Temuco city is categorized as one of the most air-polluted cities in Latin America; consequently, an air quality monitoring (AQM) program based on low-volume air samplers has been established by the Chilean government. However, AQM program does not consider any microbiological parameters to be analyzed. In this context, we firstly investigated the bacterial communities contained in particulate matter fractions (PM_{10} and $PM_{2.5}$) collected by AQM program in Temuco city. Secondly, we compared the bacterial communities collected by AQM program with those collected by an environmental monitoring (EM) sampling using a high-volume air sampler. The potential relation between bacterial abundances and some environmental parameters was also addressed. In AQM samples, significant differences of bacterial abundances between PM fractions were not revealed by qPCR, although DNA metabarcoding analysis showed significant differences in bacterial diversities between PM fractions. When both EM and AQM sampling methods were

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10453-023-09803-9.

T. Ruiz-Gil
Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile

J. I. Rilling · M. Campos · J. J. Acuña · M. A. Jorquera (✉)
Laboratorio de Ecología Microbiana Aplicada (EMALAB), Departmento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile
e-mail: milko.jorquera@ufrontera.cl

M. Campos
Laboratorio de Investigación en Salud de Precisión, Departamento de Procesos Diagnósticos y Evaluación, Facultad de Ciencias de la Salud, Universidad Católica de Temuco, Temuco, Chile

E. Carrazana
Programa de Doctorado en Ciencias Mención Biología Celular y Molecular Aplicada, Universidad de La Frontera, Temuco, Chile

S. Fujiyoshi · F. Maruyama
Microbial Genomics and Ecology, The IDEC Institute, Hiroshima University, Hiroshima, Japan

S. Fujiyoshi · F. Maruyama · J. Noda · J. J. Acuña · M. A. Jorquera
Center for Holobiome and Built Environment (CHOBE), Hiroshima University, Hiroshima, Japan

D. Tanaka · A. Sakatoku
School of Science, Academic Assembly, University of Toyama, Toyama, Japan

J. Noda
Graduate School of Veterinary Medicine, Rakuno Gakuen University, Hokkaido, Japan

J. J. Acuña · M. A. Jorquera
Network for Extreme Environment Research (NEXER), Scientific and Technological, Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile

Published online: 18 December 2023

出典　doi:org/10.1007/s10453-023-09803-9
https://link.springer.com/article/10.1007/s10453-023-09803-9
最終責任者 Milko A. Jorquera (Corresponding Authors)

動物生命科学（Animal Life Science）

Taku Miyasho

Associate Professor

准教授　宮庄　拓

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

1）Role of Macroautophagy in Mammalian Male Reproductive Physiology.
Kirat D, Alahwany AM, Arisha AH, Abdelkhalek A, **Miyasho T**.
Cells 12: 1322. 2023. doi: 10.3390/cells12091322.

II. その他　＜Others＞

1）Decreased Immunoreactivity of Hepatitis E Virus Antigen Following Treatment with Sakhalin Spruce (*Picea glehnii*) Essential Oil.
Maeda N, Horochi S, Hasegawa Y, Iwasaki T, Nakatani N, **Miyasho T**, Hagiwara K, Yokota H, Funatsu Y.
Chem Biodivers 20: e202200924. 2023. doi: 10.1002/cbdv.202200924.

cells

MDPI

Review

Role of Macroautophagy in Mammalian Male Reproductive Physiology

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

1 Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt
2 Department of Animal Physiology and Biochemistry, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Cairo, Badr City 11829, Egypt
3 Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Cairo, Badr City 11829, Egypt
4 Laboratory of Animal Biological Responses, Department of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan
* Correspondence: doaakirat@hotmail.com (D.K.); taku-m@rakuno.ac.jp (T.M.)

Abstract: Physiologically, autophagy is an evolutionarily conserved and self-degradative process in cells. Autophagy carries out normal physiological roles throughout mammalian life. Accumulating evidence shows autophagy as a mechanism for cellular growth, development, differentiation, survival, and homeostasis. In male reproductive systems, normal spermatogenesis and steroidogenesis need a balance between degradation and energy supply to preserve cellular metabolic homeostasis. The main process of autophagy includes the formation and maturation of the phagophore, autophagosome, and autolysosome. Autophagy is controlled by a group of autophagy-related genes that form the core machinery of autophagy. Three types of autophagy mechanisms have been discovered in mammalian cells: macroautophagy, microautophagy, and chaperone-mediated autophagy. Autophagy is classified as non-selective or selective. Non-selective macroautophagy randomly engulfs the cytoplasmic components in autophagosomes that are degraded by lysosomal enzymes. While selective macroautophagy precisely identifies and degrades a specific element, current findings have shown the novel functional roles of autophagy in male reproduction. It has been recognized that dysfunction in the autophagy process can be associated with male infertility. Overall, this review provides an overview of the cellular and molecular basics of autophagy and summarizes the latest findings on the key role of autophagy in mammalian male reproductive physiology.

Keywords: bulk autophagy; selective autophagy; testis; spermatogenesis; germ cells; Sertoli cells; Leydig cells; testosterone; steroidogenesis

---

出典　doi: 10.3390/cells12091322.
https://www.mdpi.com/2073-4409/12/9/1322

最終責任者　Taku Miyasho (Corresponding Author)

doi.org/10.1002/cbdv.202200924 RESEARCH ARTICLE CHEMISTRY & BIODIVERSITY

Decreased Immunoreactivity of Hepatitis E Virus Antigen Following Treatment with Sakhalin Spruce (*Picea glehnii*) Essential Oil

Naoyuki Maeda,*[a] Shouya Horochi,[a] Yasuhiro Hasegawa,[a] Tomohito Iwasaki,[a] Nobutake Nakatani,[b] Taku Miyasho,[c] Katsuro Hagiwara,[d] Hiroshi Yokota,[e] and Yasuhiro Funatsu[a]

[a] Department of Food Science and Human Wellness, Rakuno Gakuen University, Hokkaido 069-8501, Japan, e-mail: n-maeda@rakuno.ac.jp
[b] Department of Environmental Sciences, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[c] Department of Veterinary Science, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[d] Department of Veterinary Medicine, Rakuno Gakuen University, Hokkaido 069-8501, Japan
[e] The General Incorporated Association 'Pine Grace', Hokkaido 069-0834, Japan

The hepatitis E virus (HEV) causes a common infectious disease that infects pigs, wild boars, deer, and humans. In most cases, humans are infected by eating raw meat. Some essential oils have been reported to exhibit antiviral activities. In this study, in order to investigate the anti-HEV properties of essential oils, the immunoreactivities of HEV antigen proteins against the relevant antibodies were analyzed after the HEV antigens underwent treatment with various essential oils. The essential oils extracted from the tea tree, which was previously reported to exhibit antiviral activity, lavender, and lemon had strongly reduced activity. We found that treatment with the essential oil prepared from Sakhalin spruce was associated with the strongest reduction in immunoreactivity of HEV antigen protein(s) among the tested substances. The main volatile constituents of Sakhalin spruce essential oil were found to be bornyl acetate (32.30%), α-pinene (16.66%), camphene (11.14%), camphor (5.52%), β-phellandrene (9.09%), borneol (4.77%), and limonene (4.57%). The anti-HEV properties of the various components of the essential oils were examined: treatment with bornyl acetate, the main component of Sakhalin spruce oil, α-pinene, the main component of tea tree oil, and limonene, the main component of lemon oil, resulted in a strong reduction in HEV antigen immunoreactivity. These results indicate that each main component of the essential oils plays an important role in the reduction of the immunoreactivity of HEV antigen protein(s); they also suggest that Sakhalin spruce essential oil exhibits anti-HEV activity. In a formulation with the potential to eliminate the infectivity of HEV in foodborne infections, this essential oil can be applied as an inactivating agent for meat processing and cooking utensils, such as knives and chopping boards.

Keywords: hepatitis E virus, sakhalin spruce, essential oil, bornyl acetate, enzyme immunoassay.

出典　doi: 10.1002/cbdv.202200924.
https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202200924

最終責任者　Naoyuki Maeda (Corresponding Author)

Published by Graduate School of Veterinary Medicine
Rakuno Gakuen University
582 Midorimachi - Bunkyodai
Ebetsu, Hokkaido 069-8501
Japan (**Not for Sale**)

March 2024

Tel.&Fax +81-11-388-4792
e-mail: yamasita@rakuno.ac.jp