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January – December
2018



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酪農学園大学



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2018年（1月～12月）の獣医学研究科英文業績集の発刊にあたって

酪農学園大学大学院

獣医学研究科長

桐澤 力雄

獣医学研究科では獣医学群教員が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に対するモチベーションの向上、並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、2002年より業績集を刊行しています。

2018年の筆頭著者（FA）及び責任著者（CA）の論文合計は54報でした。過去5年間では、2013年：48報、2014年：51報、2015年：44報、2016年：45報、2017年：48報となっており、論文数は若干ですが上昇傾向にあるように見えます。過去4年間にFA/CA論文のない教員が13名（昨年12名）、そのうち4名（昨年3名）は全く論文がありません。昨年とほぼ同じ数の教員が長期にわたり論文が出ない状況については、何らかの対策を講じる必要があると考えております。研究を推進するには研究費が必要です。競争的資金の科研費への学群教員の申請状況をみると、2016年度：38名、2017年度：38名、2018年度：34名、2019年度：36名でした。大学院担当教員で申請されていない方がいる一方で、嘱託助手の先生で申請された方がいます（2019年度から嘱託助手も公的な競争的資金に申請できるようになりました）。獣医学群の教員の応募率は60%程度となります。この応募率は大学の研究基盤を映す鏡の一つですので、さらに上げていく必要があります。

世界に目を向けると、自然科学系の科学論文数（2014－2016年平均）のトップはアメリカで、次いで中国（僅差）、ドイツ、そして日本となっております。日本は10年前が2位でしたので相対的な低下が起きているとともに、論文数も漸減しています。Top10%補正論文数も10年前の4位から9位に後退しています。このような背景のもと、我が国の政府の科学技術予算はここ10年3兆5,000億円程度で推移していましたが、2018年度3兆8,400億円、そして2019年度は基礎研究強化・若手育成を図る方針で4兆2,670億円（11.1%増）となりました。科研費においても、近年2,200億円台で推移していましたが、2019年度は136億円増加します。これにより若手研究者の支援や優れた研究への支援が厚くなります。

2018年度の獣医学専攻博士課程の修学者総数は27名で今年度の修了認定者は4名です。獣医保健看護学専攻修士課程は修学者数1名（1年生）です。2019年度の学術振興会特別研究員のDC1・農学に本研究科博士課程から1名内定になりました。約20年ぶりのことです。全体の採択率は20%程度ですが、採択者の多くは国公立大学の大学院生で90%を占めています。

大学院の学生を多く受け入れることは研究の活性化ならびに学類学生の教育に欠かせません。2018年度に北海道大学が主幹校となって共同申請していた「One Healthフロンティア卓越大学院」が採択され、2019年度から本格始動します。この制度を大いに利用し、大学院教育のさらなる高度化を進めていきたいと考えています。

最後に関係各位には本業績集をご高覧いただき、各教員の研究内容などから共同研究の推進、さらには広範な研究体制の構築等へのご高配いただけることを願っております。そして、それらから得られる研究成果が教育・社会へ大きく還元されることを期待しております。今後とも、ご指導ご鞭撻をよろしくお願い申し上げます。

Contents (January - December 2018)

■ 獣医学類	Department of Veterinary Medicine	
浅川 満彦	教 授 (Prof. Mitsuhiro Asakawa) (Veterinary Parasitology)	1
岩野 英知	教 授 (Prof. Hidetomo Iwano) (Veterinary Biochemistry)	9
上野 博史	教 授 (Prof. Hiroshi Ueno) (Small Animal Surgery I)	21
内田 郁夫	教 授 (Prof. Ikuo Uchida) (Veterinary Bacteriology)	23
遠藤 大二	教 授 (Prof. Daiji Endoh) (Veterinary Basic Radiology)	29
及川 伸	教 授 (Prof. Shin Oikawa) (Veterinary Herd Health)	35
大杉 剛生	教 授 (Prof. Takeo Ohsugi) (Laboratory Animal Science)	37
翁長 武紀	教 授 (Prof. Takenori Onaga) (Veterinary Physiology and Nutrition)	41
加藤 敏英	教 授 (Prof. Toshihide Kato) (Large Animal Surgery)	43
廉澤 剛	教 授 (Prof. Tsuyoshi Kadosawa) (Veterinary Clinical Oncology)	45
北村 浩	教 授 (Prof. Hiroshi Kitamura) (Veterinary Physiology)	47
桐澤 力雄	教 授 (Prof. Rikio Kirisawa) (Veterinary Virology)	53
鈴木 一由	教 授 (Prof. Kazuyuki Suzuki) (Large Animal Surgery)	57
田口 清	教 授 (Prof. Kiyoshi Taguchi) (Large Animal Surgery)	65
田島 誉士	教 授 (Prof. Motoshi Tajima) (Large Animal Internal Medicine I)	67
寺岡 宏樹	教 授 (Prof. Hiroki Teraoka) (Veterinary Pharmacology)	71
中田 健	教 授 (Prof. Ken Nakada) (Theriogenology)	79
中出 哲也	教 授 (Prof. Tetsuya Nakade) (Veterinary Diagnostic Imaging)	83
萩原 克郎	教 授 (Prof. Katsuro Hagiwara) (Veterinary Virology)	85
樋口 豪紀	教 授 (Prof. Hidetoshi Higuchi) (Animal Health)	93
福本真一郎	教 授 (Prof. Shin-ichiro Fukumoto) (Veterinary Parasitology)	101
蒔田 浩平	教 授 (Prof. Kohei Makita) (Veterinary Epidemiology)	103
村松 康和	教 授 (Prof. Yasukazu Muramatsu) (Zoonotic Diseases)	115
山下 和人	教 授 (Prof. Kazuto Yamashita) (Veterinary Anesthesiology)	119
安藤 達哉	准教授 (Assoc. Prof. Tatsuya Andoh)	
	(Large Animal Internal Medicine II)	125
井坂 光宏	准教授 (Assoc. Prof. Mitsuhiro Isaka) (Small Animal Surgery I)	127
植田 弘美	准教授 (Assoc. Prof. Hiromi Ueda) (Veterinary Anatomy)	129
臼井 優	准教授 (Assoc. Prof. Masaru Usui)	
	(Food Microbiology and Food Safety)	133
大塚 浩通	准教授 (Assoc. Prof. Hiromichi Ohtsuka)	
	(Large Animal Internal Medicine I)	145
岡本 実	准教授 (Assoc. Prof. Minoru Okamoto) (Veterinary Immunopathology)	149
川本 哲	准教授 (Assoc. Prof. Satoshi Kawamoto)	
	(Large Animal Internal Medicine II)	151

高橋 優子	准教授 (Assoc. Prof. Yuko Takahashi) (Veterinary Ethics)	153
能田 淳	准教授 (Assoc. Prof. Jun Noda) (Environmental Safety)	155
林 英明	准教授 (Assoc. Prof. Hideaki Hayashi) (Veterinary Physiology)	161
平田 晴之	准教授 (Assoc. Prof. Haruyuki Hirata) (Veterinary Parasitology)	163
堀 泰知	准教授 (Assoc. Prof. Yasutomo Hori)	
	(Small Animal Internal Medicine II)	165
前原 誠也	准教授 (Assoc. Prof. Seiya Maehara) (Veterinary Ophthalmology)	171
松田 一哉	准教授 (Assoc. Prof. Kazuya Matsuda) (Veterinary Pathology)	175
安井由美子	准教授 (Assoc. Prof. Yumiko Yasui)	
	(Veterinary Physiology and Nutrition)	179
渡邊 敬文	准教授 (Assoc. Prof. Takafumi Watanabe) (Veterinary Anatomy)	181
内田 玲麻	講 師 (Lecturer Leo Uchida) (Zoonotic Diseases)	187
玉本 隆司	講 師 (Lecturer Takashi Tamamoto)	
	(Small Animal Internal Medicine II)	193
福森 理加	講 師 (Lecturer Rika Fukumori) (Veterinary Herd Health)	197
三好健二郎	講 師 (Lecturer Kenjiro Miyoshi) (Veterinary Anesthesiology)	203
村田 亮	講 師 (Lecturer Ryo Murata) (Veterinary Bacteriology)	207
権平 智	助 教 (Asst. Prof. Satoshi Gondaira) (Animal Health)	211
酒井 俊和	助 教 (Asst. Prof. Toshikazu Sakai) (Veterinary Clinical Oncology)	215
佐野 悠人	助 教 (Asst. Prof. Yuto Sano) (Veterinary Pathology)	217
杉浦 智親	助 教 (Asst. Prof. Tomochika Sugiura) (Theriogenology)	221
華園 究	助 教 (Asst. Prof. Kiwamu Hanazono) (Veterinary Basic Radiology)	225
藤木 純平	助 教 (Asst. Prof. Jumpei Fujiki) (Veterinary Biochemistry)	229

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

北澤多喜雄	教 授 (Prof. Takio Kitazawa) (Veterinary Pharmacology)	239
嶋本 良則	教 授 (Prof. Yoshinori Shimamoto) (Animal Disease Therapeutics)	247
菅野美樹夫	教 授 (Prof. Mikio Sugano) (Large Animal Nursing)	251
郡山 尚紀	准教授 (Assoc. Prof. Takanori Kooriyama)	
	(Companion Animal Behavior and Wildlife Ecology)	255
佐野 忠士	准教授 (Assoc. Prof. Tadashi Sano) (Animal Clinical Intensive Care)	257
椿下 早絵	准教授 (Assoc. Prof. Sae Tsubakishita) (Animal Physical Therapy)	261
宮庄 拓	講 師 (Lecturer Taku Miyasho) (Animal Biological Responses)	263
八百坂紀子	講 師 (Lecturer Noriko Yaosaka) (Animal Nursing)	265

獣医寄生虫病学 (Veterinary Parasitology)

Mitsuhiko Asakawa

Professor

教授 浅川 満彦

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

- 1) Redescription of *Heligmonoides vladimiri* Sadovskaja, 1952 (Nematoda: Heligmonellidae: Nippostrongylinae) parasitic in the striped field mouse, *Apodemus agrarius* (Rodentia: Muridae), of the Far East.
Asakawa M., Hasegawa H.
Biogeography. 20: 34-40. 2018.
- 2) Redescription of *Subulura (Murisubulura) andersoni* (Cobbold, 1876) (Nematoda: Subuluridae) from *Bunomys* spp. (Rodentia: Muridae) of Sulawesi, Indonesia, with special reference to *S. (M.) suzukii* and other related species in the adjacent areas.
Dewi K, Hasegawa H, Asakawa, M.
J. Vet. Med. Sci. 80: 1639-1645. 2018.
- 3) A helminthological record on free-ranging pikas and rodents collected from Tibetan Plateau, China: Preliminary results.
Tsevegmid K, Motokawa M, Zhang H, Pei J, Asahara M, Wen W-J, Liu C, Mahmut H, Wu X, Hoshino B, Asakawa M.
Ann. Clin. Cytol. Pathol. 4: 1106. 2018.
- 4) Helminths and helminthiasis in captive amphibians and reptiles: a brief overview of recent records from Wild Animal Medical Center in Rakuno Gakuen University, Japan.
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- 1) Universal nematode detection by degenerate-oligonucleotide-primed polymerase chain reaction (DOP-PCR) of purified nematode nucleic acids.
Yoshino T, **Asakawa M**, Osa Y, Kaneko M, Endoh D.
Jpn. J. Vet. Res. 66: 311-316. 2018.
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Redescription of *Heligmonoides vladimiri* Sadovskaja, 1952 (Nematoda: Heligmonellidae: Nippostrongylinae) parasitic in the striped field mouse, *Apodemus agrarius* (Rodentia: Muridae), of the Far East

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Abstract: *Heligmonoides vladimiri* Sadovskaja, 1952 (Nematoda: Heligmonellidae: Nippostrongylinae) is redescribed based on worms collected from striped field mouse, *Apodemus agrarius* (Rodentia: Muridae), of Northeastern China, South Korean Peninsula, Jeju Island, Uotsuri Island and Taiwan. It is closest to *Heligmonoides speciosus* Konno, 1958 parasitic in *Apodemus speciosus* and *Apodemus argenteus* of Japan, by having more than 25 ridges in midbody of which left lateral ones only slightly hypertrophied than the others, but is readily distinguished by having a dissymmetrical bursa copulatrix and rays 8 each with swollen basal portion and not extending beyond the dorsal ray. It was suggested that *A. agrarius* became to acquire the infection with *H. vladimiri* after extending its distribution to East Asia.

■ 論文題目

極東ロシアのセスジアカネズミ *Apodemus agrarius* に寄生するヘリグモネラ科線虫 *Heligmonoides vladimiri* Sadovskaja, 1952 の再記載

■ 要 旨

かねてから、極東ロシア産セスジアカネズミ *Apodemus agrarius* に寄生するヘリグモネラ科線虫 *Heligmonoides vladimiri* Sadovskaja, 1952 は、原記載された時点で、記載に不適な部分（特に、原記載後に体系完成した体表面の隆起線構造や交接囊肋の配置）が指摘されていた。そこで、国内外の動物生態学者からご提供頂いた、中間人民共和国および台湾などで採集され、保存されていた同線虫標本について、形態学的特徴の再記載を実施し、近縁種との比較から系統分類学および生物地理学的な検討も実施した。日本にも近縁な *Heligmonoides* 属線虫の固有種（日本列島にしか分布しない線虫種）が複数生息するが、その由来を論考する上で、本論文で得られた情報は極めて有益な知見となった。

■ 最終責任者 Mitsuhiko Asakawa (First Author)



FULL PAPER

Wildlife Science

Redescription of *Subulura* (*Murisubulura*) *andersoni* (Cobbold, 1876) (Nematoda: Subuluridae) from *Bunomys* spp. (Rodentia: Muridae) of Sulawesi, Indonesia, with special reference to *S. (M.) suzukii* and other related species in the adjacent areas

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ABSTRACT. *Subulura* (*Murisubulura*) *andersoni* (Cobbold, 1876) (Nematoda: Subuluridae) is redescribed in detail using light and scanning electron microscopy based on materials collected from *Bunomys chrysocomus* and *Bunomys andrewsi* (Rodentia: Muridae) of Sulawesi, Indonesia, to provide basic reference data for identification. Comparison was made among congeners from adjacent areas, especially *Subulura* (*Murisubulura*) *suzukii* Yagi and Kamiya, 1981 of Japan. Because morphological differences distinguishing *S. (M.) andersoni* from *S. (M.) suzukii* and *S. (M.) sipiroki* were only the thickness of female tail and length of male tail, respectively, further study using DNA sequence analysis is necessary to prove their distinctness.

KEY WORDS: *Bunomys*, Indonesia, *Murisubulura*, SEM, *Subulura*

■論文題目

インドネシア共和国スラウェシ島産固有ネズミ科 *Bunomys* 産に寄生するスブルラ科線虫 *Subulura* (*Murisubulura*) *andersoni* (Cobbold, 1876)とその周辺地域に分布する *S. (M.) suzukii* など線虫種の比較分類学と再記載

■要 旨

インドネシア共和国スラウェシ島は生物地理学的に注目されるワラシアの代表的な島嶼の一つである。この島には固有のネズミ科 *Bunomys* 産が生息し、スブルラ科線虫 *Subulura* (*Murisubulura*) *andersoni* が寄生することが知られていた。しかし、この種が、日本列島含む西太平洋各地で記載された *S. (M.) suzukii* などとの混同が生じ、分類学的な問題が醸し出されていた。そこで、詳細な比較形態学的な検討を行い、かつ生物地理学的な検討も実施した。なお、筆頭著者は本学獣医学研究で JSPS の RONPAKU 助成事業を受け博士(獣医学)の学位を受けた方で、本報告はその学位主論文の中でも一部取り扱われた情報であった。

■最終責任者 Mitsuhiko Asakawa (Corresponding Author)

Short Communication

A Helminthological Record on Free-Ranging Pikas and Rodents Collected from Tibetan Plateau, China: Preliminary Results

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Keywords

- Murids
- Nematodes
- Pikas
- Qinghai province
- Tibetan plateau

Abstract

There is little available data regarding helminths of mammals in the Tibetan Plateau, Qinghai Province, China. This study presents preliminary data obtained from the China-Japan co-operative survey, performed in the summers of 2007 and 2009. Alimentary tracts and viscera of 78 small mammals, including plateau pikas (*Ochotona curzoniae*) and rodents (*Mus musculus*, *Alticola stoliczkanus*, *Cricetulus longicaudatus*, *Meriones meridianus* and *Phodopus roborovskii*), were investigated. Thirty of these animals were negative for helminths. Among the helminth-positive samples, no platyhelminthes (including metacercariae of *Echinococcus* spp.) or acanthocephalan parasites were obtained. Four nematode genera, including *Heligmosomoides*, *Heligmosomum*, *Syphacia* and *Trichuris* were obtained from rodents. Four other nematode genera and species, including *Obbayashinem aachotani*, *Trichostrongylus retortaeformis*, *Gradiphiellochotanae* and *Cephaluris coloradensis*, were obtained from the plateau pika spp. With the exception of *C. coloradensis*, these were first recorded instances of these species in the pikas. These can be added as newly-recorded nematode parasites in plateau pikas, though none of these are new species, nor do they appear medically relevant.

INTRODUCTION

Small mammals, such as rodents and pikas, play an important role in the ecosystem of the Tibetan Plateau in the Qinghai Province of China [1]. To date, limited data have been collected regarding parasitic helminths of these species, because studies are difficult to conduct in this area [2-4]. Therefore, we present preliminary data from a China-Japan cooperative survey of mammalian species performed in the summers of 2007 and 2009 [5].

Mammals were collected at several sites in Qinghai Province, China, with taking strict rules of the euthanasia and other ethics in both China and Japan into consideration [5]. After then, the fixed alimentary tracts including esophagus, stomach, small large intestines, and viscera including liver, lung, kidney and genital organ shown below were sent to the 1st and correspondence

authors. Each location, longitude, and altitude is previously described by Motokawa et al. [5].

The alimentary tracts and viscera from a total of 78 small mammals, including rodents (*Mus musculus*, *Alticola stoliczkanus*, *Cricetulus longicaudatus*, *Meriones meridianus* and *Phodopus roborovskii*) and plateau pikas (*Ochotona curzoniae*) were stored in 70% ethanol solution and examined under a dissecting microscope. Nematodes were cleared in lactophenol solution. Cross-sections were hand-cut, using a surgery scalpel, and used to examine gradients of ridges [6]. Morphological analysis and measurements were performed using a microscope and photo camera (BH2, OLYMPUS).

Thirty of the collected small mammals were negative for helminths. Namely, we have 48 helminth positive materials. Neither platyhelminth nor acanthocephalan parasites were

Cite this article: Tsevegmid K, Motokawa M, Zhang H, Pei J, Asahara M, et al. (2018) A Helminthological Record on Free-Ranging Pikas and Rodents Collected from Tibetan Plateau, China: Preliminary Results. Ann Clin Cytol Pathol 4(4): 1106.

■論文題目

中華人民共和国チベット高原に生息する野生ナキウサギ類および齧歯類における寄生蠕虫類の保有状況(予報)

■要 旨

中華人民共和国チベット高原に生息する野生ナキウサギ類および齧歯類における寄生蠕虫類の保有状況について、特に、ナキウサギ類の線虫類に関しての分析結果を予報した。なお、筆頭著者は本学連携協定にあるモンゴル農業大学教員である。また、本調査自体は本論文共著者でもある本学環境共生学類・星野仏方教授が代表となり、獲得された JSPS 科研費の一環として実施された。

■最終責任者 Mitsuhiko Asakawa (Corresponding Author)

Helminths and Helminthiasis in Captive Amphibians and Reptiles: A Brief Overview of Recent Records from the Wild Animal Medical Center of Rakuno Gakuen University, Japan

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Abstract

The corresponding author, a manager at the Wild Animal Medical Center established at Rakuno Gakuen University (Hokkaido, northern Japan), and his students introduce some cases of helminths and helminthiasis of captive amphibians and reptiles, including the Japanese giant salamanders and variable species of tortoises, terrapins, lizards and snakes, from zoological gardens or exotic pet clinics between 2004 and 2017 based on the research activities performed at the facility. Probably, the present summarization will prove to be a tool for future research.

INTRODUCTION

In 2004, the Wild Animal Medical Center (WAMC) was established at Rakuno Gakuen University (RGU, Hokkaido, northern Japan), to provide facilities for conservation medical research and education. Research activities performed at the WAMC vary, and include veterinary medical studies of wild and/or zoo animals and epidemiological studies of infectious diseases (especially helminthiasis). The present corresponding author is a manager at the WAMC and is a wildlife helminthologist at RGU [1-3]. Additionally, in 2006, the Japanese Society of Zoo and Wildlife Medicine designated the WAMC as a wildlife helminthiasis center of the society, and this designation accelerates studies on helminths and helminthiasis of various vertebrates. The author and his co-workers including his students have published several reviews of the scientific field derived from avian and mammalian hosts [4-10]. Furthermore, several reviews and reports have been published on helminths of free-ranging amphibians and reptiles, including invasive alien species in Japan [1,4,11-13]. However, there have been no comprehensive articles that focused on captive amphibians and reptiles from zoological gardens or exotic pet clinics in Japan; although some short reports have been published in Japanese (references are shown later). Therefore, in this review the authors introduce some cases from between 2004 and 2017 based on the research activities performed at the WAMC.

JAPANESE GIANT SALAMANDERS FOR BREEDING PROJECT

The Japanese giant salamander (*Andrias japonicus*) is an endangered amphibian species kept and bred in Hiroshima City Asa Zoological Park, western Honshu, Japan. To monitor their health, a helminthological survey of carcasses and living salamanders kept in the park was performed at the WAMC. We detected four total nematode taxa, including mature *Amphibiocapillaria tritonispunctati* and immature *Spiroxya* sp., *Kathlanidae* gen. sp. and *Physalopteroides* fam. gen. sp. worms, and a mature trematode (*Liolopecopulans*) from the alimentary tracts of carcasses [14-16] (Figure 1). In addition to the internal parasites, we also detected a leech species (*Hemiclepsis marginata*) from the body surface of a living individual [15], (Figure 2). Overall, except for the leech species, the helminths obtained were previously recorded from free-ranging Japanese giant salamanders, but were not previously found on those in captivity. Consequently, we continue to monitor the relationship between any health problems of the captive salamanders and their diets, because helminth infections are derived from intermediate hosts, such as freshwater fishes. However, this is also the first record of a leech in any Japanese giant salamander, including free-ranging individuals. Because of a leech-caused local lesion on adaptive individual (Figure 3), we speculate that parasitism by leeches is intimately associated with host health.

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■論文題目

酪農学園大学野生動物医学センターWAMCに依頼のあった飼育両生類および爬虫類の寄生
蠕虫類およびその疾病の診断・疫学に関する報告概要

■要 旨

最近のエキゾチックペットの流行を反映し、酪農学園大学野生動物医学センターWAMCに依頼
される両生類および爬虫類が排出した寄生蠕虫類の鑑定依頼やその疾病診断等に関する相談
が激増した。そこでこれまでのケースの一部について概要紹介を試みた。なお、筆頭および第2
著者は浅川が指導する本学学部生で、本報告も浅川が代表として執行したJSPSの科研費
(基盤C)の一環として公表された。

■最終責任者 Mitsuhiko Asakawa (Corresponding Author)

Universal nematode detection by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) of purified nematode nucleic acids

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Abstract

In this study, we have described a modified degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) method for molecular typing of nematodes collected from wild birds. To design the DOP-PCR, we selected 50 nonamers as DOP-motifs referring the data from a nematode genome. Inverted repeats of nonamers with a 100-1,500 base interval in the reported nematode genomes on the selection of the 50 nonamers. In these nonamers, 5 nonamers showed to create ladder pattern by DOP-PCR on 9, 6, 1, 2 and 3 species of morphologically identified adult nematodes, respectively. Eleven species of nematodes were distinguishable by combining the results of the two primers. It was suggested that the nematode species could be distinguished by DOP-PCR with a combination of 2 nonamers.

■論文題目

網羅的な寄生線虫検出を目的にした縮退オリゴヌクレオチド・プライマーを用いたPCR法(DOP-PCR)の開発

■要 旨

野生動物には多様な寄生線虫が保有されるが、これを網羅的に検出・同定をするため、縮退オリゴヌクレオチド・プライマーを用いたPCR法(DOP-PCR)の開発を試みた。なお、筆頭著者は本学獣医学研究で博士(獣医学)の学位を受けた方で、本報告はその主論文の一部であった。

■最終責任者 Daiji Endoh (Corresponding Author)

Multi-locus exon-primed intron crossing (EPIC) primer design for regional birds and algorithm design for a combination of introns

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Abstract

There are a variety of purposes for identifying the different species of wild animals and birds. It needs a method that can save cost and time for the investigation of many birds. One of which is DNA barcoding that has been used for species identification from sampled muscle, feather or feces. In this study, we developed a novel nuclear exon-primed intron-crossing (EPIC) markers to distinct local birds in Hokkaido area. In birds, the size of introns is smaller than that of mammals. Many introns are within the size range that is suitable for EPIC. To design the EPIC primers, we collated the genome sequences of chicken, turkey, zebra finch and flycatcher on which genes and exons were annotated. We tested the primers for 16 introns which could amplify the introns in 12 different species birds. On the analysis of amplified introns, the size of polymorphisms has been shown in some species of birds. While an intron in TBC1D22B gene showed an interspecies difference in the sizes of amplified introns, some of the size difference was too small for reproducible observation. Since a species could not be identified with one gene, attempts were made to identify species by combining multiple genes. Resultantly, the amplified products of 3 introns have distinguished 10 species of birds. It has been noted that the EPIC method is capable of distinguishing 10 species of local birds in Hokkaido and is therefore suggested to be utilized as a convenient means for multiple species identification.

■ 論文題目

Multi-locus exon-primed intron crossing (EPIC) 法による鳥類組織を標的にした同定手法の開発

■ 要 旨

農業被害に関わる多様な野生動物、特に、鳥類の被害種同定のため、現場に残された組織片を標的に、Multi-locus exon-primed intron crossing (EPIC) 法を応用して分析する手法を開発した。

■ 最終責任者 Daiji Endoh (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) Blood biochemistry and hematological changes in rats after administration of a mixture of three anesthetic agents.

Ochiai Y, Baba A, Hiramatsu M, Toyota N, Watanabe T, Yamashita K, Yokota H, **Iwano H.**

J Vet Med Sci. 80:387-394. 2018.

- 2) Bacteriophage ΦSA012 Has a Broad Host Range against *Staphylococcus aureus* and Effective Lytic Capacity in a Mouse Mastitis Model.

Iwano H. Inoue Y, Takasago T, Kobayashi H, Furusawa T, Taniguchi K, Fujiki J, Yokota H, Usui M, Tanji Y, Hagiwara K, Higuchi H, Tamura Y. *Biology (Basel).* 7(1): 8. 2018.

- 3) Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent *Staphylococcus aureus* Bacteriophage.

Fujiki J, Nakamura T, Furusawa T, Ohno H, Takahashi H, Kitana J, Usui M, Higuchi H, Tanji Y, Tamura Y, **Iwano H.** *Pharmaceuticals (Basel).* 11(1). pii: E25. 2018.

- 4) Biotransformation of Bisphenol A and Adverse Effects on the Next Generation.

Iwano H. Inoue H, Nishikawa M, Fujiki J, Yokota H. IntechOpen, Book title: Endocrine Disruptors (Chapter 5) doi:10.5772/intechopen.78275

II. その他<Others>

- 1) Corticosterone biosynthesis in mouse clonal myoblastic C2C12 cells.

Fujiki J, Maeda N, Sato M, Ieko T, Inoue H, Iwasaki T, **Iwano H**, Yokota H. *Steroids*. 138: 64-71. 2018.

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J Vet Sci. 19: 79-87. 2018.

- 3) Identification of a novel mechanism of action of bovine IgG antibodies specific for *Staphylococcus aureus*.

Furukawa M, Yoneyama H, Hata E, **Iwano H**, Higuchi H, Ando T, Sato M, Hayashi T, Kiku Y, Nagasawa Y, Niimi K, Usami K, Ito K, Watanabe K, Nochi T, Aso H.

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FULL PAPER

Laboratory Animal Science

Blood biochemistry and hematological changes in rats after administration of a mixture of three anesthetic agents

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ABSTRACT. Currently, given the concerns regarding animal welfare, it is required that anesthesia or analgesia be used during surgery in experimental animals. Therefore, it is important to understand how anesthesia affects the health conditions of experimental animals. In this study, rat blood biochemistry and hematological changes were examined following administration of a mixture of three anesthetic agents—medetomidine, midazolam and butorphanol (MMB). One of three MMB dose combinations was subcutaneously administered to rats. After 1 hr, rats were treated with atipamezole, to reverse the anesthetic effects. Blood biochemistry and hematological parameters were assessed at 1, 4 and 24 hr post-MMB treatment. We also recorded body weight and food intake at 0, 2, 4, 6 and 24 hr post-MMB administration. Following MMB administration, transient increases were observed in glucose (GLUC) levels, hematocrit (HCT) values and hemoglobin (HGB) levels, whereas transient decreases were observed in total protein (TP) content and white blood cell (WBC) counts. Most of these parameters returned to control values 24 hr following MMB administration. Additionally, body weight and food intake decreased in MMB-treated rats. In conclusion, intermediate and high doses of MMB changed some blood biochemistry and hematological parameters, body weight and food intake. In contrast, low-dose MMB did not cause these effects. Therefore, depending on the experimental design, MMB may influence the results of studies that use laboratory animals. Consequently, anesthetic agents used in laboratory animals should be chosen based on detailed knowledge of their pharmacological effects.

KEY WORDS: anesthesia, atipamezole, butorphanol, medetomidine, midazolam

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■論文題目

ラットにおける3種混合麻酔による血液中の生化学的、血液学的変化

■要旨

近年、動物福祉に関する理解が深まり、実験動物においても手術中の麻酔の適正使用が求められている。本研究では、ラットに対する3種混合麻酔(メデトメジン、ミダゾラム、ブトロファンール)の皮下投与において、血液中の生化学的、血液学的な性状を詳細に検討した。高濃度での麻酔導入後24時間において、血中グルコース濃度、ヘマトクリットの値ヘモグロビンレベルが増加し、総タンパク、と白血球レベルが減少した。低濃度においては、そのような以上は見られなかった。本研究から実験動物における3種混合麻酔により、血液中の生化学的な数値は変動する事があり、実験の目的に応じて考慮しながら麻酔措置を行う必要があることが示された。

■最終責任者 Hidetomo Iwano (corresponding author)

Article

Bacteriophage Φ SA012 Has a Broad Host Range against *Staphylococcus aureus* and Effective Lytic Capacity in a Mouse Mastitis Model

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Abstract: Bovine mastitis is an inflammation of the mammary gland caused by bacterial infection in dairy cattle. It is the most costly disease in the dairy industry because of the high use of antibiotics. *Staphylococcus aureus* is one of the major causative agents of bovine mastitis and antimicrobial resistance. Therefore, new strategies to control bacterial infection are required in the dairy industry. One potential strategy is bacteriophage (phage) therapy. In the present study, we examined the host range of previously isolated *S. aureus* phages Φ SA012 and Φ SA039 against *S. aureus* strains isolated from mastitic cows. These phages could kill all *S. aureus* (93 strains from 40 genotypes) and methicillin-resistant *S. aureus* (six strains from six genotypes) strains tested. Using a mouse mastitis model, we demonstrated that Φ SA012 reduced proliferation of *S. aureus* and inflammation in the mammary gland. Furthermore, intravenous or intraperitoneal phage administration reduced proliferation of *S. aureus* in the mammary glands. These results suggest that broad host range phages Φ SA012 is potential antibacterial agents for dairy production medicine.

Keywords: bacteriophage; mastitis; *Staphylococcus aureus*

■ 論文題目

バクテリオファージΦSA012は黄色ブドウ球菌に対し広範な溶菌スペクトルを有し、マウス乳房炎モデルにおいて効果的な溶菌活性を示す

■ 要 旨

ウシ乳房炎は細菌感染に起因する乳腺の炎症であり、その治療のために抗生物質を連用することからも酪農産業において最も大きな経済的損失である。また、黄色ブドウ球菌

(*Staphylococcus aureus*: SA)はウシ乳房炎の主な原因菌であり、薬剤耐性化も問題となっている。この様に、細菌感染症を制御する新たな抗菌戦略が求められる中、ファージセラピーはその一つとして注目されている。本研究では、我々がこれまでに分離したバクテリオファージ(ΦSA012およびΦSA039)の乳房炎由来SAに対する宿主域を精査した。これらのバクテリオファージは、40パタンの遺伝子型に分類される93株のSAを溶菌し、さらに6パタンの遺伝子型に分類される6株のMRSAも溶菌した。さらに、マウス乳房炎モデルにおいて、ΦSA012の投与は、SAの増殖と乳腺における炎症を抑制した。これらの結果は、広範な宿主域を示すΦSA012が酪農産業における有用な抗菌性物質となる可能性を示唆している。

■ 最終責任者 Hidetomo Iwano (First Author and Corresponding Author)



Article

Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent *Staphylococcus aureus* Bacteriophage

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Abstract: Antibiotic-resistant bacteria (ARB) have spread widely and rapidly, with their increased occurrence corresponding with the increased use of antibiotics. Infections caused by *Staphylococcus aureus* have a considerable negative impact on human and livestock health. Bacteriophages and their peptidoglycan hydrolytic enzymes (endolysins) have received significant attention as novel approaches against ARB, including *S. aureus*. In the present study, we purified an endolysin, Lys-phiSA012, which harbors a cysteine/histidine-dependent amidohydrolase/peptidase (CHAP) domain, an amidase domain, and a SH3b cell wall binding domain, derived from a polyvalent *S. aureus* bacteriophage which we reported previously. We demonstrate that Lys-phiSA012 exhibits high lytic activity towards staphylococcal strains, including methicillin-resistant *S. aureus* (MRSA). Analysis of deletion mutants showed that only mutants possessing the CHAP and SH3b domains could lyse *S. aureus*, indicating that lytic activity of the CHAP domain depended on the SH3b domain. The presence of at least 1 mM Ca²⁺ and 100 μM Zn²⁺ enhanced the lytic activity of Lys-phiSA012 in a turbidity reduction assay. Furthermore, a minimum inhibitory concentration (MIC) assay showed that the addition of Lys-phiSA012 decreased the MIC of oxacillin. Our results suggest that endolysins are a promising approach for replacing current antimicrobial agents and may contribute to the proper use of antibiotics, leading to the reduction of ARB.

Keywords: antibiotic resistant; multidrug resistant; antimicrobial agent; phage therapy; bacteriophage; endolysin; staphylococci; *Staphylococcus aureus*

■ 論文題目

多価黄色ブドウ球菌ファージ由来 LysK 様エンドライシン (Lys-phiSA012) の機能解析

■ 要 旨

抗生物質の使用の増加に伴い、薬剤耐性菌は世界的に急速な広がりを見せている。特に、黄色ブドウ球菌 (*Staphylococcus aureus*: SA) は、ヒトおよび家畜の健康において甚大な被害をもたらしている。また、バクテリオファージ、およびバクテリオファージ由来溶菌酵素 (エンドライシン) は、SAを含む薬剤耐性菌に対する新たなアプローチとして大きな注目を集めている。そこで本研究では、我々がこれまでに報告した多価バクテリオファージからエンドライシン (Lys-phiSA012) を単離・精製し、その溶菌機能について解析を実施した。Lys-phiSA012は、MRSAを含む複数のSAに対して高い溶菌活性を示した。また、Lys-phiSA012が有する2つの触媒ドメイン (CHAP, Amidase ドメイン)、1つの菌体認識ドメイン (SH3b ドメイン) の欠失変異体を構築し、溶菌活性を評価した結果、SH3b ドメインに依存的なCHAPドメインによる溶菌活性が示唆された。また、1 mMのカルシウムイオンおよび100 μ Mの亜鉛イオンの添加は、Lys-phiSA012の溶菌活性を亢進させた。さらに、Lys-phiSA012の併用は、オキサシリンのMICを減少させた。以上の結果から、エンドライシンは薬剤耐性菌の抑制に貢献する有用なアプローチであることが示唆された。

■ 最終責任者 Hidetomo Iwano (Corresponding Author)

Biotransformation of Bisphenol A and Its Adverse Effects on the Next Generation

Hidetomo Iwano, Hiroki Inoue, Miyu Nishikawa,
Jumpei Fujiki and Hiroshi Yokota

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.78275>

Abstract

Although we are exposed to many chemical substances in routine daily life, the body has metabolic systems capable of detoxifying and eliminating these chemicals. Bisphenol A (BPA) is an endocrine disrupter of great concern because of its estrogenic activity, but studies have indicated no severe adverse effects in adult rodents exposed to BPA due to metabolic detoxification. BPA is metabolized by glucuronidation mediated by phase II enzymes such as UDP-glucuronosyltransferase. Numerous recent studies in rodents have indicated that maternal BPA exposure causes adverse effects in offspring. It was also shown that bisphenol analogs are efficiently absorbed via the oral route and distributed to the reproductive tract in pregnant rats, with its residue capable of crossing the placental barrier in the late stage of gestation. Both animal and human studies have demonstrated that BPA and the BPA metabolite BPA-GA are detectable in fetal and amniotic fluid, suggesting the presence of a placental transfer mechanism. In this review, we discuss the pharmacokinetics of BPA, particularly its (1) metabolism and disposition in the intestine, (2) metabolism and disposition in the liver, and (3) transfer from maternal tissues to the fetus.

Keywords: bisphenol A, UDP-glucuronosyltransferase (UGT), multidrug resistance-associated protein (MRP), organic anion-transporting polypeptide (Oatp), xenobiotic-metabolizing enzymes (XMEs), liver perfusion, β -glucuronidase

■論文題目

ビスフェノール A の生体内代謝と次世代への悪影響について

■要旨

エストロゲン活性のあるビスフェノール A (BPA) は、内分泌かく乱物質の 1 つであるが、正常な成体においては薬物代謝酵素第 II 相の UDP グルクロン酸転移酵素により容易に解毒代謝される。一方で、妊娠期の BPA の投与は、次世代に様々な悪影響をあたえること、さらに妊娠後期には BPA 代謝物が胎盤を通過し、胎仔側へ移行していることなどが報告され、BPA の代謝動態と次世代への影響の関連性が重要視されてきている。本総説では、BPA の代謝動態を中心に、特に、①小腸での移行と代謝動態、②肝臓における代謝動態、③胎仔への移行 について解説した。

■最終責任者： Hidetomo Iwano (First author and Corresponding author)



Corticosterone biosynthesis in mouse clonal myoblastic C2C12 cells

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ABSTRACT

Corticosterone (CORT), the major glucocorticoid in rodents, is secreted from the adrenal gland, affects various organs in the body and regulates energy metabolism as a stress response. Although local steroidogenesis of androgens and estrogens in skeletal muscles has been previously reported, local CORT synthesis in skeletal muscle remains unconfirmed. In the present study, we investigated steroidogenic activities in a clonal myoblastic cell line, C2C12 cells. Three enzymes involved in CORT synthesis, 3 β -hydroxysteroid dehydrogenase (3 β -HSD), cytochrome P450c21 and cytochrome P45011 β , were identified in C2C12 cells by detecting the enzymatic reaction products with LC-MS/MS analysis. Only one enzyme that mediates cholesterol cleavage was not detected in the cells. After the addition of pregnenolone-sulfate conjugates to the cell culture medium, pregnenolone was detected and increased according to the incubation time. In conclusion, CORT synthesis occurs in C2C12 cells, and it is suggested that the initial steroidogenesis substrate is the pregnenolone-sulfate conjugate.

■ 論文題目

マウス由来 C2C12 筋芽細胞におけるコルチコステロン生合成

■ 要 旨

げっ歯類における主要な糖質コルチコイドであるコルチコステロン (CORT) は、副腎皮質から分泌され、体内の様々な臓器に作用することでストレス応答としてエネルギー代謝を制御している。骨格筋におけるアンドロゲン、エストロゲンの局所的なステロイド合成が報告されている一方で、骨格筋局所的なCORT合成は未だ報告されていない。そこで本研究では、マウス由来C2C12筋芽細胞におけるステロイド合成能について解析を実施した。LC-MS/MSを用いた酵素活性試験において、CORT生合成に必須である3種類のステロイド酵素 (3 β -HSD, P450c21, P45011 β) の活性が認められた。一方で、ステロイド合成においてコレステロールの側鎖を切断しプレグネノロンを合成する酵素 (P450scc) の活性は認められなかった。また、C2C12筋芽細胞の培養上清にプレグネノロン硫酸抱合体を添加し培養した結果、培養上清から脱抱合を受けたプレグネノロンが経時的に検出された。以上の結果から、C2C12筋芽細胞においてCORTが合成され、その出発基質としてプレグネノロン硫酸抱合体が機能していることが示唆された。

■ 最終責任者 Hiroshi Yokota (Corresponding Author)

Innate immune response of bovine mammary epithelial cells to *Mycoplasma bovis*

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Mycoplasma spp. are contagious bacteria, and mycoplasmal mastitis is a serious productivity problem on dairy farms. Bovine mammary epithelial cells (bMECs) have an important role in the elimination of pathogens, but the effect of *Mycoplasma bovis* on bMECs has not been fully described. To elucidate the immune response against intramammary infection by *M. bovis*, we undertook microarray analysis to examine and profile mRNA expression in bMECs after stimulation with *M. bovis*. We also compared the effects of *M. bovis*, *Staphylococcus aureus*, and *Escherichia coli* on immune-related mRNA expression in bMECs. Transcriptome analysis indicated a significant decrease in the level of mRNA-encoding lysine-specific demethylase 4D, suggesting that the immune response is suppressed by a decrease in histone demethylase activity. Interleukin (IL)-1 β , IL-6, tumor necrosis factor alpha, toll-like receptor (TLR) 2, and TLR4 mRNA expression levels were significantly increased in bMECs stimulated with heat-killed *M. bovis*, but the expression levels were lower than those following stimulation by heat-killed *S. aureus* or *E. coli*. Our results suggest that *M. bovis* weakly affects mRNA expression in bMECs compared to the effects of *E. coli* or *S. aureus*. Moreover, live *M. bovis* may induce suppression of the immune response in bMECs.

Keywords: *Mycoplasma bovis*, bovine mammary epithelial cells, cytokines, innate immunity, microarray analysis

■ 論文題目

Mycoplasma bovis に対するウシ乳腺上皮細胞の自然免疫応答

■ 要 旨

マイコプラズマ性乳房炎は酪農場において甚大な経済的損失をもたらす疾患である。ウシ乳腺上皮細胞は病原体に対する免疫応答に重要な役割を担っている。*Mycoplasma bovis*に対するウシ乳腺上皮細胞の免疫応答を明らかにするため、*M. bovis*刺激における免疫応答に関連するmRNA発現量を定量した。また、*M. bovis*の比較対象として*Staphylococcus aureus*および*Escherichia coli*がウシ乳腺上皮細胞に及ぼす影響を検討した。*M. bovis*の生菌刺激に対するウシ乳腺上皮細胞の網羅的遺伝子発現解析結果から、ヒストン脱メチル化により免疫応答を活性化するlysine-specific demethylase 4Dの発現量が無刺激対照と比較して有意に低下した。*M. bovis*の死菌刺激によるウシ乳腺上皮細胞におけるIL-1 β 、IL-6、TNF α 、TLR 2、TLR 4のmRNA発現量は有意に増加したが、*S. aureus*および*E. coli*による刺激と比較してそれらの発現量は低値であった。以上の結果から*M. bovis*は*S. aureus*および*E. coli*と比較して弱い免疫応答性を示し、*M. bovis*の生菌はウシ乳腺上皮細胞の免疫応答を抑制することが考えられた。

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RESEARCH ARTICLE

Open Access



Identification of a novel mechanism of action of bovine IgG antibodies specific for *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a major pathogen that causes subclinical mastitis associated with huge economic losses to the dairy industry. A few vaccines for bovine mastitis are available, and they are expected to induce the production of *S. aureus*-specific antibodies that prevent bacterial adherence to host cells or promote opsonization by phagocytes. However, the efficacy of such vaccines are still under debate; therefore, further research focusing on improving the current vaccines by seeking additional mechanisms of action is required to reduce economic losses due to mastitis in the dairy industry. Here, we generated *S. aureus*-specific bovine IgG antibodies (anti-*S. aureus*) that directly inhibited bacterial growth in vitro. Inhibition depended on specificity for anti-*S. aureus*, not the interaction between Protein A and the fragment crystallizable region of the IgG antibodies or bacterial agglutination. An in vitro culture study using *S. aureus* strain JE2 and its deletion mutant JE2ΔSrtA, which lacks the gene encoding sortase A, revealed that the effect of anti-*S. aureus* was sortase-A-independent. Sortase A is involved in the synthesis of cell-wall-associated proteins. Thus, other surface molecules, such as membrane proteins, cell surface polysaccharides, or both, may trigger the inhibition of bacterial growth by anti-*S. aureus*. Together, our findings contribute insights into developing new strategies to further improve the available mastitis vaccine by designing a novel antigen on the surface of *S. aureus* to induce inhibitory signals that prevent bacterial growth.

■ 論文題目

黄色ブドウ球菌に対する特異的なウシ IgG を分離しそのメカニズムを解析した。

■ 要 旨

黄色ブドウ球菌は、症状としては示しにくい酪農産業には多大な経済損失をもたらす乳房炎を引き起こす主要な病原体である。宿主細胞への細菌の接着を妨げるか、オプソニン作用を誘導するいくつかのワクチンが開発されているが、その効果については議論されており、新たなワクチン開発が求められている。本研究では、黄色ブドウ球菌の増殖を直接阻害するIgG抗体を作り出した。この抗体は、proteinAとの相互作用により増殖を阻害しているわけではなく、また、細胞壁に関連するタンパク質などを認識しているわけではないことがあきらかとなった。つまり、このIgGは、細胞壁のタンパク質を認識しているわけではなく、糖鎖など他の分子を認識して、細菌の増殖を抑制していることが示唆された。本結果は、乳房炎ワクチン開発において、細菌増殖を直接抑える新たな抗体を誘導するワクチン開発に応用できることを示唆するものである。

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- 1) Phylogenetic characterization of *Salmonella enterica* serovar Typhimurium and its monophasic variant isolated from Food animals in Japan revealed replacement of major epidemic clones in the last 4 decades
Arai N, Sekizuka T, Tamamura Y, Tanaka K, Barco L, Izumiya H, Kusumoto M, Hinenoya A, Yamasaki S, Iwata T, Watanabe A, Kuroda M, **Uchida I.** Akiba M.
J. Clin. Microbiol. 56: e01758-17. 2018.
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Tamamura Y, **Uchida I.**
JARQ 52: 293-299. 2018.



Phylogenetic Characterization of *Salmonella enterica* Serovar Typhimurium and Its Monophasic Variant Isolated from Food Animals in Japan Revealed Replacement of Major Epidemic Clones in the Last 4 Decades

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ABSTRACT *Salmonella enterica* serovar Typhimurium (*Salmonella* Typhimurium) and its monophasic variant (*Salmonella* 4[5,12]–) are the major causes of gastroenteritis in both humans and animals. Pulsed-field gel electrophoresis and multilocus, variable-number tandem-repeat analysis have been used widely as subtyping methods for these pathogens in molecular epidemiological analyses, but the results do not precisely reflect phylogenetic information. In this study, we performed a phylogenetic analysis of these serovars using whole-genome sequencing data and identified nine distinct genotypic clades. Then, we established an allele-specific PCR-based genotyping method detecting a clade-specific single nucleotide polymorphism to rapidly identify the clade of each isolate. Among a total of 815 isolates obtained from cattle in Japan between 1977 and 2017, clades 1, 7, and 9 contained 77% of isolates. Obvious replacement of the dominant clone was observed five times in this period, and clade 9, which mostly contains *Salmonella* 4[5,12]–, is currently dominant. Among 140 isolates obtained from swine in Japan between 1976 and 2017, clades 3 and 9 contained 64% of isolates. Clade 9 is the latest clone as is the case in cattle isolates. Clade 9 is similar to an epidemic clone from Europe, which is characterized by sequence type 34 (ST34), chromosomal *Salmonella* genomic island 3, and a composite transposon containing antimicrobial resistance genes. The increased prevalence of clade 9 among food animals in Japan might be a part of the pandemic of the European *Salmonella* 4[5,12]– clone.

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Keywords: *Salmonella enterica*; Typhimurium; whole-genome sequencing; antimicrobial resistance; food safety.

■ 論文題目

日本における家畜と食品由来 *Salmonella* serovar Typhimurium およびその単相変異菌の過去 40 年間ににおける遺伝子型の変遷

■ 要 旨

近年、2 相 H 抗原が発現しない非定型 *Salmonella enterica* Typhimurium (ST) による本症の発症数が上昇傾向にあるが、本血清型の遺伝的背景は不明である。本研究は全ゲノム情報を基に ST と非定型 ST の一塩基多型を元に遺伝子型別法を構築・適用することで国内に分布する ST と非定型 ST の遺伝系統を調べることを目的とした。系統解析の結果、供試菌株は 9 つの遺伝子型に区分された。系統樹において非定型 ST は、国内外の分離株が混在する SNP9 型、2000 年代に流行した ST と近縁な SNP8 型、鞭毛相変異に関与する領域の点変異により単相化した SNP3 型の 3 つのクレードに集約された。近年我が国で顕在化している非定型 ST による家畜のサルモネラ症は、欧州株と近縁な特定の系統 (SNP9 型) によることが示唆された。

■ 最終責任者 Masato Akiba (Corresponding Author)



NOTE

Virology

A long-term animal experiment indicating persistent infection of bovine coronavirus in cattle

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ABSTRACT. A long-term animal experiment involving inoculation with bovine coronavirus (BCoV) was conducted to verify its persistent infection in cattle. Three colostrum-deprived Holstein calves were housed separately in individual rooms of a high-containment facility and inoculated with the BCoV strain Kumamoto/1/07. Until the end of the experiment (1,085, 700 and 280 days, respectively), viral RNAs were detected sporadically by RT-PCR and nested PCR from plasma, nasal discharge, and feces. Seroconversion and titer changes were validated by hemagglutination inhibition tests and neutralization tests. Among the samples, nasal discharge showed a higher viral positivity than feces, which seemed to be associated with positive detection in the plasma. These data demonstrate the existence of persistent infection of BCoV in the respiratory tissues of cattle.

KEY WORDS: bovine coronavirus, long-term animal experiment, persistent infection

■ 論文題目

牛コロナウイルスの牛体内における持続感染の検証試験

■ 要 旨

牛を用いた牛コロナウイルスの感染実験を実施した。RT-PCR および nested PCR により 3 頭の牛においてそれぞれ 1085, 700, 280 日間、血漿、鼻腔、および糞便にウイルスが排出されていることが示唆された。鼻腔中におけるウイルスの排出が糞便中の排出よりも長期間認められ、血漿中のウイルスの検出状況と一致する傾向を示した。これらの結果から牛コロナウイルスの呼吸器における持続感染が証明された。

■ 最終責任者 Toru Kanno (Corresponding Author)

REVIEW

Molecular Epidemiological Analysis of *Salmonella enterica* subsp. *enterica* Serovar Typhimurium Isolated from Cattle in Hokkaido, Japan

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Abstract

Salmonella enterica subsp. *enterica* serovar Typhimurium is one of the most common causative agents of bovine salmonellosis. This review summarizes the molecular epidemiology of *S. Typhimurium* isolated from cattle in Hokkaido, Japan. The molecular epidemiology of 545 isolates collected between 1977 and 2009 from cattle in Hokkaido was investigated using pulsed-field gel electrophoresis (PFGE). Nine main clusters (I–IX) were identified by PFGE. Clusters I and VII were dominant in 1992–2002 and 2002–2009, respectively. Next, we analyzed the function of cluster I and VII isolates. Cluster I isolates produced the ADP-ribosyltransferase toxin, ArtA/ArtB which were fatal to mice. Cluster VII isolates were multidrug resistant and shared a tetracycline-resistance plasmid. Thus, epidemiological surveillance enables early recognition of epidemic *Salmonella* strains.

Discipline: Animal health

Additional key words: bovine salmonellosis, molecular epidemiology

■ 論文題目

北海道で分離された牛由 *Salmonella enterica* subsp. *enterica* serovar Typhimurium の来分子疫学的解析

■ 要 旨

Salmonella enterica subsp. *enterica* serovar Typhimurium (ST) は牛のサルモネラ症の主な原因菌である。このレビューは北海道における牛由来 ST の分子疫学的解析に関する研究について解説したものである。1977 年から 2009 年に分離された 545 株の牛由来株についてパルスフィールド電気泳動法 (PFGE) により 9 のクラスター (I ~ IX) に分類した。1992 年から 2002 年の流行株および 2002 年から 2009 年の流行株がそれぞれ I 型および VII 型であることを明らかにした。I 型菌は ADP-リボシル化毒素 (ArtA/ArtB) を産生し、本毒素はマウスに対して致死活性を示した。一方 VII 型菌は薬剤耐性病原性プラスミドを共通して保有していた。本研究は分子疫学的解析手法によるサーベランスが *Salmonella* における流行型の迅速同定を可能にすることを示した。

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- 1) Multi-locus exon-primed intron crossing (EPIC) primer design for regional birds and algorithm design for a combination of introns.
Kumakura, K, Osanai, Y, Osa, Y, Fujii, K, Asakawa, M, Ushiyama, K, Onuma, M, Igarashi, H, **Endoh, D.**
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II. その他<Others>

Multi-locus exon-primed intron crossing (EPIC) primer design for regional birds and algorithm design for a combination of introns

Kumakura, Keiko; Osanai, Yuta; Osa, Yuichi; Fujii, Kei; Asakawa, Mitsuhiro; Ushiyama, Kii; Onuma, Manabu; Igarashi, Hiroataka; Endoh, Daiji

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KEYWORDS : EPIC; polymorphic introns; species-identification; Hokkaido; Max-covering computer program

Abstract

There are a variety of purposes for identifying the different species of wild animals and birds. It needs a method that can save cost and time for the investigation of many birds. One of which is DNA barcoding that has been used for species identification from sampled muscle, feather or feces. In this study, we developed a novel nuclear exon-primed intron-crossing (EPIC) markers to distinct local birds in Hokkaido area. In birds, the size of introns is smaller than that of mammals. Many introns are within the size range that is suitable for EPIC. To design the EPIC primers, we collated the genome sequences of chicken, turkey, zebra finch and flycatcher on which genes and exons were annotated. We tested the primers for 16 introns which could amplify the introns in 12 different species birds. On the analysis of amplified introns, the size of polymorphisms has been shown in some species of birds. While an intron in TBC1D22B gene showed an interspecies difference in the sizes of amplified introns, some of the size difference was too small for reproducible observation. Since a species could not be identified with one gene, attempts were made to identify species by combining multiple genes. Resultantly, the amplified products of 3 introns have distinguished 10 species of birds. It has been noted that the EPIC method is capable of distinguishing 10 species of local birds in Hokkaido and is therefore suggested to be utilized as a convenient means for multiple species identification.

FULL TEXT: [PDF](#), [MICROSOFT WORD XML\(SUPPLEMENTAL DATA\)](#)

■ 論文題目

地域鳥類のための Exon-Primed Intron-Crossing (EPIC) プライマー設計とイントロンの組合せのためのアルゴリズム設計

■ 要 旨

野生動物や鳥の異なる種を識別ことには多様なニーズがある。現場では多くの鳥の調査のための費用と時間を節約することが求められる。良く使われる方法として筋肉、羽毛または糞便からの種の識別に使用されてきた DNA バーコード化がある。本研究では、北海道地域の異なる地域の鳥類を対象として新規の Exon-Primed Intron-Crossing (EPIC) マーカーを開発した。鳥類では、イントロンのサイズは哺乳動物のそれよりも小さく、多くのイントロンは EPIC に適したサイズ範囲内にある。EPIC プライマーを設計するために、我々は遺伝子およびエクソンが注釈を付けられたニワトリ、シチメンチョウ、ゼブラフィンチおよびヒタキのゲノム配列を照合した。著者らは、12 種の異なる鳥においてイントロンを増幅することができる 16 のイントロンについてプライマーを試験した。増幅されたイントロンの分析において、多型のサイズはいくつかの鳥種において示されている。TBC1D22B 遺伝子のイントロンは増幅されたイントロンのサイズにおいて種間差を示したが、サイズ差のいくつかは再現可能な観察には小さすぎた。1つの遺伝子では種を特定できないため、複数の遺伝子を組み合わせて種を特定することが試みられた。結果として、3つのイントロンの増幅産物は10種の鳥を識別した。EPIC法は北海道の10種の鳥を識別することができ、多種の同定のための便利な手段として利用されることが示唆された。

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Universal nematode detection by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) of purified nematode nucleic acids

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JaLCDOI : [10.14943/jjvr.66.4.311](https://doi.org/10.14943/jjvr.66.4.311)

KEYWORDS : degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR); inverted repeat; molecular typing PCR

Abstract

In this study, we have described a modified degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) method for molecular typing of nematodes collected from wild birds. To design the DOP-PCR, we selected 50 nonamers as DOP-motifs referring the data from a nematode genome. Inverted repeats of nonamers with a 100-1,500 base interval in the reported nematode genomes on the selection of the 50 nonamers. In these nonamers, 5 nonamers showed to create ladder pattern by DOP-PCR on 9, 6, 1, 2 and 3 species of morphologically identified adult nematodes, respectively. Eleven species of nematodes were distinguishable by combining the results of the two primers. It was suggested that the nematode species could be distinguished by DOP-PCR with a combination of 2 nonamers.

FULL TEXT: [PDF](#) , [MICROSOFT WORD XML\(SUPPLEMENTAL DATA\)](#)

■ 論文題目

線虫 DNA の Degenerate Oligonucleotide-primed Polymerase Chain Reaction (DOP-PCR) による汎用線虫検出

■ 要 旨

本研究では、野鳥から収集した線虫の分子型別のための Degenerate Oligonucleotide-primed Polymerase Chain Reaction (DOP-PCR) 法を報告する。DOP-PCR を設計するために、線虫ゲノムからのデータを参照し、DOP モチーフとして 50 個の 9 量体を選択した。報告された線虫ゲノム中の 100～1,500 塩基間隔を有する 50 個の 9mer の選択に関する 9 量体の逆方向反復を選択した。これらの 9mer において、5 種の 9mer は、それぞれ 9、6、1、2 および 3 種の形態学的に同定された成体線虫に対して DOP-PCR によって PCR 増幅パターンを形成することを示した。11 種の線虫は、2 つのプライマーの結果を組み合わせることによって識別可能であった。線虫種は、2 つの 9mer の組み合わせを用いた DOP-PCR によって識別できることが示唆された。

■ 最終責任者 Daiji Endoh (Corresponding Author)

Novel synthetic nucleotides of notifiable dengue (1–4), Japanese encephalitis, yellow fever and Zika flaviviruses

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View Article

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Aim: To produce synthetic nucleotides of notifiable dengue virus (1–4 types), Japanese encephalitis, yellow fever and Zika flaviviruses. These notifiable flaviviruses, particularly dengue and Zika, are problematic mosquito-borne infections in the Philippines, as well as in those countries with tropical and subtropical climates. **Method:** An algorithmic design formulation of overlap extension – polymerase chain reaction (OE-PCR) was performed to propagate 50–60 oligomer lengths of select notifiable flaviviral RNAs to DNA nucleotides via the two-step process of OE-PCR. **Result:** Algorithmic OE-PCR design formulation efficiently produced 253–256 bp of notifiable flaviviruses. Comparing the newly designed algorithmic OE-PCR with existing executable programs demonstrated it to be efficient and useful in generating accurate sequences of synthetic flaviviral nucleotides. **Conclusion:** The efficiently and accurately produced novel synthetic nucleotides of notifiable dengue virus 1–4, Japanese encephalitis, yellow fever and Zika flaviviruses using OE-PCR is useful in understanding the dynamics of flaviviral species and holds potential for the development of synthetic nucleotide-based immunogens.

■ 論文題目

行政機関が指定するデング熱(1～4)、日本脳炎、黄熱病およびジカフラビウイルスの新規合成ヌクレオチド

■ 要 旨

行政機関が指定する(notifiable)デング熱ウイルス(1～4 種類)、日本脳炎、黄熱病およびジカフラビウイルスの遺伝子の一部の合成を試みた。これらの行政機関が指定するフラビウイルス、特にデング熱とジカウイルスは、フィリピンだけでなく熱帯や亜熱帯気候の国々で蔓延する蚊媒介感染である。方法:Overlap Extension-Polymerase Chain Reaction (OE-PCR) のアルゴリズム設計式を実行して、OE-PCR の2段階プロセスを介して50～60オリゴマー長の選択可能なフラビウイルスRNAをDNA断片として合成した。結果:OE-PCR デザインアルゴリズムは効率的に 253～256 bp の notifiable なフラビウイルスを生産した。新しく設計されたアルゴリズム的 OE-PCR を既存の実行可能プログラムと比較すると、合成フラビウイルスヌクレオチドの正確な配列を生成するのに効率的かつ有用であることが実証された。結論:OE-PCR を用いた効率的かつ正確に製造された notifiable なデング熱ウイルス 1～4、日本脳炎、黄熱病およびジカフラビウイルスの新規合成ヌクレオチドはフラビウイルス種の動態を理解するのに有用であり、防御に利用可能な免疫源を合成する方法としての可能性を示した。

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- 1) Novel small molecule SIRT2 inhibitors induce cell death in leukemic cell lines.

Kozako T, Mellini P, **Ohsugi T**, Aikawa A, Uchida YI, Honda SI, Suzuki T.

BMC Cancer 18:791. doi: 201810.1186/s12885-018-4710-1.

RESEARCH ARTICLE

Open Access

Novel small molecule SIRT2 inhibitors induce cell death in leukemic cell lines



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Abstract

Background: Sirtuin 2 (SIRT2) is a member of the sirtuin family, nicotinamide adenine dinucleotide⁺-dependent deacylases, which participates in modulation of cell cycle control, neurodegeneration, and tumorigenesis. SIRT2 expression increases in acute myeloid leukemia blasts. Downregulation of SIRT2 using siRNA causes apoptosis of HeLa cells. Therefore, selective inhibitors of SIRT2 are candidate therapeutic agents for cancer. Adult T-cell leukemia/lymphoma (ATL) is a T-cell malignancy that has a poor prognosis and develops after long-term infection with human T-cell leukemia virus (HTLV)-1. Sirtuin 1 inhibition has been shown to induce apoptosis and autophagy in HTLV-1-infected cell lines, whereas the effects of SIRT2 inhibition alone have not been elucidated.

Methods: We assessed the efficacy of our small molecule selective SIRT2 inhibitors NCO-90/141 to induce leukemic cell death. Cell viability was examined using the cell proliferation reagent Cell Count Reagent SF. Apoptotic cells were detected by annexin V-FITC and terminal deoxynucleotidyl transferase dUTP nick end labeling assays by flow cytometry. Caspase activity was detected using an APOPCYTO Intracellular Caspase Activity Detection Kit. The presence of autophagic vacuoles was assessed using a Cyto-ID Autophagy Detection Kit.

Results: Our novel small molecule SIRT2-specific inhibitors NCO-90/141 inhibited cell growth of leukemic cell lines including HTLV-1-transformed T-cells. NCO-90/141 induced apoptosis via caspase activation and mitochondrial superoxide generation in leukemic cell lines. However, a caspase inhibitor did not prevent this caspase-associated cell death. Interestingly, NCO-90/141 increased the LC3-II level together with autophagosome accumulation, indicating autophagic cell death. Thus, NCO-90/141 simultaneously caused apoptosis and autophagy.

Conclusions: These results suggest that NCO-90/141 are highly effective against leukemic cells in caspase-dependent or -independent manners via autophagy, and they may have a novel therapeutic potential for treatment of leukemias including ATL.

Keywords: Human T-cell leukemia virus-1, Adult T-cell leukemia/lymphoma, SIRT2, Apoptosis, Caspase-independent cell death

論文題目

新規低分子 SIRT2 阻害剤の白血病細胞株における細胞死誘導

要 旨

細胞周期制御、神経変性および腫瘍形成の調節に関与するサーチュイン 2 (SIRT2) は、急性骨髄性白血病芽球において異常発現しており、SIRT2 の選択的阻害剤は癌に対する治療候補剤である。成人 T 細胞白血病/リンパ腫 (ATL) は予後不良で、ヒト T 細胞白血病ウイルス (HTLV) -1 による長期感染後に発症する T 細胞悪性腫瘍であり、SIRT2 が異常発現するもその阻害剤の効果は明らかにされていない。そこで、新規開発した低分子選択的 SIRT2 阻害剤 NCO-90/141 が白血病細胞株の細胞死を誘導するか評価した。NCO-90/141 は、HTLV-1 形質転換 T 細胞を含む白血病細胞株の細胞増殖を阻害した。NCO-90/141 は白血病細胞系においてアポトーシスを誘導し、さらにオートファジー細胞死を示すオートファゴソームの蓄積とともに LC3-II レベルを増加させた。従って、NCO-90/141 はアポトーシスとオートファジーを同時に引き起こし、ATL を含む白血病の新規治療薬として有望であることを示唆する。

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- 1) Brazilian propolis ethanol extract and its component kaempferol induce myeloid-derived suppressor cells from macrophages of mice *in vivo* and *in vitro*

Kitamura H., Saito N, Fujimoto J, Nakashima K, Fujikura D.
BMC Complement. Altern. Med. 18: 138. 2018.

- 2) Establishment of protocol for preparation of gene-edited bovine ear-derived fibroblasts for somatic cell nuclear transplantation.

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- 1) Macrophages switch their phenotype by regulating Maf expression during different phases of inflammation.

Kikuchi K, Iida M, Ikeda N, Moriyama S, Hamada M, Takahashi S,
Kitamura H., Watanabe T, Hasegawa Y, Hase K, Fukuhara T, Sato H,
Kobayashi EH, Suzuki T, Yamamoto M, Tanaka M, Asano K.
J. Immunol. 201: 635-651. 2018.

RESEARCH ARTICLE

Open Access



Brazilian propolis ethanol extract and its component kaempferol induce myeloid-derived suppressor cells from macrophages of mice *in vivo* and *in vitro*

Hiroshi Kitamura^{1*}, Natsuko Saito¹, Junpei Fujimoto¹, Ken-ichi Nakashima² and Daisuke Fujikura³

Abstract

Background: Brazilian green propolis is produced by mixing secretions from Africanized honey bees with exudate, mainly from *Baccharis dracunculifolia*. Brazilian propolis is especially rich in flavonoids and cinammic acid derivatives, and it has been widely used in folk medicine owing to its anti-inflammatory, anti-viral, tumoricidal, and analgesic effects. Moreover, it is applied to prevent metabolic disorders, such as type 2 diabetes and arteriosclerosis. Previously, we demonstrated that propolis ethanol extract ameliorated type 2 diabetes in a mouse model through the resolution of adipose tissue inflammation. The aims of this study were to identify the immunosuppressive cells directly elicited by propolis extract and to evaluate the flavonoids that induce such cells.

Methods: Ethanol extract of Brazilian propolis (PEE; 100 mg/kg i.p., twice a week) was injected into lean or high fat-fed obese C57BL/6 mice or C57BL/6 ob/ob mice for one month. Subsequently, immune cells in visceral adipose tissue and the peritoneal cavity were monitored using FACS analysis. Isolated macrophages and the macrophage-like cell line J774.1 were treated with PEE and its constituent components, and the expression of immune suppressive myeloid markers were evaluated. Finally, we injected one of the identified compounds, kaempferol, into C57BL/6 mice and performed FACS analysis on the adipose tissue.

Results: Intraperitoneal treatment of PEE induces CD11b⁺, Gr-1⁺ myeloid-derived suppressor cells (MDSCs) in visceral adipose tissue and the peritoneal cavity of lean and obese mice. PEE directly stimulates cultured M1 macrophages to transdifferentiate into MDSCs. Among twelve compounds isolated from PEE, kaempferol has an exclusive effect on MDSCs induction *in vitro*. Accordingly, intraperitoneal injection of kaempferol causes accumulation of MDSCs in the visceral adipose tissue of mice.

Conclusion: Brazilian PEE and its compound kaempferol strongly induce MDSCs in visceral adipose tissue at a relatively early phase of inflammation. Given the strong anti-inflammatory action of MDSCs, the induction of MDSCs by PEE and kaempferol is expected to be useful for anti-diabetic and anti-inflammatory therapies.

Keywords: Propolis, Kaempferol, Myeloid-derived suppressor cells, Macrophage, Type 2 diabetes, Chronic inflammation

■ 論文題目

ブラジル産プロポリスとその成分であるケンフェロールは *in vivo* 及び *in vitro* でマクロファージから骨髄由来免疫細胞を誘導する。

■ 要 旨

ブラジル産プロポリスのエタノール抽出液（PEE）は内臓脂肪組織の慢性炎症を抑え、2型糖尿病の発症を抑える。本研究ではPEEが肥満マウスの脂肪組織や腹腔のM1マクロファージや培養マクロファージから、炎症を抑える機能を有する骨髄由来免疫抑制細胞（MDSC）を誘導することを見出した。またこの誘導にはPEE中のケンフェロールが関わることを示した。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)

Establishment of protocol for preparation of gene-edited bovine ear-derived fibroblasts for somatic cell nuclear transplantation

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ABSTRACT

Recently, gene-editing using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technique has attempted to utilize fibroblasts of livestock animals for somatic cell nuclear transfer. In this study, we establish the procedure for preparing skin fibroblast clones whose genes were edited by the CRISPR/Cas9 technique. After isolating fibroblasts from earlobes of Japanese Black cattle, subsequent collagenase-digestion and extensive wash procedures enabled us to avoid contamination of fungi. Electroporation using NEPA21, rather than lipofection using commercially available liposome reagents, allowed us to perform more efficient transfection of plasmid constructs. Although bovine ear-derived fibroblasts were not able to proliferate in single cell cultures in Dulbecco's modified Eagle medium containing 10% fetal calf serum, supplementation with insulin-transferrin-selenium mixture, human recombinant epidermal growth factor, or human recombinant basic fibroblast growth factor promoted proliferation of the cells, even in a single cell culture. Taking advantage of our established protocol, we eventually obtained eight ear-derived fibroblast clones with a recessive mutation in the isoleucyl-tRNA synthetase gene corrected by the CRISPR/Cas9 technique.

■ 論文題目

体細胞核移植のためのウシ耳由来線維芽細胞のゲノム編集プロトコルの確立

■ 要 旨

体細胞核移植技術とゲノム編集技術によりウシの遺伝疾患を治療するために、ゲノム編集用遺伝子コンストラクトの体細胞への導入法と編集クローンの増幅法を検討した。黒毛和種耳由来線維芽細胞にゲノム編集用の遺伝子コンストラクトを導入する際、メーカー各社が販売しているリポソーム試薬を用いたリポフェクション法より、NEPA21 による電気穿孔法が効果的であることを示した。また 10%ウシ胎児血清含有のダルベッコ改変培地では 1 細胞からの増幅が困難なウシ編集クローンも、インスリン-トランスフェリン-セレンウム混合液やヒト組換え EGF、bFGF を培地に加えると、安定的に増幅できることを示した。また、これらの手法を用いて *IARS* 異常牛の由来の線維芽細胞を短時間でゲノム編集した治療細胞を充分数得た。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)



Generation and validation of novel anti-bovine CD163 monoclonal antibodies ABM-1A9 and ABM-2D6

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ABSTRACT

The scavenger receptor CD163 is widely used as a cell signature for alternatively active "M2" macrophages in mammals. In this study, we generated two monoclonal antibodies, ABM-1A9 and ABM-2D6, against the extracellular region of bovine CD163. Conventional Western blotting using the antibodies yielded immunoreactive bands of bovine CD163 at 130 and 150 kDa in non-reduced and reduced spleen lysates, respectively. The minimum limit of detectable concentration of both antibodies was relatively lower (5.0 ng/mL) than that of the anti-human CD163 monoclonal antibody AM-3K (> 1.0 µg/mL), which has been used previously for the detection of bovine CD163. An immunohistochemical study using formalin-fixed paraffin-embedded sections revealed that ABM-1A9 and ABM-2D6 clearly stained some Iba1⁺ macrophages in the lymph nodes of cattle with mastitis. Moreover, the CD163-stained macrophages were frequently observed engulfing leukocytes. ELISA using ABM-2D6 distinguished levels of circulating soluble CD163 in healthy cattle (less than 16.9 pmol/mL) and cattle with mastitis (more than 33.7 pmol/mL). These new monoclonal antibodies can be used in the diagnosis and evaluation of inflammatory disease prognosis in cattle with immunohistological analyses and blood test applications.

■ 論文題目

新規抗ウシ CD163 モノクローナル抗体 ABM-1A9 と ABM-2D6 の樹立と評価

■ 要 旨

CD163 は広く M2 マクロファージのマーカー分子として知られる。本研究ではウシの CD163 の細胞外ドメインを認識する新たなモノクローナル抗体 ABM-1A9 と ABM-2D6 を樹立した。この抗体はウェスタンブロットでは CD163 に相当する 130kDa と 150kDa のバンドを検出し、抗ヒト CD163 抗体である AM-3K より高感度にウシ CD163 を検出した。また乳房炎牛のリンパ節のパラフィン切片において、両抗体とも Iba-1 陽性マクロファージの中で貪食を示す細胞を染色した。さらに、ABM-2D6 を用いた ELISA では健常牛より乳房炎牛で高い濃度の CD163 を定量した。以上の結果より、新たに樹立した 2 種の抗 CD163 抗体はウシの炎症性疾患の種々の検査や研究に使用できることが明らかになった。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)

Macrophages Switch Their Phenotype by Regulating Maf Expression during Different Phases of Inflammation

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Macrophages manifest distinct phenotype according to the organs in which they reside. In addition, they flexibly switch their character in adaptation to the changing environment. However, the molecular basis that explains the conversion of the macrophage phenotype has so far been unexplored. We find that CD169⁺ macrophages change their phenotype by regulating the level of a transcription factor Maf both in vitro and in vivo in C57BL/6J mice. When CD169⁺ macrophages were exposed to bacterial components, they expressed an array of acute inflammatory response genes in Maf-dependent manner and simultaneously start to downregulate Maf. This Maf suppression is dependent on accelerated degradation through proteasome pathway and microRNA-mediated silencing. The downregulation of Maf unlocks the NF-E2-related factor 2-dominant, cytoprotective/antioxidative program in the same macrophages. The present study provides new insights into the previously unanswered question of how macrophages initiate proinflammatory responses while retaining their capacity to repair injured tissues during inflammation. *The Journal of Immunology*, 2018, 201: 635–651.

■ 論文題目

異なる炎症の時期における Maf によるマクロファージの表現型の調節

■ 要 旨

マクロファージは環境に応じて表現型を変化させる可塑性を有することは広く知られるが、その分子メカニズムは明らかでない。本研究では特にCD169⁺マクロファージの表現型変化の機構について調べた。バクテリア成分に暴露されるとCD169⁺マクロファージはMaf依存的に炎症関連分子を誘導し、炎症促進型の表現型を示す。一方このとき、Mafはプロテアソームによる分解とmiRNA依存的に減少を始める。このMafの減少はNrfの脱抑制につながり、CD169⁺マクロファージを修復型・抗酸化型の表現型へと転じさせる。以上の知見より、同じマクロファージが炎症を惹起しながらも、組織修復にも関わることの分子機構を明らかにできた。

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II . その他<Others>

- 1) Evaluation of the chemiluminescent enzyme immunoassay system for the measurement of testosterone in the serum and whole blood of stallions.
Toishi Y, Tsunoda N, Nagata SI, **Kirisawa R**, Nagaoka K, Watanabe G, Yanagawa Y, Katagiri S, Taya K.
J Reprod Dev. 64: 41-47. 2018. doi: 10.1262/jrd.2017-099.
- 2) Establishment of protocol for preparation of gene-edited bovine ear-derived fibroblasts for somatic cell nuclear transplantation.
Ishino T, Hashimoto M, Amagasa M, Saito N, Dochi O, **Kirisawa R**, Kitamura H.
Biomed Res. 39: 95-104. 2018. doi: 10.2220/biomedres.39.95.

—Original Article—

Evaluation of the chemiluminescent enzyme immunoassay system for the measurement of testosterone in the serum and whole blood of stallions

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Abstract. Testosterone (T) concentration is a useful indicator of reproductive function in male animals. However, T concentration is not usually measured in veterinary clinics, partly due to the unavailability of reliable and rapid assays for animal samples. In this study, a rapid chemiluminescent enzyme immunoassay system (CLEIA system) that was developed for the measurement of T concentration in humans use was validated for stallion blood samples. First, serum T concentrations were measured using the CLEIA system and compared with those measured by a fluorimmunoassay that has been validated for use in stallions. The serum T concentrations measured by the two methods were highly correlated ($r = 0.9865$, $n = 56$). Second, to validate the use of whole blood as assay samples, T concentrations in whole blood and in the serum were measured by the CLEIA system. T concentrations in both samples were highly correlated ($r = 0.9665$, $n = 64$). Finally, to evaluate the practical value of the CLEIA system in clinical settings, T concentrations were measured in three stallions with reproductive abnormalities after the administration of human chorionic gonadotropin (hCG). Two stallions with small or absent testes in the scrotum showed an increase in T production in response to hCG administration and one stallion with seminoma did not. In conclusion, the CLEIA system was found to be a rapid and reliable tool for measuring T concentrations in stallions and may improve reproductive management in clinical settings and in breeding studs.

Key words: Chemiluminescent enzyme immunoassay, Cryptorchidism, Reproductive abnormalities, Stallions, Testosterone
(J. Reprod. Dev. 64: 41–47, 2018)

■ 論文題目

スタリオンの血清および全血中のテストステロン測定における化学発光酵素免疫法の評価

■ 要 旨

ヒトのテストステロン測定用に開発された迅速な化学発光酵素免疫反応をスタリオンのテストステロン測定に応用した。その結果、スタリオンの血清および全血中のテストステロン測定に使用できることが明らかとなった。この方法を繁殖現場に応用することにより、スタリオンの繁殖管理を向上させることが期待された。

■ 最終責任者 Seiji Katagiri (Corresponding Author)

Establishment of protocol for preparation of gene-edited bovine ear-derived fibroblasts for somatic cell nuclear transplantation

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ABSTRACT

Recently, gene-editing using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technique has attempted to utilize fibroblasts of livestock animals for somatic cell nuclear transfer. In this study, we establish the procedure for preparing skin fibroblast clones whose genes were edited by the CRISPR/Cas9 technique. After isolating fibroblasts from earlobes of Japanese Black cattle, subsequent collagenase-digestion and extensive wash procedures enabled us to avoid contamination of fungi. Electroporation using NEPA21, rather than lipofection using commercially available liposome reagents, allowed us to perform more efficient transfection of plasmid constructs. Although bovine ear-derived fibroblasts were not able to proliferate in single cell cultures in Dulbecco's modified Eagle medium containing 10% fetal calf serum, supplementation with insulin-transferrin-selenium mixture, human recombinant epidermal growth factor, or human recombinant basic fibroblast growth factor promoted proliferation of the cells, even in a single cell culture. Taking advantage of our established protocol, we eventually obtained eight ear-derived fibroblast clones with a recessive mutation in the isoleucyl-tRNA synthetase gene corrected by the CRISPR/Cas9 technique.

■ 論文題目

体細胞核移植のためのウシ耳由来線維芽細胞のゲノム編集プロトコルの確立

■ 要 旨

体細胞核移植技術とゲノム編集技術によりウシの遺伝疾患を治療するために、ゲノム編集用遺伝子コンストラクトの体細胞への導入法と編集クローンの増幅法を検討した。黒毛和種耳由来線維芽細胞にゲノム編集用の遺伝子コンストラクトを導入する際、メーカー各社が販売しているリボソーム試薬を用いたリポフェクション法より、NEPA21による電気穿孔法が効果的であることを示した。また 10%ウシ胎児血清含有のダルベッコ改変培地では 1 細胞からの増幅が困難なウシ編集クローンも、インスリン-トランスフェリン-セレンウム混合液やヒト組換え EGF、bFGF を培地に加えると、安定的に増幅できることを示した。また、これらの手法を用いて IARS 異常牛の由来の線維芽細胞を短時間でゲノム編集した治療細胞を充分数得た。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)

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II. その他<Others>

- 1) Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves.
El-Hawari SF, Sakata H, Oyama N, Tamura J, Higuchi C, Endo Y, Miyoshi K, Sano T, **Suzuki K**, Yamashita K.
J Vet Med Sci. 80: 361-367. 2018
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J Vet Med Sci. 80: 1808-1812. 2018



Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves

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ABSTRACT. The anesthetic and cardiorespiratory effects of xylazine-alfaxalone combination were evaluated in calves. Six calves (age: 6–9 months old; weight: 114–310 kg) were anesthetized with intravenous alfaxalone 15 min after administration of intramuscular saline (0.5 ml/100 kg) or xylazine (0.1 mg/kg; 0.5 ml/100 kg of a 2% xylazine solution). Anesthesia induction was smooth and orotracheal intubation was achieved in all calves. The calves anesthetized with xylazine-alfaxalone required a smaller induction dose of alfaxalone (1.23 ± 0.17 mg/kg, $P=0.010$) and accepted endotracheal intubation for a significantly longer period (16.8 ± 7.2 min, $P=0.022$) than the calves anesthetized with alfaxalone alone (2.28 ± 0.65 mg/kg 7.3 ± 1.6 min). At 5 min after induction, tachycardia (heart rate: 166 ± 47 beats/min of heart rate), hypertension (mean arterial blood pressure: 147 ± 81 mmHg) and hypoxemia (partial pressure of arterial blood oxygen [PaO_2]: 43 ± 10 mmHg) were observed in the calves anesthetized with alfaxalone alone, whereas hypoxemia (PaO_2 : 47 ± 7 mmHg) and mild hypercapnia (partial pressure of arterial blood carbon dioxide: 54 ± 5 mmHg) were observed in the calves anesthetized with xylazine-alfaxalone. Premedication with xylazine provided a sparing effect on the induction dose of alfaxalone and a prolongation of anesthetic effect. Oxygen supplementation should be considered to prevent hypoxemia during anesthesia.

KEY WORDS: alfaxalone, calf, intravenous anesthesia, xylazine

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■ 論文題目

子牛における筋肉内キシラジン前投与を伴うまたは伴わないアルファキサロン単回ボラス静脈内投与の麻酔および心肺作用

■ 要 旨

キシラジン - アルファキサロン併用投与の麻酔効果と心肺機能効果を子牛で評価した。筋肉内食塩水 (0.5ml/100kg) またはキシラジン (0.1mg/kg; 0.5ml/100kg) の投与15分後に、6頭の子牛にアルファキサロンで静脈内麻酔した。麻酔導入はスムーズで、気管挿管はすべての子牛で達成した。キシラジン - アルファキサロン麻酔導入子牛は、 α 機作論単独投与よりも麻酔時間が有意に延長した。キシラジン - アルファキサロンで麻酔した子牛には低酸素血症 (PaO_2 : 47 ± 7 mmHg) および軽度の高炭酸ガス血症 (動脈血中二酸化炭素分圧: 54 ± 5 mmHg) が観察された。キシラジン前投薬は、アルファキサロンの導入量を減少させ麻酔効果の延長をもたらした。酸素供給は、麻酔中の低酸素血症を防ぐために考慮すべきである。

■ 最終責任者 Kazuto Yamashita (First Author and Corresponding Author)



NOTE

Internal Medicine

Is bovine protothecal mastitis related to persistent infection in intestine?

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ABSTRACT. *Prototheca zopfii* is associated with bovine mastitis, which causes a reduction in milk production and secretion of thin, watery milk with white flakes. However, the source of infection and an infection route of mastitis have not been clarified. In this study, we evaluated the prevalence of *P. zopfii* genotype 2 in fecal samples from Japanese dairies with or without a history of protothecal mastitis in 2017. *P. zopfii* genotype 2 was detected in 23 of 60 (38%) fecal samples in only the herd with a history of protothecal mastitis. These results suggest that occurrence of bovine protothecal mastitis is related to persistent infection in intestine and the source of infection is feces.

KEY WORDS: bovine protothecal mastitis, genotype, intestinal flora, *Prototheca zopfii*

■ 論文題目

ウシプロトテカ乳房炎は腸内の持続感染に関連しているか?

■ 要 旨

*Prototheca zopfii*は牛乳房炎に関連し、乳量の減少と水様性乳汁を呈する。しかしながら、感染源や乳房炎の感染経路は明らかにされていない。本研究では、2017年にプロトテカ乳房炎の既往歴の有無にかかわらず、乳汁と糞便サンプルにおける*P. zopfii*遺伝子2型の有病率を評価した。*P. zopfii*遺伝子2型は、60頭の乳牛のうち23頭(38%)から検出された。これらの結果は、ウシのプロトテカ乳房炎の発生は腸内の持続感染に関連しており、感染源は糞便であることを示唆している。

■ 最終責任者 Rui Kano (Corresponding Author)



FULL PAPER

Internal Medicine

Hypoglycemia and failure of respiratory compensation are risk factors for mortality in diarrheic calves in Hokkaido, northern Japan

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ABSTRACT. The aim of present study was to identify risk factors among laboratory findings for mortality in calves with diarrhea. A retrospective analysis was conducted utilizing medical records of 221 diarrheic calves (10.4 ± 3.7 days old) with no concurrent severe disorders that were treated with intravenous fluid therapy from the initial examination. Thirty-eight of the diarrheic calves (17.2%) died within 35 days from the initial examination. Multivariate logistic regression analysis indicated that hypoglycemia (OR 3.09; 95% CI 1.22–7.87; $P=0.02$) and failure of respiratory compensation (OR 2.63; 95% CI 1.05–6.62; $P=0.04$) were the major risk factors associated with a negative outcome in diarrheic calves. According to the Kaplan-Meier analysis, diarrheic calves with hypoglycemia and/or failure of respiratory compensation had a significantly shorter survival than calves without these factors.

KEY WORDS: calf, diarrhea, mortality, risk factor

■ 論文題目

北海道において低血糖と呼吸不全下痢性子牛における死亡の危険因子である

■ 要 旨

本研究の目的は下痢の子牛の死亡率の危険因子を検査所見から識別することであった。初診時に静脈内輸液療法が行われた合併症のない 221 頭の下痢性子牛（10.4 ± 3.7 日齢）の臨床記録簿に基づいて回顧的に分析を行った。初診から 35 日以内に 38 頭（17.2%）の下痢性子牛が死亡した。多変量ロジスティック回帰分析は、低血糖症（OR 3.09、95% CI 1.22～7.87、 $P = 0.02$ ）と呼吸不全（OR 2.63、95%CI 1.05～6.62、 $P = 0.04$ ）が主な危険因子であることを示した。下痢性子牛の死亡イベントは、 Kaplan-Meier 分析によれば、低血糖症および／または呼吸不全を伴う下痢性子牛で、これらの要因を持たない子牛よりも有意に短い生存期間を有していた。

■ 最終責任者 Kenji Tsukano (Corresponding Author)

Original Article

Next-generation Sequencing Analysis of Bacterial Flora in Bovine *Prototheca* Mastitic Milk

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ABSTRACT

Prototheca zopfii is an achlorophyllic algae that causes bovine mastitis, resulting in a reduction in milk production and the secretion of thin, watery milk with white flakes. The aim of the present study was to evaluate the bacterial flora in the udder environment in protothecal mastitis. We used metagenomic next-generation sequencing (NGS) analysis to identify 16S rRNA genes from bacterial flora present in milk samples from protothecal mastitic dairy cows.

Seven clinical strains of *P. zopfii* genotype 2 were isolated from 7 milk samples from 7 cases (Holstein cow) of protothecal mastitis; another 9 milk samples were obtained from 9 normal Holstein cows. The samples were collected in 2017 from cows in one dairy located in the Kushiro region in Hokkaido, Japan, which had a history of protothecal mastitis infection.

The NGS produced 10,000 to 15,000 sequences in each DNA sample. To facilitate comparison, we grouped the sequencing results according to the culture-based protothecal mastitis diagnosis; sequences derived from the milk samples obtained from healthy cows were grouped separately.

Sequences classified as *Streptococcus* spp., *Pseudomonas* spp., and *Sphingomonas* spp., *Caulobacter segnis*, *Macroccoccus caseolyticus*, *Methylobacterium tarhaniae*, and *Sphingomonas leidyi* were the main sequences detected in the groups of samples from cows characterized by culture as having protothecal mastitis. Notably, *Calothrix desertica* (a cyanobacterium) sequences showed higher prevalence in these samples.

To our knowledge, this is the first study to report that *C. desertica* sequences, effectively absent in the samples derived from healthy cows, are detected at high prevalence in samples from protothecal mastitic animals.

Key words : bacterial flora, bovine *Prototheca* mastitis, next-generation sequencing, *Prototheca zopfii*

■ 論文題目

ウシプロトテカ乳房炎牛の乳中細菌叢の次世代配列解析

■ 要 旨

*Prototheca zopfii*は、牛乳房炎の原因となるクロロフィル藻類である。原発性乳腺炎を有すると特徴付けられたウシ由来のサンプル群において検出された主要な配列は、スフィンゴモナス属種、カウロバクター・セグニス、マクロコッカス・カセリティカス、メチロバクター・タルハニアエ、およびスフィンゴモナス・レイディイであった。特に、*Calothrix desertica*（シアノバクテリア）配列はこれらのサンプルでより高い有病率を示した。



NOTE

Internal Medicine

Advantage of parenteral nutrition for diarrheic calves

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ABSTRACT. This study assessed the advantages of dextrose and amino acid mixture solution as parenteral nutrition (PN) therapy for diarrheic calves. Thirty diarrheic calves were randomly assigned to receive PN (PN group, n=15) or only dextrose solution (Dex group, n=15). The treatment period for the PN group (4.0 days; min-max, 2–10 days) was significantly shorter than that for the Dex group (6.0 days; min-max, 3–21 days) ($P<0.01$). The PN therapy tended to improve plasma diamine oxidase activity compared with traditional therapy. One potential association between PN therapy and shortened treatment period may be the repair of damaged intestinal villi. Although our proposal has limitations, PN therapy suggested the potential for new treatment of diarrheic calves.

KEY WORDS: amino acid, calf, cryptosporidiosis, diarrhea, supportive therapy

■ 論文題目

下痢症子牛に対する非経口栄養輸液の有用性

■ 要 旨

本研究は下痢子牛のための非経口栄養（PN）療法としてのブドウ糖とアミノ酸混合溶液の利点を評価した。30頭の下痢子牛をランダムにPN（PN群、n = 15）またはデキストロース溶液（Dex群、n = 15）のみを投与する群に割り当てた。PN群の治療期間（4.0日）はDex群の治療期間（6.0日）より有意に短かった（ $P<0.01$ ）。PN療法は、従来法と比較して血漿ジアミンオキシダーゼ活性を改善する傾向があったため、PN両方は損傷した腸絨毛の修復効果が推察できた。PN療法は下痢子牛の新たな治療法の可能性を示唆した。

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生産動物内科学 I (Large Animal Internal Medicine I)

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- 1) Radiographic evaluation of the lateral angle of the metacarpophalangeal joint and the distal interphalangeal joint in metacarpophalangeal flexural deformities in calves

Sato A, Ishii O, **Tajima M.**

Vet. Rec. Open 5: e000271. 2018. doi:10.1136/vetreco-2017-000271

II. その他<Others>

Radiographic analysis of the angle in the lateromedial projection of the metacarpophalangeal joint and the distal interphalangeal joint in metacarpophalangeal flexural deformities in calves

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ABSTRACT

The bone alignment of the metacarpophalangeal joint (MPJ) of the distal interphalangeal joint (DIPJ) in metacarpophalangeal flexural deformity (MPFD) in calves was evaluated by radiography. This study was designed by retrospective study of radiographs. Lateral to medial radiographs of distal forelimbs were taken from 19 MPFD affected calves (35 forelimbs) and 21 normal calves (42 forelimbs). Based on the radiographs, the lateral angles of MPJ were measured from the metacarpal bone axis and proximal phalanx axis, and lateral angles of DIPJ were measured from the middle phalanx axis and distal phalanx axis. Mean lateral angle of MPJ in the normal limbs was 175.9 (95% CI 174.5 to 177.4). Mean lateral angles of MPJ in MPFD were as follows: mild: 167.1 (158.9–175.2), moderate: 165.1 (158.5–171.7) and severe: 150.6 (146–155.1). MPJ angle in MPFD limbs was narrower than that in the normal limbs (mild, moderate and severe: $P=0.017$, $P=0.003$ and $P<0.001$, respectively). Mean lateral angle of DIPJ in the normal limbs was 211.9 (210.7–213.2). Mean lateral angles of DIPJ in moderate: 200.6 (195.2–206.1) and severe: 204.9 (203.3–206.5) MPFD were narrower than that in the normal limbs (both $P<0.001$). There was no significant difference between the normal limbs and mild: 210.3 (206.9–213.7) MPFD limbs ($P=0.7$). The clinical severity of MPFD corresponded well with the lateral angle of MPJ. The flexion of DIPJ in moderate and severe MPFD was similar to the flexion of MPJ in MPFD. This suggested that the lateral to medial radiographs accurately reflected the MPJ flexion and the DIPJ in MPFD in calves, providing useful information for the treatment of MPFD.

INTRODUCTION

Congenital metacarpophalangeal flexural deformities (MPFD) in calves present most commonly in the forelimbs.¹ A diagnosis can be made based on the anatomical construction of limbs examined by inspection and palpation.^{2,3} Most cases are mild and recover naturally, whereas moderate and severe cases can be treated with non-surgical treatments

such as splints or foot extensions.^{4–6} However, some severe cases do not recover and require surgery, in which MPFD is treated by sequentially transecting the superficial digital flexor tendon (SDFT), deep digital flexor tendons (DDFT) and suspensory ligament until remission of the deformity.^{7–15} The surgeon decides intraoperatively which tendons or ligament to transect, and thus an estimation of the deformity is helpful for treatment.

In horses, distal limb flexural deformities present as carpus flexural deformities, MPFD or distal interphalangeal flexural deformities (clubfoot, DIPFD).^{7–11} The DDFT, SDFT and suspensory ligament may all contribute to the occurrence of MPFD in horses.^{10,12,13} In contrast, DIPFD involves only the DDFT.^{9,12,13} For identification of the pathogenic origin in such cases, radiography is useful for assessing joint disorders and their prognoses.^{7,8,11,14–18} Lateral to medial and 45°–60° dorsopalmar views of the foot are used for the diagnosis of DIPFD and MPFD in foals.

In the bovine cases, criteria for the choice of treatment have not been described. The objective of this study was to evaluate the changes of bone alignment on the metacarpophalangeal joint (MPJ) and the distal interphalangeal joint (DIPJ) in MPFD in calves.

MATERIALS AND METHODS

Animals

This study was conducted according to the guidelines of the Experimental Animal Research Committee of Rakuno Gakuen University.

Nineteen calves (14±10 days old), 17 Holstein and two Brown Swiss, with MPFD were hospitalised at the Animal Medical

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論文題目

子牛突球の球節関節角度のレントゲン像による解析

要 旨

子牛の先天性異常である突球の、病態把握と予後判定のためのレントゲン像の有用性を検討した。解析のための撮像方法を設定し、症状の程度に応じた基準角度を呈示した。さらに、病態に関与する趾屈腱の影響を実験的に確認し、レントゲン像と病態との関連性を解析した。突球の処置において、レントゲン像の解析によって客観的に治療や予後判定が可能となることが明らかとなった。

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Hiroki Teraoka

Professor

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- 1) Contamination Status of Seven Elements in Hooded Cranes Wintering in South-West Kyushu, Japan: Comparison with Red-Crowned Cranes in Hokkaido, Japan.
Teraoka H., Miyagi H, Haraguchi Y, Takase K, Kitazawa T, Noda J.
Arch Environ Contam Toxicol. 75: 557-565. 2018.
- 2) Limited expression of functional cytochrome p450 2c subtypes in the liver and small intestine of domestic cats.
Ono Y, Sugiyama S, Matsushita M, Kitazawa T, Amano T, Uno Y, Ikushiro S, **Teraoka H.**
Xenobiotica. 13: 1-9. 2018.
- 3) Comparing time-series of chemical concentrations in zebrafish (*Danio rerio*) embryos/larvae exposed to teratogens with different hydrophobicity; caffeine, sodium valproate, and diethylstilbestrol.
Nawaji T, Mizoguchi N, Ono M, Matuura T, Seki M, **Teraoka H.**
J Toxicol Sci. 43: 267-273. 2018.
- 4) Aroclor 1254 and BDE-47 inhibit dopaminergic function manifesting as changes in locomotion behaviors in zebrafish embryos.
Tanaka Y, Fujiwara M, Shindo A, Yin G, Kitazawa T, **Teraoka H.**
Chemosphere. 193: 1207-1215. 2018.

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- 1) Alpha1B-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atrium.
Zhang S, Takahashi R, Yamashita N, **Teraoka H.**, Kitazawa T.

Eur J Pharmacol. 839: 82-88. 2018.

- 2) Structural determination, distribution, and physiological actions of ghrelin in the guinea pig.

Okuhara Y, Kaiya H, **Teraoka H**, Kitazawa T.

Peptides. 99: 70-81. 2018.



Contamination Status of Seven Elements in Hooded Cranes Wintering in South-West Kyushu, Japan: Comparison with Red-Crowned Cranes in Hokkaido, Japan

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Abstract

The hooded crane is designated as an endangered species. The cranes breed primarily in wetlands in southeast Russia and China in summer. Most of the hooded crane population winters in the Izumi plain in Japan. It is difficult to know the contamination status of their habitat because of their vast breeding area. We determined the levels of Cd, Pb, As, (total) Hg, Se, Zn, and Cu in the liver, kidney, and muscle of hooded cranes that were found dead in Izumi in the periods 2003–2006 and 2014–2015 compared with the levels in red-crowned cranes in Hokkaido, Japan, as the only cranes in which these elements had been studied extensively. There were no notable differences between levels of the seven elements in the two periods. Overall, tissue levels of the elements examined in hooded cranes were comparable to those in red-crowned cranes except for Hg and Se. Tissue levels of Hg and Se were clearly lower in hooded cranes than in red-crowned cranes that were found dead from 2000. One lead poisoning case was confirmed. The results suggest that Hooded cranes wintering in Izumi are not extensively contaminated with the seven elements examined.

■ 論文題目

九州南西部で越冬するナベヅルにおける 7 元素の汚染状況：北海道に生息するタンチョウとの比較

■ 要 旨

ナベヅルは主にアムール川流域を含むロシア、中国、モンゴルで繁殖し、大部分が出水（鹿児島）で越冬する渡り鳥である。アムール川やその周辺は重金属を含む環境汚染が問題になっているが、ナベヅルおよびその営巣地の汚染状況に関する情報は全く得られていない。本研究では、2003–06年、2014–15年（以下2000年代、2010年代）に出水で死亡したナベヅルの肝臓、腎臓、骨格筋の代表的有害元素（Hg、Cd、Pb、As）と一部の必須元素含量（Se、Cu、Zn）を測定した。2000年代のナベヅルの肝臓で鉛中毒と思われる成鳥が1羽確認されたが、Hg、Cd、Pb、Asとも清浄値の範囲であった。タンチョウと比較すると、総じてほぼ同程度であったが、Hgについてのみナベヅルはいずれの臓器とも顕著に低かった。2000年代から2010年代にかけてナベヅル臓器でHgやCd含量の僅かな変化が認められたが他の元素とも問題と考えられるレベルではなかった。今後とも継続的な検討が望まれる。

■ 最終責任者 Hiroki Teraoka (First Author and Corresponding Author)

RESEARCH ARTICLE



Limited expression of functional cytochrome p450 2c subtypes in the liver and small intestine of domestic cats

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ABSTRACT

1. Compared to information for herbivores and omnivores, knowledge on xenobiotic metabolism in carnivores is limited. The cytochrome P450 2C (CYP2C) subfamily is recognized as one of the most important CYP groups in human and dog. We identified and characterized CYP2C isoforms and variants in cat, which is an obligate carnivore.
2. Quantitative RT-PCR and immunoblot analyses were carried out to evaluate the expression of CYP2C in the liver and small intestine. A functional CYP2C isoform was heterologously expressed in yeast microsomes to determine the enzymatic activity.
3. Cat had two CYP2C genes, 21 and 41, in the genome; however, CYP2C21P was a pseudogene that had many stop codons. Three splicing variants of CYP2C41 were identified (v1–v3), but only one of them (v1) showed a complete deduced amino acid sequence as CYP2C protein. Transcripts of feline CYP2C41v1 were detected but the amounts were negligible or very small in the liver and small intestine. Immunoreactivity to an antihuman CYP2C antibody was confirmed in the recombinant feline CYP2C41v1 but not in the feline liver.
4. Recombinant feline CYP2C41v1 metabolized several substrates, including dibenzylfluorescein that is specific to human CYP2C.
5. The results suggest a limited role of functional CYP2C isoforms in xenobiotic metabolism in cat.

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Carnivore; cytochrome P450 2C; domestic cats; xenobiotics

■ 論文題目

酵素活性をもつシトクローム P450 2C はネコ肝臓にほとんど発現していない

■ 要 旨

シトクロームP450 (CYP) 2Cはヒトやイヌにおいて、ワルファリンやトルブタミド、NSAIDsなどの薬物代謝において重要な酵素である。本研究では、ネコのCYP2C分子種を初めて同定し、臓器における発現量と代謝酵素活性を検討した。その結果、CYP2C21PとCYP2C41の2種類を新規に同定した。CYP2C41は3種のスプライスバリエントであり、v1のみ代謝酵素活性を示した。CYP2C41v1は肝臓や小腸で発現していたが、CYP他の分子種に比べて発現量は極めて少なかった。ネコCYP2C21v1に反応するヒトCYP2C抗体を用いたリコンビナント免疫ブロットでもCYP2Cを検出できなかった。ネコの薬物代謝におけるCYP2Cの関与は少ないことが示唆された。このことはネコのNSAIDs代謝能が低いことと関係があるかもしれない。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)

Letter

**Comparing time-series of chemical concentrations
in zebrafish (*Danio rerio*) embryos/larvae exposed to
teratogens with different hydrophobicity; caffeine, sodium
valproate, and diethylstilbestrol**

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ABSTRACT — Developmental toxicity is an adverse developmental outcome, i.e., death, malformation, growth retardation, or functional deficiency. Recently, alternative methods of assessing developmental toxicity using zebrafish (*Danio rerio*) as a preliminary screening have attracted attention because of their low cost and high throughput. However, most toxicity evaluations have been based on a chemical concentration in an aqueous solution, and the chemical concentrations in embryos/larvae and their temporal behavior have in most cases been unclear, regardless of differences of chemical hydrophobicity. In the present study, we selected three teratogens with different hydrophobicities (caffeine, CA, log K_{ow} -0.07; sodium valproate, VA, log K_{ow} 0.26 (pH 7.4); and diethylstilbestrol, DES, log K_{ow} 5.07), and we measured their concentrations in embryos/larvae exposed to these chemicals every 24 hr post-fertilization (hpf) until 144 hpf. Kinetic analysis based on a one-compartment fish model that yields first order kinetics for CA and VA revealed that concentrations of both CA and VA in embryos/larvae increased gradually and became saturated by around 100 hpf. In contrast, DES concentrations in embryos/larvae reached a maximum at 48 or 72 hpf and then decreased gradually. The present study suggests that the temporal pattern of chemical concentrations is a function of the hydrophobicity of the chemicals.

Key words: Zebrafish, Developmental toxicity, Concentration in embryos/larvae, Hydrophobicity, Lipid concentration

■ 論文題目

発達中ゼブラフィッシュに暴露した異なる疎水性を示す3種の化学物質（カフェイン、バルプロ酸、ジエチルスチルベステロール）の全身濃度の変遷

■ 要 旨

ゼブラフィッシュ胚/稚魚は毒性試験や薬理試験のスクリーニングとして世界的に広く使用されているが化合物動態についての知識は極めて乏しい。本研究では脂溶性（オクタノール/水 分配比率、log K_{ow} ）の異なる3種の化合物を発生段階のゼブラフィッシュに継続暴露した場合、ほかの2つは徐々に全身濃度が増加したのに対して、脂溶性が極めて高いジエチルスチルベステロール（DES）では低下することを確認した。脂質を大量に含む卵黄嚢の縮小との関連が示唆された。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)



Aroclor 1254 and BDE-47 inhibit dopaminergic function manifesting as changes in locomotion behaviors in zebrafish embryos

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HIGHLIGHTS

- Aroclor 1254 and BDE-47 caused hyperactivities in zebrafish embryos.
- Inhibition of tyrosine hydroxylase and VMAT2 also induced hyperactivities.
- Hyperactivities by these treatments were inhibited by precursors of dopamine synthesis.
- Organohalogen reduced dopamine contents and increased the DOPAC/dopamine ratio.

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ABSTRACT

Contamination with polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the environment is a major concern due to their persistent bioaccumulative toxicity that can disturb neurobehavioral functions including movements. Recently, it was reported that some PBDE including BDE-47 stimulates locomotor activities of zebrafish embryos by unknown mechanism. In this study, motor movements of the zebrafish embryo were used as a model system to evaluate the neuronal toxicity of a non-coplanar PCB-dominant mixture (Aroclor 1254) and BDE-47. Both organohalogen increased tail shaking and rotation of embryos in a concentration-dependent manner. Chemical inhibition and gene knock-down of tyrosine hydroxylase and vesicular monoamine transporter 2 (VMAT2) also induced hyperactivities. Hyperactivities induced by these treatments were all inhibited by supplementation of L-tyrosine and L-dopa, precursors of dopamine synthesis. Both organohalogen reduced dopamine contents and increased the 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine ratio in whole embryos. The results suggest that functional inhibition of dopaminergic neurons is involved in hyperactivities of zebrafish embryos caused by Aroclor 1254 and BDE-47.

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■ 論文題目

PCBs と臭素化難燃物はドーパミン神経機能を抑制して発達中ゼブラフィッシュの運動活性を変化させる

■ 要 旨

PCBsおよびポリ臭素化難燃物（PBDEs）などのポリハロゲン化難燃物は有毒な難分解性化学物質であることから、使用が禁止または制限されているが、その毒性機序の多くは不明である。本研究では、ポリハロゲン化難燃物がドーパミン合成を低下させることにより、発生初期のゼブラフィッシュの自発運動を促進させることを示した。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)



Research Article

Structural determination, distribution, and physiological actions of ghrelin in the guinea pig

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Growth hormone secretagogue receptor 1a (GHS-R1a)

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ABSTRACT

We identified guinea pig ghrelin (gp-ghrelin), and examined its distribution and physiological actions in the guinea-pig. Gp-ghrelin is a 28-amino acid peptide (GASFR SPEHH SAQQR KESRK LPAKI QPR); seven amino acids are different from that of rat ghrelin at positions 2, 5, 10, 11, 19, 21, and 25, which include the conserved region known in mammals. The third serine residue is mainly modified by *n*-decanoyl acid. Both gp-ghrelin and rat ghrelin increased intracellular Ca^{2+} concentration of HEK293 cells expressing guinea pig growth hormone secretagogue receptor 1a (GHS-R1a), and the affinity of gp-ghrelin was slightly higher than that of rat ghrelin. In addition, gp-ghrelin was also effective in CHO cells expressing rat GHS-R1a with similar affinity to that of rat ghrelin. Gp-ghrelin mRNA was predominantly expressed in the stomach, whereas the expression levels in other organs was low. High levels of GHS-R1a mRNA expression were observed in the pituitary, medulla oblongata, and kidney, while medium levels were noted in the thalamus, pons, olfactory bulb, and heart. Immunohistochemistry identified gp-ghrelin-immunopositive cells in the gastric mucosa and pancreas. Intraperitoneal injection of gp-ghrelin increased food intake in the guinea pig. Gp-ghrelin did not cause any mechanical responses in isolated gastrointestinal smooth muscles *in vitro*, similar to rat ghrelin. In conclusion, the N-terminal structures that are conserved in mammals were different in gp-ghrelin. Moreover, the functional characteristics of gp-ghrelin, other than its distribution, were dissimilar from those in other Rodentia.

■ 論文題目

モルモットグレリンの同定とその分布、生理的役割に関する研究

■ 要 旨

本論文ではこれまでアミノ酸構造が同定されていなかったモルモットグレリンを分子生物学的手法と database を用いて解析した。構造は GASFR SPEHH SAQQR KESRK LPAKI QPR であり、28 個のアミノ酸からなり、3 位のセリンはデカン酸により修飾を受けていた。モルモットグレリンは他の動物種と同様に胃粘膜に最も発現が認められた。合成モルモットグレリンは、モルモットおよびラットの GHS-R1a をほぼ同じ親和性で興奮させた。摘出モルモット消化管標本ではグレリンによって著明な収縮が引き起こされなかった。その理由としては消化管の GHS-R1a 発現量が極めて低いことが原因と考えられた。グレリンによりモルモットで摂餌量と血中グルコースの増加が認められた。今回同定したモルモットグレリンは他の哺乳動物と異なる N 末端構造（特に 10 位まで）を有しており、今後モルモットにおけるグレリン研究に有用であると考えられた。

■ 最終責任者 Takio Kitazawa (Corresponding Author)



Cardiovascular pharmacology

Alpha_{1B}-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atriumShuangyi Zhang^a, Rena Takahashi^b, Natsumi Yamashita^a, Hiroki Teraoka^a, Takio Kitazawa^{a,b,*}^a Veterinary Pharmacology, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan^b Comparative Animal Pharmacology, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

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ABSTRACT

Modulation of cardiac contractility by α -adrenoceptor is well known in several mammals. Mice are useful experimental animals, but α -adrenoceptor-mediated responses have been examined only in the ventricles. To determine function of α -adrenoceptors in the atrium, effects of α -adrenoceptor agonists on spontaneous contraction and electrical-field stimulation (EFS)-induced contraction were examined. In addition, expression of α_{1A} , α_{1B} , α_{1D} and β_1 -adrenoceptor mRNAs were examined. In the right atrium, noradrenaline and phenylephrine caused positive inotropic and positive chronotropic actions. However, methoxamine, clonidine and xylazine caused positive inotropic actions, but contractile frequency was decreased at high concentrations. Phenylephrine-induced positive inotropic and chronotropic actions were partially decreased by propranolol, and both actions remained in the presence of propranolol were inhibited by phentolamine or prazosin. A low concentration of silodosin (< 100 nM) did not but a high concentration (1 μ M) decreased the phenylephrine-induced chronotropic actions. Negative chronotropic actions of clonidine and xylazine were insensitive to propranolol and phentolamine. The EFS-induced contraction of the left atrium was potentiated by noradrenaline, phenylephrine and methoxamine but was not changed by clonidine or xylazine. Propranolol partially decreased the actions of phenylephrine, and prazosin caused additional inhibition. Expression of β_1 , α_{1A} , α_{1B} and α_{1D} -adrenoceptor mRNAs was found in the atrium, and the expression level of β_1 -adrenoceptor was the highest. Of α_1 -adrenoceptors, the expression level of α_{1B} was higher than that of α_{1A} and α_{1D} . In conclusion, α_{1B} -adrenoceptors are expressed in the mouse atrium and mediate both positive chronotropic and inotropic actions. In contrast, the α_2 -adrenoceptor is not functional in the isolated atrium.

■ 論文題目

Alpha_{1B}-アドレナリン受容体を介したマウス心房の陽性変力および陽性変時作用

■ 要 旨

マウス心房筋収縮に影響を与える α 受容体について薬理学的な解析を行った。Alpha-1 受容体作動薬の phenylephrine は右心房標本の収縮高と心拍数を濃度依存性に増加させた。一方、 α -2 受容体作動薬の clonidine, xylazine は高濃度では収縮頻度を低下させ、収縮高を増大させた（反応は α , β いずれの遮断薬でも抑制されなかった）。Phenylephrine の作用は一部、propranolol で抑制されたが、残存する作用は prazosin で減弱した。このことは、 α -1 受容体を介した収縮高、心拍数増大機構がマウス心房筋に存在することを示している。Alpha-1 受容体の遺伝子発現をマウス心臓の各部位（右心房、左心房、心室）で比較すると $1B \gg 1A = 1D$ の順となった。Silodosin は、低濃度では phenylephrine の作用(propranolol 処置下)に影響を与えなかったが、高濃度(1 μ M)では phenylephrine の作用を抑制した。このことから、収縮高・心拍数の増大に關与する α -1 受容体は、1B タイプであることが示唆された。

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- 1) Diagnostic and prognostic significance of serum gastrin and pepsinogen in dairy cows with displaced abomasum.

Khalaphallah A., Elmeligy E., Aamer A., AbdelAll T., Oikawa S. and **Nakada K.**

Bulg. J. Vet. Med. 21: 67-75. 2018.

- 2) Changes in clinical and blood lipid metabolism parameters in holstein dairy cattle during the transition period.

Khalaphallah A., Aamer A., AbdelAll T., Elmeligy E., Oikawa S. and **Nakada K.**

Bulg. J. Vet. Med. 21: 420-428. 2018.



DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF SERUM GASTRIN AND PEPSINOGEN IN DAIRY COWS WITH DISPLACED ABOMASUM

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Summary

Khalphallah, A., E. Elmeligy, A. A. Amer, T. AbdelAll, S. Oikawa & K. Nakada, 2018. Diagnostic and prognostic significance of serum gastrin and pepsinogen in dairy cows with displaced abomasum. *Bulg. J. Vet. Med.*, 21, No 1, 67–75.

The study aimed to describe the changes in clinical findings and serum levels of gastrin, pepsinogen and chloride (Cl) in dairy cattle with displacement of the abomasum (DA) from day 0 until day 30 after surgery and to evaluate their diagnostic and prognostic value in evaluation of the abomasal function. The study was conducted on DA cattle (n=25) belonging to dairy farms in Hokkaido area, Japan. Cows were examined and sampled at days 0 (surgery), 7 and 30. Based on blood β -hydroxybutyric acid (BHBA) at day 0, DA cows were classified into three categories; DA only (<1.2 mmol/L), DA with subclinical ketosis (DA SCK: 1.2–2.4 mmol/L) and DA with clinical ketosis (DA CK: $\geq$ 2.5 mmol/L). All DA groups had higher serum gastrin than their physiological reference values in cattle both before or after surgery. Serum gastrin was significantly increased ($P<0.05$) in DA and DA SCK groups particularly at day 30 vs day 0. Serum pepsinogen and chlorides were not remarkably changed in any of the three diseased groups compared to reference values. Serum pepsinogen showed no significant within- and inter-group changes. The surgery and the 30-day follow-up period were not sufficient to serum gastrin to return to its physiological levels. In conclusion, further future studies may be required to investigate serum gastrin levels change in DA cattle. A longer follow up period up to 45 day is suggested.

Key words: dairy cattle, displaced abomasum, gastrin, pepsinogen

■論文題目

第四胃変位に罹患した乳用牛の血清ガストリンおよびペプシノーゲン測定の診断および予察の意義

■要 旨

第四胃変位に罹患した乳牛の手術時 (0)、7 日後、30 日後の血清ガストリン、ペプシノーゲン、塩素濃度の測定を行った。第四胃変位の牛を、第四胃変位単独、潜在性ケトosis 併発、臨床型ケトosis 併発の 3 群に区分し、比較を行った。術後の変化ならびに、3 群間のそれらの濃度に違いは認められなかった。測定時期の延長等、今後さらなる研究を行う必要がある。

■最終責任者 Arafat Khalphallah (Corresponding Author)



CHANGES IN CLINICAL AND BLOOD LIPID METABOLISM PARAMETERS IN HOLSTEIN DAIRY CATTLE DURING THE TRANSITION PERIOD

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Summary

Khalphallah, A., A. A. Aamer, T. AbdelAll, E. Elmeligy, S. Oikawa & K. Nakada, 2018. Changes in clinical and blood lipid metabolism parameters in Holstein dairy cattle during the transition period. *Bulg. J. Vet. Med.*, 21, No 4, 420–428.

The transition period is a critical period in the production cycle of the dairy cow. This study aimed to describe the clinical and biochemical changes, particularly lecithin: cholesterol acyltransferase (LCAT) and apolipoprotein B-100 (ApoB-100), during the transition period in dairy cattle. It was conducted on Holstein dairy cattle (n=80) classified into control cows (n=61) and diseased cows (n=19). They belonged to dairy farms in Ebetsu city, Hokkaido area, Japan. Body condition score (BCS) was significantly reduced in postfresh control cows when they were compared to those of close-up control group. Serum LCAT and apoB-100 were remarkably increased ($P<0.05$) at close-up period control group when they compared with those in close-up period diseased one. LCAT activities were also remarkably increased ($P<0.05$) while apoB-100 showed no significant changes at postfresh control cattle when they were compared to their values at post-fresh diseased cattle. Although apoB-100 was within the normal physiological reference values in the control and diseased cattle, it was lower than physiological reference values in 9.83% of the control cattle and in 26.32% of the diseased group. Blood glucose and packed cell volume were still within the physiological reference range in all cows. The study concluded that LCAT activities were a highly sensitive diagnostic marker of the transition period and of higher predictive value and diagnostic significance than the other lipid profile indicators of transition period disorders.

Key words: dairy cattle, fat metabolism, lecithin:cholesterol acyltransferase, transition period

■論文題目

移行期のホルスタイン種乳牛の血液脂質代謝マーカーの変化

■要 旨

移行期の乳牛の血中脂質代謝マーカー、LCAT、アポリポ蛋白質 B-100 の測定を行った。乳用牛を分娩後に臨床症状を呈する疾患を発症したグループと、正常の2群に区分けして比較した。クローズアップ期のLCATは分娩後に疾病を伴った群よりも、正常群で明らかな増加が認められた。このことからLCAT活性値の変化は、移行期における分娩後疾病発生の子察マーカーとなる可能性が示唆された。

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Prev Vet Med. 157: 105-114. 2018.
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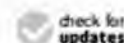
Article

Regulation of Constitutive Interferon-Stimulated Genes (ISGs) in Tumor Cells Contributes to Enhanced Antitumor Response of Newcastle Disease Virus-Infected Tumor Vaccines

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Abstract: Newcastle disease virus (NDV) is an oncolytic virus. As immunogenicity of tumor cells is enhanced by NDV infection, recombinant NDV-infected tumor vaccines (rNDV-TV) are effective methods for inducing specific immunity. However, several tumor cells resist NDV infection, and tumor specific immunity is not sufficiently induced by rNDV-TV. Therefore, we clarified the factor contributing to the suppression of NDV infection and attempted to improve rNDV-TV. Initially we investigated the correlation between the NDV infection rate and interferon-related gene expression in six murine tumor cell lines. A significant negative correlation was observed between the constitutive gene expression of Interferon-stimulated genes (ISGs) and NDV infectivity. The NDV infection rate was examined in each tumor cell treated with the Janus kinase (JAK) inhibitor ruxolitinib (Rux). Furthermore, we evaluated the Th1 response induction by Rux-treated rNDV-TV (rNDV-TV-Rux). In Rux-treated tumor cells, *Oasl2* gene expression was significantly decreased and viral infectivity was increased. In immunized mice, the number of CD8⁺ cells, and those expressing the IFN- γ gene, were significantly increased as compared with Rux-untreated rNDV-TV. The infectivity of the virus was dependent on the degree of ISGs expression in tumor cells. To remedy for this problem, rNDV-TV-Rux was expected to have a Th1 immune response.

Keywords: Newcastle disease virus; tumor; vaccines; ISG; ruxolitinib

■ 論文題目

インターフェロン誘導性遺伝子 (ISG) 制御によるニューカッスル病ウイルス腫瘍免疫効果の促進

■ 要 旨

ニューカッスル病ウイルス (NDV) は、腫瘍溶解性であり、腫瘍ワクチンとしても有用である。しかし、その効果は腫瘍細胞におけるウイルス感染性がネックとなることから、腫瘍細胞 JAK 抑制剤で処理する方法で様々な腫瘍に対して腫瘍ワクチン応用による抗腫瘍効果を得ることが示された。

■ 最終責任者 Katsuro Hagiwara (Corresponding Author)



NOTE

Public Health

Survey of tick-borne zoonotic viruses in wild deer in Hokkaido, Japan

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ABSTRACT. Tick-borne encephalitis (TBE) and severe fever with thrombocytopenia syndrome (SFTS) are both tick-borne zoonotic diseases caused by TBE virus (TBEV) and SFTS phlebovirus (SFTSV). In 2016, a second domestic TBE case was reported in Hokkaido, Japan, after an absence of 23 years. We conducted IgG ELISA for TBEV and SFTSV on 314 deer (*Cervus nippon yesoensis*) serum samples collected from 3 places in Hokkaido. There were 7 seropositive samples for TBEV but none for SFTSV by ELISA. The specificity of the 7 positive samples was confirmed by neutralization tests against TBEV, and 5 sera showed 320 to 640 of 50% focus reduction endpoint titers. Our results provide information about the infectious status of TBEV in wild deer in Hokkaido, Japan.

KEY WORDS: serosurvey, severe fever with thrombocytopenia syndrome virus, tick-borne encephalitis virus, wild deer, zoonotic disease

■ 論文題目

北海道エゾシカにおけるダニ媒介性人獣共通感染症調査

■ 要 旨

北海道の3地域に生息する野生シカ314頭を対象に、Tick-borne encephalitis(TBE)と severe fever with thrombocytopenia syndrome(SFTS)に対する ELISA 抗体調査を実施した。調査の結果、7頭で TBE 陽性が確認され、北海道に生息する野生シカの TBE 感染状況を明らかにすることができた。

■ 最終責任者 Katsuro Hagiwara (Corresponding Author)



Influence of changes in the intestinal microflora on the immune function in mice

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ABSTRACT. The composition of the intestinal microbiota is related to the health and immune function of the host. Administration of antibiotics affects the composition of the intestinal microbiota. However, the effects of immune function on the composition of the intestinal microbiota are still unclear. In this study, we investigated the lymphocyte composition and determined the relationships between lymphocyte function and the intestinal microbiota following antibiotic treatment in mice. To change the composition of the intestinal microbiota, mice were treated with or without antibiotics. Analysis of intestinal microbiota was performed by metagenomic analysis targeting 16S rRNA. Lymphocyte subsets of splenocytes were measured by flow cytometry. For functional analysis of T cells, splenocytes were stimulated with concanavalin (Con A), and cytokine gene expression was measured by real-time polymerase chain reaction. Firmicutes were predominant in the control group, whereas Bacteroidetes predominated in the antibiotic-treated group, as determined by metagenomic analysis. The diversity of the microbiota decreased in the antibiotic-treated group. Analysis of lymphocyte subsets showed that CD3⁺ cells decreased, whereas CD19⁺ cells increased in the antibiotic-treated group. All cytokine genes in splenocytes treated with Con A were downregulated in the antibiotic-treated group; in particular, genes encoding interferon- γ , interleukin (IL)-6, and IL-13 significantly decreased. Taken together, these results revealed that changes in the composition of the intestinal microbiota by antibiotic treatment influenced the population of lymphocytes in splenocytes and affected the immune response.

KEY WORDS: cytokine, immune response, intestinal microbiota, metagenomics analysis, T cell

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■ 論文題目

マウスにおける腸内細菌層の違いによる免疫機能変化

■ 要 旨

腸内細菌叢の異なるマウスを抗生剤投与により作出し、腸内細菌叢構成をメタゲノム解析により調べ、各マウスのリンパ球サブセットやサイトカイン遺伝子発現レベルを比較考察した。その結果、無処置では腸内細菌叢のうち Firmicutes が優位であったが抗生剤処理すると Bacteroidetes が優位種となり、構成菌の多様性が低下した。リンパ球では、CD3⁺ 細胞が低下し CD19⁺細胞割合が増加する傾向を示した。ConA 刺激における発現は、抗生剤処置により低下し、特に interferon- γ , interleukin (IL)-6, and IL-13 が特徴的であった。以上から、腸内細菌叢の変化はリンパ球割合やサイトカインの刺激応答性に影響することを示した。

■ 最終責任者 Katsuro Hagiwara (Corresponding Author)

Short Communication

Zoonotic Enterobacterial Pathogens Detected in Wild Chimpanzees

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Abstract: Infectious diseases including those acquired through direct or indirect contact with people and livestock threaten the survival of wild great apes. Few studies have reported enterobacterial pathogens in chimpanzees. We used multiplex PCR to screen faeces of chimpanzees sharing a landscape with villagers and livestock in Bulindi, Uganda for *Salmonella* spp., enterohemorrhagic *Escherichia coli* (*E. coli*) and *Shigella* spp./enteroinvasive *E. coli*. All three potentially zoonotic pathogens were detected. Individual prevalence ranged between 7 and 20%, with most infections observed in mature male chimpanzees. These preliminary findings suggest detailed investigation of enterobacterial infections in people, primates and livestock in this ecosystem is warranted.

Keywords: *Salmonella*, *Shigella*, *Escherichia coli*, Pan troglodytes, Health monitoring, Pathogen screening

■ 論文題目

野生チンパンジーにおける人獣共通消化管感染病原体の検出

■ 要 旨

ウガンダの村人の住居と接して生息する野生のチンパンジーの群れを対象に、糞便中に排泄される病原性細菌 (*Salmonella* spp., enterohemorrhagic *Escherichia coli* (*E. coli*) and *Shigella* spp./enteroinvasive *E. coli*) のモニターをPCR法で検査した。その結果、全ての病原体が調査個体のいずれかに感染していることが示され、人との居住関係のエコシステムを考慮するうえで重要な点であることを指摘した。

■ 最終責任者 Matthew R. McLennan (Corresponding Author)

Article

Bacteriophage Φ SA012 Has a Broad Host Range against *Staphylococcus aureus* and Effective Lytic Capacity in a Mouse Mastitis Model

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Abstract: Bovine mastitis is an inflammation of the mammary gland caused by bacterial infection in dairy cattle. It is the most costly disease in the dairy industry because of the high use of antibiotics. *Staphylococcus aureus* is one of the major causative agents of bovine mastitis and antimicrobial resistance. Therefore, new strategies to control bacterial infection are required in the dairy industry. One potential strategy is bacteriophage (phage) therapy. In the present study, we examined the host range of previously isolated *S. aureus* phages Φ SA012 and Φ SA039 against *S. aureus* strains isolated from mastitic cows. These phages could kill all *S. aureus* (93 strains from 40 genotypes) and methicillin-resistant *S. aureus* (six strains from six genotypes) strains tested. Using a mouse mastitis model, we demonstrated that Φ SA012 reduced proliferation of *S. aureus* and inflammation in the mammary gland. Furthermore, intravenous or intraperitoneal phage administration reduced proliferation of *S. aureus* in the mammary glands. These results suggest that broad host range phages Φ SA012 is potential antibacterial agents for dairy production medicine.

Keywords: bacteriophage; mastitis; *Staphylococcus aureus*

■ 論文題目

バクテリオファージ Φ SA012 の *Staphylococcus aureus* に対する感染多様性とマウス乳房炎モデルにおける有用な溶菌効果

■ 要 旨

バクテリオファージ Φ SA012 を用いて、乳房炎から分離した 6 株の *Staphylococcus aureus* を用いて溶菌効果を検討し、マウス乳房炎モデルにおいても増菌抑制が確認された。以上から、 Φ SA012 は多様な菌株に対応できるものであることを示した。

■ 最終責任者 Hidetomo Iwano (Corresponding Author)



Socio-economic factors associated with voluntary rabies control measures in Vietnam

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ABSTRACT

Rabies is a fatal zoonosis, and in Vietnam, it remains problematic despite the availability of dog rabies vaccination. The purpose of this study was to clarify the socio-economic factors associated with voluntary rabies control measures among the general population using a “Knowledge, Attitudes, and Practice” framework to provide health and veterinary authorities in Vietnam with baseline information for better planning of policy support.

A questionnaire survey with interviews was conducted in 495 households (64 mountainous and 431 plain-area households) in Thai Nguyen Province in September 2016. After the survey, uni- and multivariable analyses were performed to detect factors associated with the practices of dog rabies vaccination and tethering dogs. Structural equation modelling (SEM) was performed to understand the structures associated with practice decisions. Contingent valuation was performed to calculate willingness-to-pay for vaccination.

Vaccination coverage was 77.4% (724/935 dogs), and was significantly lower in mountainous areas dominated by ethnic minorities (53.5%, 67/105) than in plains (79.2%, 657/830, $\chi^2 = 11.7$, $df = 1$, $p < 0.001$). Mean estimation of willingness-to-pay for a vaccination was 2.30 USD (51,959 Vietnamese dong), which was more than double the current price. The willingness-to-pay in mountainous areas was 2.16 USD, while that in plain areas was 2.32 USD. The proportion that never confined dogs was significantly higher in mountainous areas (65.6%, 42/64 households) than in plain areas (26.5%, 114/430, $\chi^2 = 37.7$, $df = 1$, $p < 0.001$). Despite the low proportion of households tethering dogs, the majority answered that they would confine (479/489, 98.0%) or leash while walking (482/491, 98.2%) if such orders were enforced. SEM result showed that higher social status promoted better knowledge (β , the coefficient, = 0.75, $se = 0.05$, $p < 0.001$), better knowledge positive attitudes ($\beta = 1.0$, $se = 0.0$, p value not calculated), and positive attitudes better practices of confinement ($\beta = 0.4$, $se = 0.05$, $p < 0.001$), vaccination ($\beta = 0.52$, $se = 0.06$, $p < 0.001$), and medication ($\beta = 0.11$, $se = 0.04$, $p < 0.001$).

Our study suggested that rabies education targeted to mountainous areas using local languages for ethnic minority groups as well as the national language, and higher subsidies on dog rabies vaccination for poor households may improve vaccination coverage. Dog management may be improved by promotion campaigns.

■ 論文題目

ベトナムの狂犬病対策に関連する社会経済的要因

■ 要 旨

狂犬病管理対策に関連する社会経済的要因を明らかにするために、2016年9月にタイグエン省の495世帯（64の山岳地帯と431の平地世帯）でインタビュー調査を実施した。調査結果は、単変量および多変量解析が行われ、犬狂犬病予防接種の実施に関連する要因を調べた。さらに、ワクチン接種に対する支払意思額を調査し予防接種の支払意思額の平均見積もりは2.30 USD（51,959ベトナムドン）で、現在の価格の2倍以上でした。平地のそれは2.32 USDだった一方山岳地帯での支払いへの意欲は2.16 USDであり、狂犬病予防に関する地域別要因を提案した。

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

- 1) Innate immune response of bovine mammary epithelial cells to *Mycoplasma bovis*.
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Innate immune response of bovine mammary epithelial cells to *Mycoplasma bovis*

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Mycoplasma spp. are contagious bacteria, and mycoplasmal mastitis is a serious productivity problem on dairy farms. Bovine mammary epithelial cells (bMECs) have an important role in the elimination of pathogens, but the effect of *Mycoplasma bovis* on bMECs has not been fully described. To elucidate the immune response against intramammary infection by *M. bovis*, we undertook microarray analysis to examine and profile mRNA expression in bMECs after stimulation with *M. bovis*. We also compared the effects of *M. bovis*, *Staphylococcus aureus*, and *Escherichia coli* on immune-related mRNA expression in bMECs. Transcriptome analysis indicated a significant decrease in the level of mRNA-encoding lysine-specific demethylase 4D, suggesting that the immune response is suppressed by a decrease in histone demethylase activity. Interleukin (IL)-1 β , IL-6, tumor necrosis factor alpha, toll-like receptor (TLR) 2, and TLR4 mRNA expression levels were significantly increased in bMECs stimulated with heat-killed *M. bovis*, but the expression levels were lower than those following stimulation by heat-killed *S. aureus* or *E. coli*. Our results suggest that *M. bovis* weakly affects mRNA expression in bMECs compared to the effects of *E. coli* or *S. aureus*. Moreover, live *M. bovis* may induce suppression of the immune response in bMECs.

Keywords: *Mycoplasma bovis*, bovine mammary epithelial cells, cytokines, innate immunity, microarray analysis

■ 論文題目

Mycoplasma bovis に対するウシ乳腺上皮細胞の自然免疫応答

■ 要 旨

マイコプラズマ性乳房炎は酪農場において甚大な経済的損失をもたらす疾患である。ウシ乳腺上皮細胞は病原体に対する免疫応答に重要な役割を担っている。*Mycoplasma bovis* に対するウシ乳腺上皮細胞の免疫応答を明らかにするため、*M. bovis* 刺激における免疫応答に関連するmRNA発現量を定量した。また、*M. bovis* の比較対象として *Staphylococcus aureus* および *Escherichia coli* がウシ乳腺上皮細胞に及ぼす影響を検討した。*M. bovis* の生菌刺激に対するウシ乳腺上皮細胞の網羅的遺伝子発現解析結果から、ヒストン脱メチル化により免疫応答を活性化する lysine-specific demethylase 4D の発現量が無刺激対照と比較して有意に低下した。*M. bovis* の死菌刺激によるウシ乳腺上皮細胞における IL-1 β 、IL-6、TNF α 、TLR 2、TLR 4 の mRNA 発現量は有意に増加したが、*S. aureus* および *E. coli* による刺激と比較してそれらの発現量は低値であった。以上の結果から *M. bovis* は *S. aureus* および *E. coli* と比較して弱い免疫応答性を示し、*M. bovis* の生菌はウシ乳腺上皮細胞の免疫応答を抑制することが考えられた。

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Prevalence and risk factors of *Mycoplasma bovis* infection in dairy farms in northern Japan

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ABSTRACT

Mycoplasma mastitis is a dairy herd health problem with growing concern in Japan. To complement the lack of epidemiological knowledge of the disease, we conducted estimation of herd-level prevalence and risk factor analysis for *Mycoplasma bovis* (*M. bovis*) mastitis using data collected from Tokachi region, one of the nation's largest milk producing area, in Hokkaido Prefecture in 2015. The herd-level prevalence was estimated at 3.8% (95% confidence interval (CI): 2.6%, 5.4%) across the region with municipality-specific prevalence ranging from 0% to 14.3%.

In this study, identified risk factors for the presence of with *M. bovis* infection on farms were corporation-type farms and purchased cattle, after controlling for the herd size. Corporation-type farms may reflect higher frequencies of moving cows and fomites to and from other farms, which increases the risk of pathogen introduction. Purchased cattle was considered as one of the major pathways of the disease incursion, and this finding highlighted the importance of more stringent separation or quarantine protocols when introducing cattle from outside in Tokachi region. Due to the limited information available in this study and inherent nature of the study designs, these results should be interpreted with caution and further research is needed.

■ 論文題目

北日本の酪農場における *Mycoplasma bovis* 感染症の分布とリスクファクター

■ 要 旨

2015年に北海道で実施した *Mycoplasma bovis* 感染症の分布状況調査により、北海道における本病の想定浸潤率は3.8% (0–14.3%)と試算された。また、法人経営農場では市場導入等によるウシの移動が危険因子として位置づけられたことから、新規導入牛の一時的な別離飼育等の必要が示唆された。

■ 最終責任者 Hidetoshi Higuchi (Corresponding Author)



Identification of a novel mechanism of action of bovine IgG antibodies specific for *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a major pathogen that causes subclinical mastitis associated with huge economic losses to the dairy industry. A few vaccines for bovine mastitis are available, and they are expected to induce the production of *S. aureus*-specific antibodies that prevent bacterial adherence to host cells or promote opsonization by phagocytes. However, the efficacy of such vaccines are still under debate; therefore, further research focusing on improving the current vaccines by seeking additional mechanisms of action is required to reduce economic losses due to mastitis in the dairy industry. Here, we generated *S. aureus*-specific bovine IgG antibodies (anti-*S. aureus*) that directly inhibited bacterial growth in vitro. Inhibition depended on specificity for anti-*S. aureus*, not the interaction between Protein A and the fragment crystallizable region of the IgG antibodies or bacterial agglutination. An in vitro culture study using *S. aureus* strain JE2 and its deletion mutant JE2ΔSrtA, which lacks the gene encoding sortase A, revealed that the effect of anti-*S. aureus* was sortase-A-independent. Sortase A is involved in the synthesis of cell-wall-associated proteins. Thus, other surface molecules, such as membrane proteins, cell surface polysaccharides, or both, may trigger the inhibition of bacterial growth by anti-*S. aureus*. Together, our findings contribute insights into developing new strategies to further improve the available mastitis vaccine by designing a novel antigen on the surface of *S. aureus* to induce inhibitory signals that prevent bacterial growth.

■ 論文題目

S. aureus 特異的ウシ IgG 機能に関する新規メカニズムの同定

■ 要 旨

S. aureus は潜在性乳房炎の主要な病原体であり、本病による経済損失は甚大である。これまでの *S. aureus* ワクチンは *S. aureus* に特異的な抗体を誘導し、病原体への付着やオプソニン化によりその効果を発現すると考えられてきた。しかし、より効果的なワクチン開発にはさらなる詳細な解明が必要である。本研究では sortaseA（膜蛋白質）の遺伝子欠損株を用いて解析を行ったところ、*S. aureus* 特異的抗体の効果に、本蛋白質は関与しないことが示された。このことから、他の膜蛋白質や膜多糖体が関与している可能性が示唆された。

■ 最終責任者 Tomonori Nochi (Corresponding Author)



Article

Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent *Staphylococcus aureus* Bacteriophage

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Abstract: Antibiotic-resistant bacteria (ARB) have spread widely and rapidly, with their increased occurrence corresponding with the increased use of antibiotics. Infections caused by *Staphylococcus aureus* have a considerable negative impact on human and livestock health. Bacteriophages and their peptidoglycan hydrolytic enzymes (endolysins) have received significant attention as novel approaches against ARB, including *S. aureus*. In the present study, we purified an endolysin, Lys-phiSA012, which harbors a cysteine/histidine-dependent amidohydrolase/peptidase (CHAP) domain, an amidase domain, and a SH3b cell wall binding domain, derived from a polyvalent *S. aureus* bacteriophage which we reported previously. We demonstrate that Lys-phiSA012 exhibits high lytic activity towards staphylococcal strains, including methicillin-resistant *S. aureus* (MRSA). Analysis of deletion mutants showed that only mutants possessing the CHAP and SH3b domains could lyse *S. aureus*, indicating that lytic activity of the CHAP domain depended on the SH3b domain. The presence of at least 1 mM Ca²⁺ and 100 μM Zn²⁺ enhanced the lytic activity of Lys-phiSA012 in a turbidity reduction assay. Furthermore, a minimum inhibitory concentration (MIC) assay showed that the addition of Lys-phiSA012 decreased the MIC of oxacillin. Our results suggest that endolysins are a promising approach for replacing current antimicrobial agents and may contribute to the proper use of antibiotics, leading to the reduction of ARB.

Keywords: antibiotic resistant; multidrug resistant; antimicrobial agent; phage therapy; bacteriophage; endolysin; staphylococci; *Staphylococcus aureus*

1. Introduction

Antibiotic-resistant bacteria (ARB) have spread rapidly worldwide and are an important global health issue. ARB often result from the inappropriate use of antibiotics [1–3] and the occurrence of ARB is correlated with the amount of antibiotics used [4]. In the United States, about half of antibiotics are used to treat humans annually and the other half to treat livestock and for other agricultural applications [5]. Notably, antibiotics are administered to livestock for not only preventive and therapeutic approaches

■ 論文題目

多価黄色ブドウ球菌ファージ由来 LysK 様エンドライシン (Lys-phiSA012) の機能解析

■ 要 旨

抗生物質の使用の増加に伴い、薬剤耐性菌は世界的に急速な広がりを見せている。特に、黄色ブドウ球菌 (*Staphylococcus aureus*: SA) は、ヒトおよび家畜の健康において甚大な被害をもたらしている。また、バクテリオファージ、およびバクテリオファージ由来溶菌酵素 (エンドライシン) は、SAを含む薬剤耐性菌に対する新たなアプローチとして大きな注目を集めている。そこで本研究では、我々がこれまでに報告した多価バクテリオファージからエンドライシン (Lys-phiSA012) を単離・精製し、その溶菌機能について解析を実施した。Lys-phiSA012は、MRSAを含む複数のSAに対して高い溶菌活性を示した。また、Lys-phiSA012が有する2つの触媒ドメイン (CHAP, Amidase ドメイン)、1つの菌体認識ドメイン (SH3b ドメイン) の欠失変異体を構築し、溶菌活性を評価した結果、SH3b ドメインに依存的なCHAPドメインによる溶菌活性が示唆された。また、1 mMのカルシウムイオンおよび100 μMの亜鉛イオンの添加は、Lys-phiSA012の溶菌活性を亢進させた。さらに、Lys-phiSA012の併用は、オキサシリンのMICを減少させた。以上の結果から、エンドライシンは薬剤耐性菌の抑制に貢献する有用なアプローチであることが示唆された。

■ 最終責任者 Hidetomo Iwano (Corresponding Author)

Bacteriophage Φ SA012 Has a Broad Host Range against *Staphylococcus aureus* and Effective Lytic Capacity in a Mouse Mastitis Model

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Abstract: Bovine mastitis is an inflammation of the mammary gland caused by bacterial infection in dairy cattle. It is the most costly disease in the dairy industry because of the high use of antibiotics. *Staphylococcus aureus* is one of the major causative agents of bovine mastitis and antimicrobial resistance. Therefore, new strategies to control bacterial infection are required in the dairy industry. One potential strategy is bacteriophage (phage) therapy. In the present study, we examined the host range of previously isolated *S. aureus* phages Φ SA012 and Φ SA039 against *S. aureus* strains isolated from mastitic cows. These phages could kill all *S. aureus* (93 strains from 40 genotypes) and methicillin-resistant *S. aureus* (six strains from six genotypes) strains tested. Using a mouse mastitis model, we demonstrated that Φ SA012 reduced proliferation of *S. aureus* and inflammation in the mammary gland. Furthermore, intravenous or intraperitoneal phage administration reduced proliferation of *S. aureus* in the mammary glands. These results suggest that broad host range phages Φ SA012 is potential antibacterial agents for dairy production medicine.

Keywords: bacteriophage; mastitis; *Staphylococcus aureus*

論文題目

バクテリオファージ Φ SA012 は黄色ブドウ球菌に対し広範な溶菌スペクトルを有し、マウス乳房炎モデルにおいて効果的な溶菌活性を示す

要 旨

ウシ乳房炎は細菌感染に起因する乳腺の炎症であり、その治療のために抗生物質を連用することからも酪農産業において最も大きな経済的損失である。また、黄色ブドウ球菌 (*Staphylococcus aureus*: SA) はウシ乳房炎の主な原因菌であり、薬剤耐性化も問題となっている。この様に、細菌感染症を制御する新たな抗菌戦略が求められる中、ファージセラピーはその一つとして注目されている。本研究では、我々がこれまでに分離したバクテリオファージ (Φ SA012 および Φ SA039) の乳房炎由来 SA に対する宿主域を精査した。これらのバクテリオファージは、40 パタンの遺伝子型に分類される 93 株の SA を溶菌し、さらに 6 パタンの遺伝子型に分類される 6 株の MRSA も溶菌した。さらに、マウス乳房炎モデルにおいて、 Φ SA012 の投与は、SA の増殖と乳腺における炎症を抑制した。これらの結果は、広範な宿主域を示す Φ SA012 が酪農産業における有用な抗菌性物質となる可能性を示唆している。

■ 最終責任者 Hidetomo Iwano (First Author and Corresponding Author)



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Generation and validation of novel anti-bovine CD163 monoclonal antibodies ABM-1A9 and ABM-2D6

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ELISA

ABSTRACT

The scavenger receptor CD163 is widely used as a cell signature for alternatively active “M2” macrophages in mammals. In this study, we generated two monoclonal antibodies, ABM-1A9 and ABM-2D6, against the extracellular region of bovine CD163. Conventional Western blotting using the antibodies yielded immunoreactive bands of bovine CD163 at 130 and 150 kDa in non-reduced and reduced spleen lysates, respectively. The minimum limit of detectable concentration of both antibodies was relatively lower (5.0 ng/mL) than that of the anti-human CD163 monoclonal antibody AM-3K (> 1.0 µg/mL), which has been used previously for the detection of bovine CD163. An immunohistochemical study using formalin-fixed paraffin-embedded sections revealed that ABM-1A9 and ABM-2D6 clearly stained some Iba1⁺ macrophages in the lymph nodes of cattle with mastitis. Moreover, the CD163-stained macrophages were frequently observed engulfing leukocytes. ELISA using ABM-2D6 distinguished levels of circulating soluble CD163 in healthy cattle (less than 16.9 pmol/mL) and cattle with mastitis (more than 33.7 pmol/mL). These new monoclonal antibodies can be used in the diagnosis and evaluation of inflammatory disease prognosis in cattle with immunohistological analyses and blood test applications.

■ 論文題目

新規の抗牛CD163モノクローナル抗体ABM-1A9およびABM-2D6の作製と検証

■ 要 旨

スカベンジャー受容体CD163は、哺乳動物における選択的に活性な「M2」マクロファージに対するセルシグナチャーとして広く使用されている。本研究では、ウシCD163の細胞外領域に対する2つのモノクローナル抗体、ABM-1A9とABM-2D6を作製した。抗体を用いたウェスタンブロッティングは、非還元および還元それぞれの脾臓ライセート中に130および150kDaのウシCD163免疫反応性バンドを生じた。両抗体の検出可能な最小限界濃度は、ウシのCD163検出のために以前に使用されていた抗ヒトCD163モノクローナル抗体AM-3Kの濃度 (> 1.0 µg/mL) よりも相対的に低かった (5.0ng/mL)。ホルマリン固定パラフィン包埋切片を用いた免疫組織化学的研究により、ABM-1A9とABM-2D6は、乳房炎の牛のリンパ節にあるいくつかのIba1 +マクロファージを明確に染色することが判明し、さらにCD163に染色されたマクロファージは白血球の取り込みが頻繁に観察された。ABM-2D6を用いたELISAは、健康な牛 (16.9pmol / mL未満) と乳房炎の牛 (33.7pmol / mL以上) の循環血液中の可溶性CD163のレベルを識別した。これらの新しいモノクローナル抗体は、免疫組織学的分析および血液検査において、ウシの炎症性疾患の予後の診断および評価に使用することができる。

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Article

Simulating Cross-Contamination of Cooked Pork with *Salmonella enterica* from Raw Pork through Home Kitchen Preparation in Vietnam

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Abstract: Pork is the most commonly consumed meat in Vietnam, and *Salmonella enterica* is a common contaminant. This study aimed to assess potential *S. enterica* cross-contamination between raw and cooked pork in Vietnamese households. Different scenarios for cross-contamination were constructed based on a household survey of pork handling practices (416 households). Overall, 71% of people used the same knife and cutting board for both raw and cooked pork; however, all washed their hands and utensils between handling raw and cooked pork. The different scenarios were experimentally tested. First, *S. enterica* was inoculated on raw pork and surfaces (hands, knives and cutting boards); next, water used for washing and pork were sampled to identify the presence and concentration of *S. enterica* during different scenarios of food preparation. Bootstrapping techniques were applied to simulate transfer rates of *S. enterica* cross-contamination. No cross-contamination to cooked pork was observed in the scenario of using the same hands with new cutting boards and knives. The probability of re-contamination in the scenarios involving re-using the cutting board after washing was significantly higher compared to the scenarios which used a new cutting board. Stochastic simulation found a high risk of cross-contamination from raw to cooked pork when the same hands, knives and cutting boards were used for handling raw and cooked pork (78%); when the same cutting board but a different knife was used, cross-contamination was still high (67%). Cross-contamination between was not seen when different cutting boards and knives were used for cutting raw and cooked pork. This study provided an insight into cross-contamination of *S. enterica*, given common food handling practices in Vietnamese households and can be used for risk assessment of pork consumption.

Keywords: cross-contamination; pork; *Salmonella enterica*; simulation; Vietnam

■ 論文題目

ベトナムの家庭キッチンでの生豚肉から調理済豚肉への交差汚染シミュレーション

■ 要 旨

ベトナムの一般家庭416戸への調査と、豚肉調理におけるサルモネラ交差汚染実験から、まな板と包丁の洗浄、使い分けの効果についてコンピュータシミュレーションを実施した。結果から洗浄しても同じまな板を使った場合交差汚染が容易に起こることが明らかとなった。

■ 最終責任者 Kohei Makita (Corresponding Author)

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Estimation of circulating bovine leukemia virus levels using conventional blood cell counts

S. Nakada, J. Kohara, K. Makita 

✱ PlumX Metrics

DOI: <https://doi.org/10.3168/jds.2018-14609>

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Abstract Full Text Images References

ABSTRACT

Bovine leukemia virus (BLV) is widely prevalent in Japanese dairy farms. To control BLV infections in Japan, segregating or managing cows according to their proviral load (PVL) is a rational strategy. This study was conducted to establish a quantitative procedure for estimating blood PVL per microliter using a statistical model to offer a cost-effective alternative to the conventional quantitative real-time PCR method. In total, 250 Holstein cows infected with BLV were identified from 10 commercial dairy farms. Information on age was collected and blood samples were tested for white blood cell and lymphocyte counts and PVL using PCR. Generalized linear models with quasi-Poisson errors were used to estimate PVL by selecting age, logarithm of lymphocyte count, and their interaction term as explanatory variables. To evaluate the model, blood samples of 92 BLV-infected Holstein cows from 2 other commercial dairy farms were tested, and measured PVL values were compared with estimated PVL values by the model. The logPVL per microliter was modeled by positive associations with log lymphocyte count and age and a negative association with the interaction term. In the evaluation, measured PVL values had a strong correlation with estimated PVL values (Spearman's $\rho = 0.87$). In conclusion, our model provides a cost-effective and more rapid alternative to the conventional method to facilitate test and segregation or management of BLV-suspected cows.

Key words:

[bovine leukemia virus](#), [proviral load](#), [statistical model](#), [dairy cow](#)

■ 論文題目

従来の血球数を用いた血中牛白血病ウイルスレベルの推定

■ 要 旨

乳用種牛の年齢と白血球数カウントを用いて血中の牛白血病ウイルス・プロウイルス量を推定する計算式を求めた。本方法は、定量的PCR検査に供する高リスク牛の安価な選抜に有用な手段となりうる。

■ 最終責任者 Kohei Makita (Corresponding Author)



Herd-level risk factors associated with *Brucella* sero-positivity in cattle, and perception and behaviours on the disease control among agro-pastoralists in Tanzania

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 Brucellosis
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 Item count technique
 Tanzania
 Willingness to pay for vaccination

ABSTRACT

Brucellosis is endemic in Tanzania, particularly in agro-pastoral areas. This study investigated the herd-level sero-prevalence and risk factors for *Brucella* sero-positivity in cattle, and perception and behaviours associated with brucellosis control among agro-pastoralists in Morogoro Region, Tanzania. A cross-sectional study involving herd milk diagnosis by indirect enzyme-linked immunosorbent assay and questionnaire survey was conducted in 124 farms. Questions included potential risk factors, knowledge of brucellosis, willingness-to-pay for cattle vaccination, and item count technique (ICT) for selling behaviour of cows that experienced abortion. Risk factor analysis for *Brucella* sero-positivity in cattle and analysis of factors associated with willingness-to-pay were conducted using classical tests and generalised linear models. Most farmers had little knowledge about brucellosis (disease name: 13.7%, symptoms: 3.2%, transmission from cattle to human: 2.4%, and *Brucella* vaccine: 2.4%). The proportion of *Brucella* sero-positive herd was 44.4% (55/124, 95%CI: 35.5–53.5). No risk factors for *Brucella* sero-positivity were identified; however, using a veterinary service was identified as a preventive factor (OR = 0.39, 95%CI: 0.18–0.84, $p = 0.02$). For scenarios of vaccinating all cattle and only calves, 59.7% and 89.5% of farmers were willing to pay for vaccination, respectively. Being a Maasai tribe member was a hesitating factor for vaccinating all cattle (OR = 0.39, 95%CI: 0.19–0.83, $p = 0.01$) and using a veterinary service was an encouraging factor for vaccinating calves (OR = 4.0, 95%CI: 1.2–13.0, $p = 0.02$). The ICT found that 45.1% of farmers sold cows that experienced abortion. This estimate was not statistically different from that obtained by direct questioning (34.1%, SE = 7.5%, binomial p value = 0.27, factor score = 1.32), suggesting that farmers did not hesitate to sell such cows. The Maasai conducted more risky behaviours for human infection such as drinking raw milk ($p = 0.06$) or blood ($p < 0.01$) and helping delivery with bare hands ($p = 0.03$) than other tribes. Community-based brucellosis control programmes with calf vaccination may be feasible in the study areas. A One Health approach including the promotion of health education and expansion of veterinary services is crucial for disease control.

■ 論文題目

タンザニア国農業放牧混合農家における農場レベル牛ブルセラ病血清陽性リスク因子と疾病制御に関する考えと行動

■ 要 旨

モロゴロ州の農業放牧混合地域の牛農場の44.4%はブルセラ病陽性であった。獣医サービスの利用が農場レベル感染の防除因子であった。牛へのワクチン接種は、子牛のみへのワクチン接種では89.5%が支払い可能と回答し、コミュニティ主体のワクチン接種による疾病制御の実現可能性が示唆された。

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Original Contribution

Brucellosis Risk in Urban and Agro-pastoral Areas in Tanzania

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Abstract: Epidemiology of human and animal brucellosis may depend on ecological conditions. A cross-sectional study was conducted to compare prevalence and risk factors of bovine brucellosis, and risky behaviours for the human infection between urban and agro-pastoral areas in Morogoro region, Tanzania. Cattle blood sampling and interviews using a structured questionnaire were conducted with farmers. Rose-Bengal test was conducted for the cattle sera, and positive samples were confirmed with competitive ELISA. Farm-level sero-prevalences were 0.9% (1/106, 95% CI 0.0–5.9%) and 52.9% (9/17, 95% CI 28.5–76.1%) in urban and agro-pastoral areas, respectively. The animal-level-adjusted prevalences were 0.2% (1/667, 95% CI 0.0–1.1%) and 7.0% (28/673, 95% CI 5.7–8.4%) in those areas. The final farm-level model including both areas found two risk factors: history of abortion in the herd ($P < 0.01$) and cattle grazing ($P = 0.07$). The animal-level risk factors in agro-pastoral areas were age ($P = 0.04$) and history of abortion ($P = 0.03$). No agro-pastoral farmer knew about *Brucella* vaccine. Agro-pastoralists generally had poorer knowledge on brucellosis and practiced significantly more risky behaviours for human brucellosis such as drinking raw milk (17.6%, $P < 0.01$) and blood (35.3%, $P < 0.01$), and helping cattle birth (100%, $P = 0.04$) than urban farmers (0, 0 and 79.2%, respectively). Intervention programs through education including both human and animal health particularly targeting agro-pastoralists would be needed.

Keywords: Brucellosis, Bovine, Risk factor, Urban, Agro-pastoral, Tanzania

■ 論文題目

タンザニアの都市部および農業放牧混合地域におけるブルセラ病リスク

■ 要 旨

タンザニア国モロゴロ州では、農場レベルのブルセラ病有病率は都市部で 0.9%、農業放牧混合地域で 52.9%と大きく異なり、リスク因子は牛群内の過去の流産と放牧であった。動物レベルでは高齢な牛、流産経験牛で有病率が高かった。生産者のブルセラ病の知識は低く、生の牛乳や牛血液を飲むなど高い感染リスクを示唆する行動が目立った。

■ 最終責任者 Kohei Makita (Corresponding Author)



Socio-economic factors associated with voluntary rabies control measures in Vietnam

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ARTICLE INFO

Keywords:
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ABSTRACT

Rabies is a fatal zoonosis, and in Vietnam, it remains problematic despite the availability of dog rabies vaccination. The purpose of this study was to clarify the socio-economic factors associated with voluntary rabies control measures among the general population using a “Knowledge, Attitudes, and Practice” framework to provide health and veterinary authorities in Vietnam with baseline information for better planning of policy supports.

A questionnaire survey with interviews was conducted in 495 households (64 mountainous and 431 plain-area households) in Thai Nguyen Province in September 2016. After the survey, uni- and multivariable analyses were performed to detect factors associated with the practices of dog rabies vaccination and tethering dogs. Structural equation modelling (SEM) was performed to understand the structures associated with practice decisions. Contingent valuation was performed to calculate willingness-to-pay for vaccination.

Vaccination coverage was 77.4% (724/935 dogs), and was significantly lower in mountainous areas dominated by ethnic minorities (63.8%, 67/105) than in plains (79.2%, 657/830, $\chi^2 = 11.7$, $df = 1$, $p < 0.001$). Mean estimation of willingness-to-pay for a vaccination was 2.30 USD (51,959 Vietnamese dong), which was more than double the current price. The willingness-to-pay in mountainous areas was 2.16 USD, while that in plain areas was 2.32 USD. The proportion that never confined dogs was significantly higher in mountainous areas (65.6%, 42/64 households) than in plain areas (26.5%, 114/430, $\chi^2 = 37.7$, $df = 1$, $p < 0.001$). Despite the low proportion of households tethering dogs, the majority answered that they would confine (479/489, 98.0%) or leash while walking (482/491, 98.2%) if such orders were enforced. SEM result showed that higher social status promoted better knowledge (β , the coefficient, = 0.75, $se = 0.05$, $p < 0.001$), better knowledge positive attitudes ($\beta = 1.0$, $se = 0.0$, p value not calculated), and positive attitudes better practices of confinement ($\beta = 0.4$, $se = 0.05$, $p < 0.001$), vaccination ($\beta = 0.52$, $se = 0.06$, $p < 0.001$), and sterilization ($\beta = 0.11$, $se = 0.04$, $p < 0.001$).

Our study suggested that rabies education targeted to mountainous areas using local languages for ethnic minority groups as well as the national language, and higher subsidies on dog rabies vaccination for poor households may improve vaccination coverage. Dog management may be improved by promotion campaigns.

■ 論文題目

ベトナムにおける自主的狂犬病対策実施に関わる社会経済学的因子

■ 要 旨

ベトナム国タイ・ングイエン省にて495戸の家計で質問票調査を実施した。狂犬病ワクチン接種率は77.4%で、少数民族が暮らす山岳地帯で有意に接種率が低かった。調査家計全体では狂犬病ワクチン支払い意欲は2.3ドルと現行の値段の二倍であった。構造化推定方程式により、暮らしぶりは狂犬病の知識量に影響し、知識は狂犬病に対する意識に、高い意識が自主的対策の実施に繋がっていた。

■ 最終責任者 Kohei Makita (Corresponding Author)

The risk of rabies spread in Japan: a mathematical modelling assessment

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Original Paper

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Mathematical modelling; rabies (animal); veterinary epidemiology

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Abstract

Rabies was eliminated from Japan in 1957. In the 60 years since elimination, vaccination coverage has declined and dog ownership habits have changed. The purpose of this study was to assess the current risk of rabies spread in Japan. A spatially explicit transmission model was developed at the 1 km² grid scale for Hokkaido and Ibaraki Prefectures. Parameters associated with dog movement and bite injuries were estimated using historical records from Japan, and were used with previously published epidemiological parameters. The final epidemic size, efficacy of rabies contingency plans and the influence of dog owner responses to incursions were assessed by the model. Average outbreak sizes for dog rabies were 3.1 and 4.7 dogs in Hokkaido and Ibaraki Prefectures, respectively. Average number of bite injury cases were 4.4 and 6.7 persons in Hokkaido and Ibaraki Prefectures, respectively. Discontinuation of mandatory vaccination increased outbreak sizes in these prefectures. Sensitivity analyses showed that higher chance of unintentional release of rabid dogs by their owners (from 0.5 to 0.9 probability) increased outbreak size twofolds. Our model outputs suggested that at present, incursions of rabies into Japan are very unlikely to cause large outbreaks. Critically, the reaction of dog owners to their dogs developing rabies considerably impacts the course of outbreaks. Contingency measures should therefore include sensitisation of dog owners.

■ 論文題目

日本における狂犬病拡散リスク:数理モデルによる評価

■ 要 旨

コンピュータシミュレーションを用いて、北海道と茨城県をモデルに、狂犬病感染犬が侵入した場合の拡散様式を再現した。現在のワクチン接種状況で、北海道では平均 3.1 頭、茨城県では 4.7 頭の犬が狂犬病に感染・発症した。発症犬による人の咬傷数はそれぞれの道県で平均 4.4 人と 6.7 人であった。最も感度の高い因子は飼い主が発症犬を制御できず犬が逃げってしまうことであった。

■ 最終責任者 Kohei Makita (Corresponding Author)



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The impacts of aflatoxin standards on health and nutrition in sub-Saharan Africa: The case of Kenya

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ARTICLE INFO

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Food safety
Food security
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ABSTRACT

Human food and animal feed can contain many different hazards, which may be biological, chemical, or physical. In most countries, there are regulations that limit the levels of these hazards permitted in food and feed so as to protect consumers. Optimally, the levels specified in the standards should make the food safe enough for everyone to consume, and often this is done by carrying out a risk assessment, based on scientific evidence of the levels that can be considered safe and the amount of contaminated products consumed. However, for some substances, especially carcinogens, it is difficult to calculate how much is safe to consume and some groups of people, such as small children or pregnant women, may be more sensitive than the population at large. While imposition of standards is motivated by health benefits, standards also have costs. These include the costs of compliance and verification, which translate into increased costs of purchase and reduction of the products available.

In this paper we summarize current standards in sub-Saharan Africa related to aflatoxins, a priority hazard, and discuss their coherence and evidence-base. Next, using our recent research findings, we estimate the health risks of consuming foods contaminated with aflatoxins in Kenya. We also estimate the negative health and economic effects that would arise from strict application of different standards for aflatoxins. We discuss the results in light of health and nutrition goals.

■ 論文題目

サハラ以南アフリカの健康と栄養へのアフラトキシン・スタンダードのインパクト: ケニヤの場合

■ 要 旨

アフラトキシンの摂取によるリスクは、小児や妊娠女性など特定の人口での高感受性の可能性や長期摂取による腫瘍発生のリスクなど定量的評価にはいまだよく知られていない困難な課題である。本研究では、ケニヤでの研究成果から定量的リスク評価を実施し、健康と栄養、そして経済の面から論じた。

■ 最終責任者 Johanna Lindahl (Corresponding Author)



Knowledge, attitudes, and practices (KAP) related to brucellosis and factors affecting knowledge sharing on animal diseases: a cross-sectional survey in the dry zone of Sri Lanka

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Abstract

Farmers' lack of knowledge is assumed to have affected the presence of brucellosis in Sri Lanka for decades. This study, carried out in the Ampara district in the dry zone of Sri Lanka, revealed that there is a significant knowledge gap for brucellosis compared to foot and mouth disease (FMD) ($p < 0.001$). Only 8.3% of farmers knew that brucellosis causes cattle abortions. Only 2.6% knew that it is zoonotic. The difference in knowledge of the symptoms and transmission of brucellosis and FMD was significant ($p < 0.001$). Farmers' attitudes and practices related to the spread of the disease were poor. Farmers' education and spoken language had a negative influence on knowledge. Young people and those with strong social relationships were efficient in knowledge sharing. It can be concluded that brucellosis knowledge, attitudes, and practices are poor; thus, there is a need for more attention in disease control policymaking. Backward farmer groups should be the focus in animal health extension programs.

Keywords Brucellosis knowledge · Social factors · Health extension

■ 論文題目

ブルセラ病に関連した知識・態度・実践と家畜疾病の知識共有に影響する因子:スリランカの乾燥地帯における横断研究

■ 要 旨

スリランカ国アンパラ県におけるブルセラ病の知識は口蹄疫に比べて有意に低く、ブルセラ病が流産を起こすこと、また人獣共通感染症であることを知っている酪農家の割合はそれぞれ 8.3%と 2.6%と低かった。若い人、社会的に影響力のある人は知識共有に長けていた。家畜衛生施策には小規模農家をターゲットにする必要が示唆された。

■ 最終責任者 Hiroichi Kono (Corresponding Author)



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Exponential random graph models to evaluate the movement of backyard chickens after the avian influenza crisis in 2004–2005, Thailand

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ABSTRACT

The aim of this study was to use exponential random graph models (ERGMs) to explain networks of movement of backyard chickens in provinces which had been hotspots for avian influenza outbreaks in Thailand during 2004–2005. We used structured questionnaires to collect data for the period January to December 2009 from participants who were involved in the backyard chicken farming network in three avian influenza hotspots (Ratchaburi, Suphan Buri, and Nakhon Pathom provinces) in Thailand. From 557 questionnaires, we identified nodes, points of entry and exit from nodes, and activities relating to backyard chicken farming and movement of chickens, and generated ERGMs based on non-festive periods (Model 1) and the Chinese New Year period (Model 2). In Model 1, *k*-star (the central node is connected to *k* other nodes) connections were predominant ($P < 0.001$). In Model 2, the frequency of movement increased by 10.62 times, *k*-star connections were still predominant ($P < 0.001$), and the model was scale-free. Hubs were formed from owners/observers in the arenas/training fields, farmers who raised chickens for consumption only, and traders. In conclusion, our models indicated that, if avian influenza was introduced during non-festive periods, the authorities would need to regularly restrict the movement of chickens. However, during high-frequency periods of movement of backyard chickens, authorities would also need to focus on the network hubs. Our research can be used by the relevant authorities to improve control measures and reduce the risk or lessen the magnitude of disease spread during an avian influenza epidemic.

■ 論文題目

指数無作為グラフモデルを用いた 2004–2005 年タイの鳥インフルエンザ危機後の裏庭養鶏の移動の評価

■ 要 旨

2009 年 1 月から 12 月に掛けて、2004–2005 年鳥インフルエンザ危機におけるホットスポットであったタイ国 3 県 557 鶏生産者への質問票調査の結果を用いて、指数無作為グラフモデルを用いてアウトブレイクリスクを評価した。旧正月以外の通常の状態では鶏の移動制限で効果的に鳥インフルエンザアウトブレイクを制御できた。一方旧正月には、鶏の移動制限に加えて、ネットワークハブのコントロールが必要であることが明らかとなった。

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II. その他 <Others>

1) Survey of tick-borne zoonotic viruses in wild deer in Hokkaido, Japan.

Uchida L, Hayasaka D, Ngwe Tun MM, Morita K, **Muramatsu Y**,
Hagiwara K.

J. Vet. Med. Sci. 80: 985-988. 2018.



NOTE

Public Health

Survey of tick-borne zoonotic viruses in wild deer in Hokkaido, Japan

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ABSTRACT. Tick-borne encephalitis (TBE) and severe fever with thrombocytopenia syndrome (SFTS) are both tick-borne zoonotic diseases caused by TBE virus (TBEV) and SFTS phlebovirus (SFTSV). In 2016, a second domestic TBE case was reported in Hokkaido, Japan, after an absence of 23 years. We conducted IgG ELISA for TBEV and SFTSV on 314 deer (*Cervus nippon yesoensis*) serum samples collected from 3 places in Hokkaido. There were 7 seropositive samples for TBEV but none for SFTSV by ELISA. The specificity of the 7 positive samples was confirmed by neutralization tests against TBEV, and 5 sera showed 320 to 640 of 50% focus reduction endpoint titers. Our results provide information about the infectious status of TBEV in wild deer in Hokkaido, Japan.

KEY WORDS: serosurvey, severe fever with thrombocytopenia syndrome virus, tick-borne encephalitis virus, wild deer, zoonotic disease

■ 論文題目

北海道の野生ジカにおけるダニ媒介性人獣共通感染症ウイルスの調査

■ 要 旨

ダニ媒介脳炎 (TBE) と重症熱性血小板減少症候群 (SFTS) は、それぞれTBEウイルス (TBEV)、SFTSウイルス (SFTSV) によってダニ媒介性人獣共通感染症を引き起こす。2016年には23年ぶりにTBEの国内2番目の症例が北海道で報告された。我々は北海道の3地域から集めたエゾシカ血清314検体を用いて、ELISAによるTBEVおよびSFTSVに対するIgG抗体測定を行った。7検体がTBEV抗体陽性で、SFTSVに対する抗体陽性検体は認められなかった。7検体の抗体特異性は中和試験により確認し、うち5検体は320から640倍の抗体価を示した。我々の調査結果は、北海道における野生ジカの、TBEV感染状況に関する情報を提供するものである。

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- 1) Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs.

Oyama N., Sano T, Yamamori M, Tamura J, Umar MA, Endo Y, Ishikawa Y, Itoh A, Miyoshi K, Yamashita K.

J. Vet. Med. Sci. 80: 1450-1455. 2018.

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J. Vet. Med. Sci. 80:361-367. 2018.

II. その他<Others>

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Ochiai Y, Baba A, Hiramatsu M, Toyota N, Watanabe T, Yamashita K. Yokota H, Iwano H.

J. Vet. Med. Sci. 80:387-394. 2018.



Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs

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■ 論文題目

犬に皮下投与したロベナコキシブの分布に対する機械的調節呼吸と静脈内輸液を実施したセボフルラン麻酔の影響

■ 要 旨

健康なビーグル犬 10 頭 (1-11 歳, 体重 9.3-14.3kg, 雄 6 頭, 雌 4 頭) に覚醒時または調節呼吸と静脈内輸液を実施したセボフルラン全身麻酔下でロベナコキシブ 2mg/kg を皮下投与し、血漿ロベナコキシブ濃度の推移を観察した。覚醒時にロベナコキシブを投与した犬における最大血漿ロベナコキシブ濃度 ($C_{\max}$) は $2.2 \mu\text{g/mL}$ [1.2-4.6] (中央値[最小-最大]) であり、投与後の $C_{\max}$ 到達時間 ($T_{\max}$) は 90 分 [60-120] であった。これに対して、セボフルラン麻酔下の犬では $C_{\max}$ は有意に低下し ($1.3 \mu\text{g/mL}$ [0.8-1.4], $P=0.008$)、 $T_{\max}$ は有意に延長した (120 分 [120-240], $P=0.018$)。調節呼吸と静脈内輸液を実施したセボフルラン麻酔は、犬におけるロベナコキシブの薬物動態に影響を及ぼした。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



FULL PAPER

Surgery

Clinical outcome of canine cardiopulmonary resuscitation following the RECOVER clinical guidelines at a Japanese nighttime animal hospital

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■ 論文題目

札幌夜間動物病院においてコンセンサスを得た犬猫の心肺蘇生ガイドライン(RECOVERガイドライン)を臨床例に適応した際の治療成績

■ 要 旨

2012年に公表された犬猫のコンセンサスを得た心肺蘇生(CPR)ガイドライン(RECOVERガイドライン)を臨床例に適応し、その治療成績を検討した。2012年1月～2015年12月までに札幌夜間動物病院に来院し、CPRを実施した犬68頭に従来のCPR(TRADITIONAL群)、73頭にRCOVERガイドラインに従ったCPR(RECOVER群)を実施した。心拍動の再開(ROSC)はTRADITIONAL群で12頭(17%)およびRECOVER群32頭(48%)で得られ、ROSC達成率はRECOVER群で有意に高かった($P<0.001$)。しかしながら、生存退院はTRADITIONAL群0頭(0%)およびRECOVER群4頭(5%)であり、依然として低かった。RECOVERガイドラインは犬のCPRにおけるROSC達成率を劇的に改善したが、治療成績の向上には、ROSC達成後の集中治療を進歩させる必要がある。

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Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves

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■ 論文題目

子牛におけるキシラジン-アルファキサロン併用注射麻酔の麻酔効果と心肺機能への影響

■ 要 旨

健康な子牛 6 頭 (6-9 ヶ月齢, 体重 114-310 kg) を繰り返し用い、アルファキサロン単独静脈内投与 (IV) またはキシラジン 0.1mg/kg 筋肉内投与 15 分後にアルファキサロンを to effect IV 投与し、麻酔効果と心肺機能の変化を比較した。気管挿管可能となる麻酔導入量は、キシラジン-アルファキサロンでアルファキサロン単独投与より有意に低く (1.23 ± 0.17 vs 2.28 ± 0.65 mg/kg, $P=0.010$)、気管挿管許容時間も有意に長かった (16.8 ± 7.2 vs 7.3 ± 1.6 分間, $P=0.022$)。アルファキサロン投与 5 分後には、アルファキサロン単独投与で頻脈 (166 ± 47 回/分)、高血圧 (平均血圧 147 ± 81 mmHg)、および低酸素血症 (動脈血酸素分圧 [PaO₂] 43 ± 10 mmHg) を認めたが、キシラジン-アルファキサロンでは低酸素血症 (PaO₂ 47 ± 7 mmHg) のみを認めた。キシラジンによる麻酔前投薬は、アルファキサロンの麻酔導入量を減少し、麻酔時間を延長したが、低酸素血症を防ぐために酸素吸入を考慮すべきである。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



FULL PAPER

Laboratory Animal Science

Blood biochemistry and hematological changes in rats after administration of a mixture of three anesthetic agents

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■ 論文題目

三種混合麻酔実施後のラットにおける血液検査及び血清生化学検査所見の変化

■ 要 旨

高用量、中用量、および低用量のメデトミジン-ミダゾラム-ブトルファノール（MMB）を用いた三種混合麻酔をラットに実施し、MMB 投与後 1 時間目にアチパメゾールで拮抗して、麻酔後の血液検査ならびに血清生化学検査所見の変化を観察した。高用量および中用量の MMB 投与後には、一過性の血糖値の上昇、ヘマトクリット値の増加、およびヘモグロビン濃度の増加を認めた。一方、血漿総タンパク濃度の低下、総白血球数の減少を認めた。ほとんどの検査項目は、MMB 投与 24 時間以内に投与前値に回復した。また、低用量の MMB では、このような変化は認められなかった。

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1) Fibrillar architecture at three different sites of the bovine superficial digital flexor tendon.

Naoki Takahashi, Takuya Hirose, Jun A. Minaguchi, **Hiromi Ueda**, Prasarn Tangkawattana, Kazushige Takehana.

J. Vet. Med. Sci. 80: 405-412. 2018.



FULL PAPER

Anatomy

Fibrillar architecture at three different sites of the bovine superficial digital flexor tendon

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ABSTRACT. Superficial digital flexor tendon (SDFT) of the bovine hindlimb originates from the caudolateral aspect of the distal femur and finally inserts onto the plantar aspect of the middle phalanges. In the present study, morphology and morphometry of the bovine SDFT at the muscle-tendon junction (MTJ), middle metatarsus (mM) and tendon-bone interface (TBI) were investigated. Cross-sectional morphology at the three regions of SDFT were oval, semioval and ring-formed, respectively. Significant difference in cross-sectional area was found only between MTJ-mM and mM-TBI ($P<0.05$). Functional compression and friction from the adjacent structures could be the most potential interactions affecting such appearances. Morphometric data of tenocyte number, water content, and glycosaminoglycan (GAG) length and angle were found increasing in the proximodistal direction, except the fibril diameter and collagen fibril index (CFI). Statistical analyzes could reveal significant differences in average number of tenocytes ($P<0.0001$), CFI (between MTJ-mM and MTJ-TBI, $P<0.05$), water content (between MTJ-TBI, $P<0.05$), length of GAG chains (between MTJ-TBI, $P<0.05$), and angle of GAG chains ($P<0.0001$), respectively. The fibrillar characteristics at the three different areas, including fibril diameter distribution and interfibrillar distance, existed in conforming to the tensional axes in situ. In addition, length and angle of GAG chains were relevant to moving directions of the collagen fibrils.

KEY WORDS: bovine, collagen fibril, glycosaminoglycans, morphometry, superficial digital flexor tendon

■ 論文題目

ウシ浅趾屈筋腱の異なる 3 部位のコラーゲン細線維の構造

■ 要 旨

ウシ浅趾屈筋腱の部位ごとのコラーゲン細線維を中心とした微細構造を観察するために、ウシ浅趾屈筋腱を肉眼、光学顕微鏡、透過型電子顕微鏡を用いて観察した。肉眼的形状は各部位の機能構造を反映していた。近位から遠位に向うにつれコラーゲン細線維は太く、密に存在し、その間を埋める GAG 鎖や水分含有量是对应し変化していった。これらの結果はコラーゲン細線維を中心とする微細構造は、腱の張力を反映するものであった。

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- 1) High prevalence of *mcr-1*, *mcr-3*, and *mcr-5* in *Escherichia coli* derived from diseased pigs in Japan.

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Letter to the Editor

High prevalence of *mcr-1*, *mcr-3* and *mcr-5* in *Escherichia coli* derived from diseased pigs in Japan

Sir,



histories of the pigs were unavailable, however COL is typically used to treat porcine diarrhoea in Japan. These results suggest that the high rates of COL resistance in diseased pigs are caused by high selective pressure in veterinary practice [1].

Among the 120 isolates derived from diseased pigs, the *mcr-1*,

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■ 論文題目

日本の病豚由来大腸菌において *mcr* 遺伝子は高頻度に分布する

■ 要 旨

日本の病豚由来大腸菌において、*mcr* 遺伝子が高頻度に分布していることを明らかにした。

■ 最終責任者 Masaru Usui, Yutaka Tamura (Corresponding Authors)



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Research Letter

RESEARCH LETTER – Environmental Microbiology

Co-harboring of cephalosporin (*bla*)/colistin (*mcr*) resistance genes among Enterobacteriaceae from flies in Thailand

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One sentence summary: In both human and livestock habitats in Thailand, a relatively high proportion of flies carry cephalosporin-resistant Enterobacteriaceae harboring co-transmissible cephalosporin (*bla*) and colistin (*mcr*) resistance genes.

Editor: Seamus Fanning

■ 論文題目

タイのハエから分離された腸内細菌科細菌のセファロスポリン/コリスチン耐性遺伝子について

■ 要 旨

タイのハエから分離された腸内細菌科細菌のセファロスポリン/コリスチン耐性遺伝子の分布について、頻度やその性状を明らかにした。

■ 最終責任者 Masaru Usui (Corresponding Author)

Stenotrophomonas maltophilia is highly prevalent among houseflies (*Musca domestica*)

Akira Fukuda,¹ Masaru Usui,^{1,*} Hideyuki Wakao,¹ Chanchai Boonla² and Yutaka Tamura¹

Abstract

Stenotrophomonas maltophilia is capable of surviving in a wide variety of environments and is considered to be among the antimicrobial-resistant bacteria of greatest public health concern in hospital settings. To clarify the role of houseflies (*Musca domestica*) in disseminating this bacterium, we collected 99 individuals from 15 locations (9 farms and 6 urban areas) in Thailand. *S. maltophilia* was isolated from 39% (39/99) of these houseflies, with the isolation rates being similar in farms and urban areas. Multiple-antimicrobial resistance was evident among the *S. maltophilia* isolates obtained. Of note, the rate of resistance to trimethoprim/sulfamethoxazole (TMP/SMX), the recommended first-line antimicrobial for *S. maltophilia* infection, was relatively high (30%). Almost all of the isolates had a different PFGE pattern. These results suggest that houseflies ingest and host *S. maltophilia* from several different environmental sources. In conclusion, houseflies may facilitate the spread of antimicrobial-resistant (including TMP/SMX-resistant) *S. maltophilia* from environmental sources to humans.

■ 論文題目

Stenotrophomonas maltophilia はイエバエから高頻度に分離される

■ 要 旨

医療関連感染に重要である *Stenotrophomonas maltophilia* は多剤耐性を示す。イエバエを捕獲し、カルバペネム耐性菌を分離したところ、*S. maltophilia* が高頻度に分離された。

■ 最終責任者 Masaru Usui (Corresponding Author)

*2017 年報告漏れ

Tigecycline Susceptibility of *Klebsiella pneumoniae* Complex and *Escherichia coli* Isolates from Companion Animals: The Prevalence of Tigecycline-Nonsusceptible *K. pneumoniae* Complex, Including Internationally Expanding Human Pathogenic Lineages

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Yutaka Tamura³ and Shin-ichi Yokota¹

Transmission of tigecycline-nonsusceptible pathogenic Enterobacteriaceae from companion animals to human should be a concern because tigecycline is a last-line drug for treating multidrug-resistant Enterobacteriaceae in human medicine. However, tigecycline susceptibility of Enterobacteriaceae isolated from companion animals has not been investigated. In this study, we investigated the tigecycline susceptibility of *Klebsiella pneumoniae* complex and *Escherichia coli* isolates from dogs and cats, and evaluated their human pathogenicity potential. Tigecycline susceptibility of *K. pneumoniae*, including *Klebsiella quasipneumoniae* ($n=86$) and *E. coli* ($n=100$) strains isolated from dogs and cats was investigated. The antimicrobial susceptibility, capsular serotype, multi-locus sequence type, *ompK36* group, presence of virulence genes, and serum resistance of tigecycline-nonsusceptible isolates were evaluated. All *E. coli* isolates were susceptible to tigecycline. Two *K. pneumoniae* (minimum inhibitory concentration [MIC], 4 mg/L) and one *K. quasipneumoniae* (MIC, 8 mg/L) isolates were tigecycline resistant. Sixteen *K. pneumoniae* and one *K. quasipneumoniae* isolates were tigecycline intermediate (2 mg/L). All tigecycline-nonsusceptible isolates ($n=20$) were also ciprofloxacin nonsusceptible. These isolates harbored five to nine virulence genes; 16 isolates were resistant to the human serum. In addition, STs of 13 *K. pneumoniae* isolates were reported to be found in strains isolated from human; isolates considered high-risk clones in human (ST11, ST15, and ST147) were also identified. In conclusion, the isolation of tigecycline-nonsusceptible *K. pneumoniae* from companion animals is an impact from the viewpoint of One Health approach to antimicrobial resistance that companion animals are a reservoir of human pathogenic lineages.

■ 論文題目

伴侶動物由来 *Klebsiella pneumoniae* および *Escherichia coli* におけるチゲサイクリン感受性

■ 要 旨

伴侶動物から分離された *Klebsiella pneumoniae* と *Escherichia coli* のチゲサイクリンに対する感受性を明らかにした。中には、人の医療で重要な病原性を示す MLST 型を示す株も存在した。

■ 最終責任者 Toyotaka Sato (Corresponding Author)

Article

Bacteriophage Φ SA012 Has a Broad Host Range against *Staphylococcus aureus* and Effective Lytic Capacity in a Mouse Mastitis Model

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Abstract: Bovine mastitis is an inflammation of the mammary gland caused by bacterial infection in dairy cattle. It is the most costly disease in the dairy industry because of the high use of antibiotics. *Staphylococcus aureus* is one of the major causative agents of bovine mastitis and antimicrobial resistance. Therefore, new strategies to control bacterial infection are required in the dairy industry. One potential strategy is bacteriophage (phage) therapy. In the present study, we examined the host range of previously isolated *S. aureus* phages Φ SA012 and Φ SA039 against *S. aureus* strains isolated from mastitic cows. These phages could kill all *S. aureus* (93 strains from 40 genotypes) and methicillin-resistant *S. aureus* (six strains from six genotypes) strains tested. Using a mouse mastitis model, we demonstrated that Φ SA012 reduced proliferation of *S. aureus* and inflammation in the mammary gland. Furthermore, intravenous or intraperitoneal phage administration reduced proliferation of *S. aureus* in the mammary glands. These results suggest that broad host range phages Φ SA012 is potential antibacterial agents for dairy production medicine.

論文題目

バクテリオファージ Φ SA012 は黄色ブドウ球菌に対し広範な溶菌スペクトルを有し、マウス乳房炎モデルにおいて効果的な溶菌活性を示す

要 旨

これまでに分離したバクテリオファージ（ Φ SA012 および Φ SA039）の乳房炎由来 SA に対する宿主域を精査した。これらのバクテリオファージは、40 パタンの遺伝子型に分類される 93 株の SA を溶菌し、さらに 6 パタンの遺伝子型に分類される 6 株の MRSA も溶菌した。さらに、マウス乳房炎モデルにおいて、 Φ SA012 の投与は、SA の増殖と乳腺における炎症を抑制した。これらの結果は、広範な宿主域を示す Φ SA012 が酪農産業における有用な抗菌性物質となる可能性を示唆している。

最終責任者 Hidetomo Iwano (Corresponding Author)



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Note

Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinary staff in small animal hospitals in Sapporo, Japan, between 2008 and 2016: A follow up study



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ABSTRACT

The aim of the present study was to determine and compare the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and their molecular characteristics among veterinary staff in Sapporo in 2008 and 2016. We isolated MRSA from veterinarians (Vet; n = 91), veterinary technicians (VT; n = 113), and other staff members (n = 24) from 45 small animal hospitals (animal hospitals), as well as from surface swabs (n = 123) obtained from 37 animal hospitals, in 2016. MRSA was observed in 14 Vets (15%), 7 VTs (6%), 2 other staff members (8%), and 6 environmental samples (5%). The prevalence of MRSA among veterinary staff tended to decrease, in comparison to 2008. All the MRSA isolates were classified as CC5/SCCmecII, which is commonly observed in medical settings in Japan. Upon performing pulse-field gel electrophoresis, with *Sma*I and *Eag*I, and *dfb* sequence typing, it was observed that 16 of the MRSA isolates from 2016 were highly similar to those obtained in 2008. This suggests that some MRSA isolates persisted throughout 8 years, although their origins remain unclear. The continuation of education and monitoring of MRSA is necessary for the prevention and control of infection in these settings.

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■ 論文題目

動物病院スタッフからの MRSA の分離(2008 年の follow up study)

■ 要 旨

伴侶動物を対象にした動物病院スタッフにおける MRSA の分布を明らかにした。本調査は、2008 年にも実施されており、そのフォローアップスタディとして 2016 年に同様の調査を行なった。MRSA 分離率は 2008 年と比較して減少傾向を示した。

■ 最終責任者 Yutaka Tamura (Corresponding Author)



Article

Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent *Staphylococcus aureus* Bacteriophage

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Abstract: Antibiotic-resistant bacteria (ARB) have spread widely and rapidly, with their increased occurrence corresponding with the increased use of antibiotics. Infections caused by *Staphylococcus aureus* have a considerable negative impact on human and livestock health. Bacteriophages and their peptidoglycan hydrolytic enzymes (endolysins) have received significant attention as novel approaches against ARB, including *S. aureus*. In the present study, we purified an endolysin, Lys-phiSA012, which harbors a cysteine/histidine-dependent amidohydrolase/peptidase (CHAP) domain, an amidase domain, and a SH3b cell wall binding domain, derived from a polyvalent *S. aureus* bacteriophage which we reported previously. We demonstrate that Lys-phiSA012 exhibits high lytic activity towards staphylococcal strains, including methicillin-resistant *S. aureus* (MRSA). Analysis of deletion mutants showed that only mutants possessing the CHAP and SH3b domains could lyse *S. aureus*, indicating that lytic activity of the CHAP domain depended on the SH3b domain. The presence of at least 1 mM Ca²⁺ and 100 μM Zn²⁺ enhanced the lytic activity of Lys-phiSA012 in a turbidity reduction assay. Furthermore, a minimum inhibitory concentration (MIC) assay showed that the addition of Lys-phiSA012 decreased the MIC of oxacillin. Our results suggest that endolysins are a promising approach for replacing current antimicrobial agents and may contribute to the proper use of antibiotics, leading to the reduction of ARB.

■ 論文題目

多価黄色ブドウ球菌ファージ由来 LysK 様エンドライシン (Lys-phiSA012) の機能解析

■ 要 旨

多価バクテリオファージからエンドライシン (Lys-phiSA012) を単離・精製し、その溶菌機能について解析を実施した。Lys-phiSA012 は、MRSA を含む複数の SA に対して高い溶菌活性を示した。また、Lys-phiSA012 が有する 2 つの触媒ドメイン (CHAP, Amidase ドメイン)、1 つの菌体認識ドメイン (SH3b ドメイン) の欠失変異体を構築し、溶菌活性を評価した結果、SH3b ドメインに依存的な CHAP ドメインによる溶菌活性が示唆された。また、1 mM のカルシウムイオンおよび 100 μM の亜鉛イオンの添加は、Lys-phiSA012 の溶菌活性を亢進させた。さらに、Lys-phiSA012 の併用は、オキサシリンの MIC を減少させた。以上の結果から、エンドライシンは薬剤耐性菌の抑制に貢献する有用なアプローチであることが示唆された。

■ 最終責任者 Hidetomo Iwano (Corresponding Author)



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Letter to the Editor

Isolation of a *mcr-1*-harbouring *Escherichia coli* isolate from a human clinical setting in Sapporo, Japan



harboured only four (*iroN*, *cvaC*, *fliA* and *ETTT* type III secretion system) of 18 tested virulence genes. Collectively, these observations suggested that the *mcr-1* gene was harboured by a commensal *E. coli* strain.

Recently, two *mcr-1*-harbouring *E. coli* strains were isolated.

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■ 論文題目

札幌の医療施設での *mcr-1* 陽性大腸菌の検出

■ 要 旨

札幌の医療施設の患者から、コリスチンに耐性を示す *mcr-1* 陽性大腸菌が検出された。

■ 最終責任者 Toyotaka Sato (Corresponding Author)



Contribution of Novel Amino Acid Alterations in PmrA or PmrB to Colistin Resistance in *mcr*-Negative *Escherichia coli* Clinical Isolates, Including Major Multidrug-Resistant Lineages O25b:H4-ST131-H30Rx and Non-x

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ABSTRACT Colistin is a last-line drug for multidrug-resistant Gram-negative bacteria. We previously reported four plasmid-mediated colistin resistance (*mcr*) gene-negative colistin-resistant *Escherichia coli* clinical isolates, including the major pathogenic and fluoroquinolone-resistant strains O25b:H4-ST131-H30Rx (isolates SRE34 and SRE44; MIC for colistin = 16 mg/liter, non-x (SME296; MIC = 8 mg/liter), and O18-ST416 (SME222; MIC = 4 mg/liter). In this study, we investigated the colistin resistance mechanism and identified novel amino acid substitutions or deletions in the PmrAB two-component system that activates *epiA* (encoding a phosphoethanolamine transferase) and *arnT* (encoding an undecaprenyl phosphate- α -4-amino-4-deoxy-L-arabinose arabinosyl transferase) in all colistin-resistant isolates. SRE34 possessed deletion $\Delta 27-45$ (USVPWLWHST EQQLF3) in PmrB, SRE44 possessed substitution L105P in PmrA, and both SME222 and SME296 included substitution G206D in PmrB. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry revealed that lipid A is modified with phosphoethanolamine in all four isolates. Deletion of *pmrAB* decreased colistin MICs to 0.5 mg/liter and lowered *epiA* and *arnT* expression. Chromosomal replacement of mutated *pmrA* or *pmrB* in colistin-susceptible O25b:H4-ST131 strain SME98 (colistin MIC = 0.5 mg/liter) increased the colistin MIC to that of the respective parent colistin-resistant isolate. In addition, SME98 mutants in which *pmrAB* was replaced with mutated *pmrAB*s showed no significant differences in bacterial growth and competition culture from the parent strain, except for the mutant with L105P in PmrA, whose growth was significantly suppressed in the presence of the parent strain. In conclusion, some O25b:H4-ST131 strains appear to acquire colistin resistance via phosphoethanolamine modification of lipid A through amino acid changes in PmrAB, and the amino acid changes in PmrB do not influence bacterial growth.

KEYWORDS: antimicrobial resistance, bacterial infection, PmrAB, lipid A, *Escherichia coli*

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■ 論文題目

多剤耐性を示す大腸菌での染色体性コリスチン耐性機序の解明

■ 要 旨

コリスチンを含む多剤に対して耐性を示す大腸菌が *mcr* を保有していなかった。この株の詳細を調べたところ、染色体上の遺伝子である、*pmrA*, *pmrB* に変異が認められた。それらの変異は、コリスチンの感受性に大きく影響していた。

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NOTE

Species distribution, virulence factors and antimicrobial resistance of *Acinetobacter* spp. isolates from dogs and cats: a preliminary study

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ABSTRACT

In this study, the prevalence of virulence factors and antimicrobial resistance among 67 *Acinetobacter* spp. isolates, consisting of 21 *Acinetobacter baumannii* and 46 non-*baumannii* *Acinetobacter* obtained from companion animals, was investigated. PCR analysis showed that the most prevalent virulence gene was *afa/draBC* (29.9%), followed by *papC* (22.4%) and *cvaC* (20.9%). Antimicrobial susceptibility testing revealed that resistance to gentamicin (14.9%) and ciprofloxacin (11.9%) was relatively prevalent. Five gentamicin- and/or ciprofloxacin-resistant *A. baumannii* strains were assigned to ST25, ST149, ST164, ST203 and ST1198. All ciprofloxacin-resistant isolates harbored point mutations in *gyrA* and/or *parC*. To the best of our knowledge, this is the first report of preliminary monitoring of animal-origin *Acinetobacter* spp. in Japan.

■ 論文題目

伴侶動物由来 *Acinetobacter* における薬剤感受性とその分子疫学背景

■ 要 旨

伴侶動物から分離されて *Acinetobacter* について薬剤感受性や病原因子を調べ、分離株の分子疫学解析を実施した。

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- 1) Effect of Pasteurella Multocida soluble Antigen stimulation on the in vitro Response of Peripheral Blood mononuclear Cells of Holstein Calves.

Ohtsuka, H., Inoue, M., Maeda, Y., Tanabe, T., Tajima, M.

Acta Veterinaria 68: 201–210. 2018.

II. その他<Others>

- 1) Heme-binding of bovine lactoferrin: the potential presence of a heme-binding capacity in an ancestral transferrin gene.

Saito, N., Iio, T., Yoshikawa, Y, **Ohtsuka, H.**, Orino, K.

Biometals 31: 131-138. 2018.

EFFECT OF *PASTEURELLA MULTOCIDA* SOLUBLE ANTIGEN STIMULATION ON THE *IN VITRO* RESPONSE OF PERIPHERAL BLOOD MONONUCLEAR CELLS OF HOLSTEIN CALVES

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The expressions of cytokines mRNA, including interleukin-4 (IL-4), interleukin-17A (IL-17A) and interferon-gamma (IFN- γ), their master regulatory transcription factors, and signal transducers and activator of transcription (STAT) stimulated in vitro with *Pasteurella* (P) multocida soluble antigen were examined in peripheral blood mononuclear cells (PBMC) from Holstein calves. The healthy Holstein calves were divided into three groups; 2 weeks old (2W Group, N=8), 6 weeks old (6W Group, N=8), and 10 weeks old (10W Group, N=8). PBMC were stimulated in vitro by soluble antigen of *P. multocida*. There were significantly lower expressions of IFN- γ , IL-4, and STAT-6 mRNA of PBMC stimulated with *P. multocida* soluble antigen in the 2W Group compared to that in the 10W Group. Expression of IL-17A and IFN- γ in PBMC stimulated with *P. multocida* soluble antigen were significantly higher compared with the PBMC without stimulation in the 6W groups. The results of the present study demonstrated that 2W old calves had decreased cytokine expression of PBMC when in vitro stimulated with *P. multocida* soluble antigen in vitro.

Key words: calves, cytokine, immune function, *Pasteurella multocida*

■ 論文題目

培養系におけるホルスタイン種子牛の末梢単核球に対するパスツレラ・マルトシダ可溶化抗原刺激による反応

■ 要 旨

ホルスタイン種子牛の末梢単核球 (PBMC) に対するパスツレラ・マルトシダ可溶化抗原刺激によるIL-4、IL-17A、IFN- γ とSTAT遺伝子発現を解析した。健康なホルスタイン種子牛を2、6と10週齢（各々2W群、6W群、10W群、各8頭）の子牛を対象とした。PBMCはパスツレラ・マルトシダ可溶化抗原にて刺激した。可溶化抗原刺激における2W群のIL-4、IFN- γ とSTAT6のmRNA発現量は10W群に比べて明らかに低かった。6W群において刺激の有無によってPBMCのIL-17AおよびIFN- γ 発現量に差がみられた。本研究から、出生2週齢の子牛のPBMCは培養系においてパスツレラ・マルトシダ可溶化抗原刺激によるサイトカイン遺伝子発現が低値であることを確認した。

■ 最終責任者 Hiromichi Ohtsuka (First Author and Corresponding Author)



Heme-binding of bovine lactoferrin: the potential presence of a heme-binding capacity in an ancestral transferrin gene

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Abstract Lactoferrin (Lf) and transferrin (Tf) are iron-binding proteins that can bind various metal ions. This study demonstrates the heme-binding activity of bovine Lf and Tf using biotinylated hemin. When both proteins were coated on separate plate wells, each directly bound biotinylated hemin. On the other hand, when biotinylated hemin was immobilized on an avidin-coated plate, soluble native Lf bound to the immobilized biotinylated hemin whereas native Tf did not, suggesting that a conformational change triggered by coating on the plate allows the binding of denatured Tf with hemin. Incubation of Lf with hemin-agarose resulted in negligible binding of Lf with biotinylated hemin. Lf in bovine milk also bound to immobilized biotinylated hemin. These results demonstrate that bovine Lf has specific heme-binding activity, which is

different from Tf, suggesting that either Tf lost heme-binding activity during its evolution or that Lf evolved heme-binding activity from its Tf ancestral gene. Additionally, Lf in bovine milk may bind heme directly, but may also bind heme indirectly by interaction with other milk iron- and/or heme-binding proteins.

Keywords Bovine · Iron · Heme · Lactoferrin · Transferrin

Introduction

Iron is an essential element for both prokaryotes and

■ 論文題目

ウシラクトフェリンのヘム結合：潜在的存在 祖先トランスフェリン遺伝子におけるヘム結合能力の解析

■ 要 旨

ラクトフェリン (Lf) およびトランスフェリン (Tf) は、様々な金属イオンと結合することができる鉄結合タンパク質である。この研究では、ビオチン化ヘミンを用いたウシLfおよびTfのヘム結合活性を実証した。双方のタンパク質を別々のプレートウェルに被覆した場合、それぞれビオチン化ヘミンに直接結合した。一方、ビオチン化ヘミンをアビジン被覆プレートに固定化すると、可溶性Lfは固定化ビオチン化ヘミンに結合したのに対し、未変性Tfは結合しなかったことから、プレート上に被覆する立体配座変化が、ヘミンとの変性Tfの結合を可能にすることを示唆している。ヘミン - アガロースとのLfの反応は、ビオチン化ヘミンとのLfのわずかな結合であった。ウシ牛乳中のLfも固定化ビオチン化ヘミンに結合した。これらの結果は、ウシLfがTfとは異なる特異的なヘム結合活性を有し、Tfがその進化中にヘム結合活性を失うか、またはLfがそのTf祖遺伝子からヘム結合活性を促進することを示唆する。さらに、牛乳中のLfはヘムに直接結合することができるが、他の乳中鉄および/またはヘム結合タンパク質との相互作用によって間接的にTfと結合するかもしれない。

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Noda J., Izumi K, Tamura Y.

Jpn J Vet Res. 66: 57-62. 2018.

II. その他 <Others>

- 1) Large-Scale Dust Event in East Asia in May 2017: Dust Emission and Transport from Multiple Source Regions.

Minamoto Y, Nakamura K, Wang, M, Kawai K, Ohara K, **Noda J.**, Davaanyam E, Sugimoto N, Kai K.

SOLA. 14: 31-34. 2018.

- 2) Contamination Status of Seven Elements in Hooded Cranes Wintering in South-West Kyushu, Japan: Comparison with Red-Crowned Cranes in Hokkaido, Japan..

Teraoka H, Miyagi H, Haraguchi Y, Takase K, Kitazawa T, **Noda J.**

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Investigation of chromated copper arsenate-treated waste wood used for bedding material in the Hokkaido area

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Abstract

Chromated copper arsenate (CCA)-treated wood has prompted public concern regarding the contamination of livestock-rearing environment. We investigated the current status of CCA-treated waste wood used as bedding material for livestock in the Hokkaido area. Two-step surveys with structured question and purposive sampling were conducted to clarify the current status of wood-based bedding materials for contamination of CCA compounds in farms. The analyses of CCA compounds with an atomic absorption spectrometer indicated unusually high concentrations of chromium, copper, and arsenic in some bedding materials. These findings suggest that ca. 2 % (n = 84) of bedding materials for livestock are most likely contaminated with CCA-treated waste wood in the Hokkaido area.

Key Words: Bedding material; CCA-treated woods; Toxic elements

■ 論文題目

北海道における家畜用敷料中 CCA 処理廃木材成分の実態調査

■ 要 旨

クロム、銅、ヒ素剤 (CCA) で処理された廃木材の家畜飼育環境での使用が懸念されている。本研究では、北海道における CCA 処理廃木材が、敷料としてどれぐらい使用されているか現状調査を行った。方法としては、第一段階の質問アンケートにて家畜の種類、飼養形態、木質敷料の使用有無を調査した。その中から木質の敷料を使用している農家に意図的サンプル提供のお願いを行った。測定の前処理として、硝酸と熱で敷料成分の分解を行い、それから原子吸光光度計にて測定した。結果として、全体 (n=84) のうち、約 2 % にて高いクロム、銅、ヒ素の値が測定されたことから、CCA 処理廃木材を活用した家畜用敷料の流通、汚染が示唆された。

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Large-Scale Dust Event in East Asia in May 2017: Dust Emission and Transport from Multiple Source Regions

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Abstract

A large-scale dust event occurred in East Asia during early May 2017, and transported dust was measured all over Japan. We performed an analysis of the entire dust event using multiple sources: a local ceilometer measurement, measurements from an optical particle counter in the Gobi Desert (Dalanadgad, Mongolia), a study of Dust RGB imagery obtained from Himawari-8, lidar measurements from Japan, and meteorological data. Our results show that three extratropical low pressure systems occurred consecutively in Mongolia and generated dust storms in the Gobi Desert. The dust generated by the third low pressure system was transported to Japan by a cold front and two pressure troughs, which were associated with the low pressure system. Remarkably, the Dust RGB imagery shows both the occurrence and the transportation of the dust, and was able to detect two dust outbreaks in the Horqin Sandy Land of Northern China and their transportation to eastern Japan; this shows that the Horqin Sandy Land was one of the source regions of this dust event.

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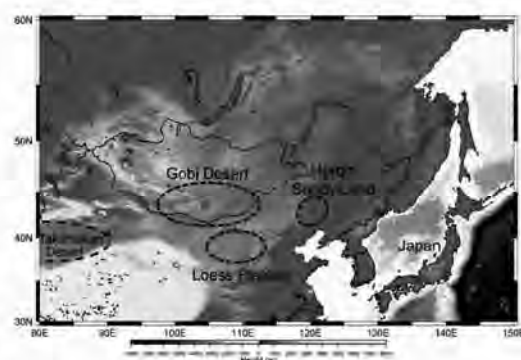


Fig. 1. Topographic map of the main arid and semiarid regions in East Asia. The red circle represents the location of Dalanzadgad.

Japan reported the presence of transported dust on 7 May 2017. This was the largest dust event that occurred in Japan since the event on 21 March 2010. Furthermore, it was the only dust trans-

論文題目

東アジアにおける大規模ダストイベント 2017 年 5 月: 複数のソース地域からのダスト発生と移動

要 旨

2017 年 5 月に発生した東アジアでの大規模ダストイベントの影響で、日本各地でも黄砂が観測された。本研究では解析を複数の観測データソースとしてゴビ砂漠でのシーロメーター観測、光散乱式粒子計の測定値から行い、地上ライダー測定を日本から、また気象衛星「ひまわり 8 号」のダスト RGB データからダストストームを高時空間分解能で検出した。この期間に 3 つの温帯低気圧がモンゴルにて連続して発生し、1, 2 番目は低気圧の前線として、3 番目は寒冷前線として、ダストストームを発生させた。ダスト RGB イメージからはダストストームの発生と、移動を検知することができ、中国北部のホルチン砂漠地帯から発生したダストストームは日本の東部までの移動が確認できた点が際立った結果である。

■ 最終責任者 Yuki Minamoto (Corresponding Author)



Contamination Status of Seven Elements in Hooded Cranes Wintering in South-West Kyushu, Japan: Comparison with Red-Crowned Cranes in Hokkaido, Japan

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Abstract

The hooded crane is designated as an endangered species. The cranes breed primarily in wetlands in southeast Russia and China in summer. Most of the hooded crane population winters in the Izumi plain in Japan. It is difficult to know the contamination status of their habitat because of their vast breeding area. We determined the levels of Cd, Pb, As, (total) Hg, Se, Zn, and Cu in the liver, kidney, and muscle of hooded cranes that were found dead in Izumi in the periods 2003–2006 and 2014–2015 compared with the levels in red-crowned cranes in Hokkaido, Japan, as the only cranes in which these elements had been studied extensively. There were no notable differences between levels of the seven elements in the two periods. Overall, tissue levels of the elements examined in hooded cranes were comparable to those in red-crowned cranes except for Hg and Se. Tissue levels of Hg and Se were clearly lower in hooded cranes than in red-crowned cranes that were found dead from 2000. One lead poisoning case was confirmed. The results suggest that Hooded cranes wintering in Izumi are not extensively contaminated with the seven elements examined.

■ 論文題目

九州南西部で越冬するナベヅルにおける 7 元素の汚染状況：北海道に生息するタンチョウとの比較

■ 要 旨

ナベヅルは主にアムール川流域を含むロシア、中国、モンゴルで繁殖し、大部分が出水（鹿児島）で越冬する渡り鳥である。アムール川やその周辺は重金属を含む環境汚染が問題になっているが、ナベヅルおよびその営巣地の汚染状況に関する情報は全く得られていない。本研究では、2003–06年、2014–15年（以下2000年代、2010年代）に出水で死亡したナベヅルの肝臓、腎臓、骨格筋の代表的有害元素（Hg、Cd、Pb、As）と一部の必須元素含量（Se、Cu、Zn）を測定した。2000年代のナベヅルの肝臓で鉛中毒と思われる成鳥が1羽確認されたが、Hg、Cd、Pb、Asとも清浄値の範囲であった。タンチョウと比較すると、総じてほぼ同程度であったが、Hgについてのみナベヅルはいずれの臓器とも顕著に低かった。2000年代から2010年代にかけてナベヅル臓器でHgやCd含量の僅かな変化が認められたが他の元素とも問題と考えられるレベルではなかった。今後とも継続的な検討が望まれる。

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Effects of the angiotensin-converting enzyme inhibitor alacepril in dogs with mitral valve disease

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ABSTRACT: Alacepril is a relatively novel angiotensin-converting enzyme inhibitor; however, the safety, tolerance, and efficacy of alacepril in terms of cough suppression in dogs with mitral valve disease (MVD) remain unknown. The aim of this study was to investigate the safety, tolerance, and cough suppression efficacy of alacepril in dogs with MVD. This was a multi-center, prospective study. Forty-two dogs with echocardiographic or radiographic evidence of cardiac enlargement in addition to cough were enrolled. Dogs were treated with alacepril (1.0–3.0 mg/kg/day) for at least 4 weeks. One dog (2.4%) developed complications, including appetite loss, lethargy, and vomiting. Thirty-six dogs were re-evaluated after 4 weeks of treatment. Cough resolved or improved in 20 dogs (55.6%) after treatment. Based on the efficacy of alacepril, the dogs were divided into an effective group (n=20) and an ineffective group (n=16). After treatment, the left ventricular end-diastolic internal diameter corrected for body weight was significantly increased from baseline in the ineffective group but was significantly decreased in the effective group. Univariate binomial logistic regression analyses showed that high atrial natriuretic peptide level, N-terminal pro-B-type natriuretic peptide level, and E wave velocity at baseline were significantly correlated with alacepril inefficacy. Alacepril as treatment for MVD is well tolerated in most dogs, and different conditions of cardiac loading may influence the effect of the drug. Alacepril is expected to improve the quality of life of dogs with early stage MVD.

KEY WORDS: alacepril, canine, cough, heart failure, vasodilator

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■ 論文題目

僧帽弁閉鎖不全症犬におけるアンギオテンシン変換酵素阻害薬アラセプリルの効果

■ 要 旨

新規アンギオテンシン変換酵素阻害薬アラセプリルの僧帽弁閉鎖不全症(MVD)に対する有効性を解析した。42頭の症例にアラセプリル(1.0-3.0 mg/kg/日)を処方した。1頭では食欲低下、元気消失、嘔吐がみられた。36頭は4週間後に再検査を行った。MVDに関連した発咳は20頭(55.6%)で改善し、有効群では左室内径の縮小が認められた。アラセプリルはMVD犬において副作用が少なく、初期のMVDに対するQOLの改善が期待できる。

■ 最終責任者 Yasutomo Hori (First Author and Corresponding Author)



Diagnostic accuracy of plasma atrial natriuretic peptide concentrations in cats with and without cardiomyopathies

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KEYWORDS

Cardiac biomarker;
Feline;
Heart disease;
Heart failure;

Hypertrophic cardio-
myopathy

Abstract Objectives: Plasma atrial natriuretic peptide (ANP) levels have been reported to be elevated in cats with cardiomyopathy. We investigated the diagnostic accuracy of plasma ANP concentration as an indicator of the severity of cardiomyopathies.

Animals: This study included 78 control cats and 83 cats with various types of cardiomyopathy.

Methods: This was a prospective multicentre study. Control cats were determined to have a normal heart, and diseased cats were diagnosed by echocardiography. Diseased cats were divided into asymptomatic cats without left atrial dilation (LAD), asymptomatic cats with LAD, and cats with heart failure. Plasma C-terminal ANP concentrations were measured using chemiluminescence.

Results: The median plasma ANP concentration in controls was 43.3 (interquartile range, 33.0–56.3) pg/mL. Plasma ANP values were significantly higher in the cardiomyopathic cats with LAD and heart failure, but the values in cats without LAD were comparable to those in control cats. To distinguish cats with cardiomyopathy from controls, a plasma ANP concentration >77.5 pg/mL afforded sensitivity of 66.3% and specificity of 84.6%. Use of plasma ANP concentration >110.9 pg/mL to identify cats with LAD had a sensitivity of 73.6% and specificity of 93.5%. The areas under the receiver-operating characteristic curve were 0.80 and 0.86.

Conclusions: Plasma ANP concentrations were higher in cats with more advanced cardiomyopathy. Although assaying the ANP concentration alone may not help to diagnose cardiac disease, measuring provides additional information that is useful for assessing the severity of cardiomyopathies.

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■ 論文題目

血漿心房性ナトリウム利尿ペプチド濃度を用いた心筋症猫の診断精度

■ 要 旨

心房性ナトリウム利尿ペプチド(ANP)を用いて心筋症猫の重症度評価を行うための診断精度を解析した。研究には 78 頭の健康猫と 83 頭の心筋症猫を対象とした。血漿 ANP は心筋症猫の重症度に応じて上昇していた。心筋症の有無を診断するためのカットオフ値は 77.5 pg/ml であり、感度・特異度はそれぞれ 66.3%と 84.6%であった。心拡大の有無を診断するためのカットオフ値は 110.9 pg/ml であり、感度・特異度はそれぞれ 73.6%と 93.5%であった。ANP の測定は心筋症猫の重症度評価に有用であることが示唆された。

■ 最終責任者 Yasutomo Hori (Corresponding Author)

Diagnostic utility of cardiac troponin I in cats with hypertrophic cardiomyopathy

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Background: Cardiac troponin I (cTnI) is useful for assessing hypertrophic cardiomyopathy (HCM) in cats.

Objective: To measure plasma cTnI concentrations in healthy cats and evaluate the clinical utility of cTnI in determining the severity of HCM.

Animals: Clinically healthy cats ($n = 88$) and cats with HCM ($n = 93$).

Methods: Multicenter prospective study. Cats with HCM, including hypertrophic obstructive cardiomyopathy at various stages, were diagnosed using echocardiography. Plasma cTnI concentrations were analyzed by a commercial laboratory. Receiver operating characteristic curve analysis was used to evaluate the accuracy of plasma cTnI concentrations to detect HCM.

Results: The median cTnI concentration was 0.027 ng/mL (interquartile range, 0.012–0.048 ng/mL) in healthy cats. Concentrations were significantly higher in diseased cats than in healthy controls, and concentrations were significantly higher in cats with heart failure than in asymptomatic cats. A plasma cTnI concentration of 0.163 ng/mL had a sensitivity of 62.0% and specificity of 100% when used to distinguish normal cats from asymptomatic HCM cats without left atrial dilation. A cutoff of 0.234 ng/mL had high sensitivity (95.0%) and specificity (77.8%) for assessing heart failure. The areas under the receiver operating characteristic curves were 0.65 and 0.93, respectively.

Conclusions and Clinical Importance: Increased cTnI concentrations reflect the severity of HCM. If other causes of cardiac injury are ruled out, plasma cTnI concentration may be useful for predicting the severity of HCM in cats.

KEYWORDS

biomarker, cTnI, feline, heart failure, hyperphosphorylation

論文題目

肥大型心筋症猫における心筋トロポニン I の診断的有用性

要 旨

心筋トロポニン I (cTnI)を用いて肥大型心筋症の猫における診断的有用性を評価した。研究には 88 頭の健康猫と 93 頭の肥大型心筋症猫を対象とした。健康猫における cTnI 濃度は 0.027 (0.012–0.048) ng/ml であった。血漿 cTnI は肥大型心筋症猫の重症度に応じて上昇していた。肥大型心筋症の有無を診断するためのカットオフ値は 0.163 ng/ml であり、感度・特異度はそれぞれ 62.0%と 100%であった。心不全の有無を診断するためのカットオフ値は 0.234 ng/ml であり、感度・特異度はそれぞれ 95.0%と 77.8%であった。cTnI の測定は肥大型心筋症の重症度評価に有用であることが示唆された。

■ 最終責任者 Yasutomo Hori (First Author and Corresponding Author)



FULL PAPER

Internal Medicine

Relationship between indirect blood pressure and various stages of chronic kidney disease in cats

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ABSTRACT. Chronic kidney disease (CKD) is a common cause of secondary systemic hypertension in cats. We investigated the relationship between indirect blood pressure and the prevalence of systemic hypertension in various CKD stages in cats. Client-owned cats (24 control cats and 77 cats with CKD) were included. Biochemical examinations of plasma were conducted by a commercial laboratory. Diseased cats were divided into two groups based on the International Renal Interest Society (IRIS) guidelines (II and III-IV). Indirect blood pressure was measured using an oscillometric technique. Severe hypertension was diagnosed if systolic blood pressure (SBP) was ≥ 180 mmHg. Indirect blood pressures were significantly higher in IRIS stage III-IV than in the control cats. Of 77 cats with CKD, 25 (32.5%) had severe hypertension. The frequency of severe hypertension increased with an increase in IRIS stage; 0% in the controls, 27.6% in the IRIS stage II, and 47.4% in the IRIS stage III-IV, respectively. The indirect SBP was weakly correlated with urea nitrogen ($r=0.27$) and creatinine ($r=0.23$) concentrations in plasma. Binary logistic regression analysis showed that if plasma creatinine concentration is >3.7 mg/dl, cats with CKD had an increased risk for developing severe hypertension ($P<0.001$). Our results suggest that indirect blood pressure was correlated with the severity of CKD, and the prevalence of severe hypertension increased in cats with severe CKD. The risk of severe hypertension may be high in cats with severe CKD.

KEY WORDS: blood pressure, cat, chronic kidney disease, hypertension, oscillometry

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■ 論文題目

猫における非観血的血圧と慢性腎不全の重症度の関係

■ 要 旨

猫において慢性腎不全(CKD)は高血圧の1因であることから、非観血的血圧とCKDの重症度の関係を解析した。研究には24頭の健康猫と77頭のCKD猫を対象とした。重度な腎不全猫(IRIS stage III-IV)の血圧は健康猫よりも有意に上昇していた。重度な高血圧(>180 mmHg)はIRIS stage III-IVの猫の47.4%で診断され、収縮期血圧はBUNならびにクレアチニンと相関を示した。このことは重度なCKDでは高血圧を併発するリスクの高いことを示している。

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- 1) Measurement of visual acuity in Beagle dog by visual evoked potential with pattern stimulation.

Maehara S., Itoh Y, Ito Y, Hayashi M and Masuko A.

J. Vet. Med. Sci. 80: 1758-1761. 2018.

II. その他<Others>

- 1) Synchysis scintillans of the anterior chamber in a dog.

Sano Y, Okamoto M, Hayashi M, Sato T, **Maehara S** and Matsuda K.

J. Vet. Med. Sci. 80: 1733-1736. 2018.



NOTE

Surgery

Measurement of visual acuity in Beagle dog by visual evoked potential with pattern stimulation

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ABSTRACT. We tried measurement of visual acuity in laboratory beagle using pattern stimulus visual evoked potential (P-VEP). We recorded P-VEP in 6 beagles which were corrected refractive power. The stimulus pattern size was 1.22 mm. The testing distance were 0.5, 1.0, and 2.0 m. The visual angles and spatial frequency were calculated from stimulus pattern size and distance. In all subjects, P-VEP was clearly recorded in all testing distance, and this result means that the eye could recognized grid pattern on the stimulus monitor. When stimulus monitor was set up 2.0 m, spatial frequency was 14.29 cpd. From our results, it was founded that the visual acuity in laboratory beagle which was corrected refractive power was 14.29 cpd and more.

KEY WORDS: canine, visual acuity, visual evoked potential with pattern stimulation

■ 論文題目

パターン刺激視覚誘発電位によるビーグル犬の視力測定

■ 要 旨

正常ビーグル犬において、パターン刺激による視覚誘発電位を用いて視力測定を行った。パターン刺激モニターの格子模様の1辺のサイズを1.22mmとし、刺激モニターから被検眼までの距離を2.0mに設定して視覚誘発電位を記録したところ、対象全眼で視覚誘発電位が記録された。この時、格子模様のサイズと被検眼までの距離から計算すると、視角は 2.10 arc/min となり、正常ビーグル犬は、少なくとも 14.29 cycle per degree 以上の視力を有することが明らかとなった。

■ 最終責任者 Seiya Maehara (Corresponding author)



NOTE

Pathology

Synchysis scintillans of the anterior chamber in a dog

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ABSTRACT. Intraocular cholesterol granuloma (CG) associated with synchysis scintillans (SS) was diagnosed in a 5-year-old spayed Shetland sheepdog. During the initial clinical examination, the patient exhibited SS in the anterior chamber. Canine SS is usually found in the vitreous cavity, and SS in the anterior chamber has not been described. Since canine SS has been reported to be a non-progressive condition, and its long-term clinical course has not been adequately documented. The present case report describes the long-term clinical course of a case of canine SS, in which SS occurred in the anterior chamber, leading to intraocular CG formation, and eventually glaucoma.

KEY WORDS: anterior chamber, dog, intraocular cholesterol granuloma, synchysis scintillans

■ 論文題目

犬における前房閃輝性融解の1例

■ 要 旨

5歳齢、避妊雌のシェットランド・シープドッグが、閃輝性融解に伴う眼内コレステリン肉芽腫と診断された。症例の臨床経過では、前房の閃輝性融解が認められていた。犬の閃輝性融解は通常硝子体腔に認められる疾患であり、前房の閃輝性融解の報告はなされていない。また、犬の閃輝性融解は非進行性疾患として報告されており、長期におよぶ臨床経過は十分に示されていない。本論文では、前房閃輝性融解に引き続き生じた眼内コレステリン肉芽腫の形成と、それにより緑内障となった長期におよぶ犬の閃輝性融解の症例を報告する。

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II. その他 <Others>

1) Synchysis scintillans of the anterior chamber in a dog.

Sano Y, Okamoto M, Hayashi M, Sato T, Maehara S, **Matsuda K.**

J Vet Med Sci. 80: 1733-1736. 2018.



NOTE

Pathology

Synchysis scintillans of the anterior chamber in a dog

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ABSTRACT. Intraocular cholesterol granuloma (CG) associated with synchysis scintillans (SS) was diagnosed in a 5-year-old spayed Shetland sheepdog. During the initial clinical examination, the patient exhibited SS in the anterior chamber. Canine SS is usually found in the vitreous cavity, and SS in the anterior chamber has not been described. Since canine SS has been reported to be a non-progressive condition, and its long-term clinical course has not been adequately documented. The present case report describes the long-term clinical course of a case of canine SS, in which SS occurred in the anterior chamber, leading to intraocular CG formation, and eventually glaucoma.

KEY WORDS: anterior chamber, dog, intraocular cholesterol granuloma, synchysis scintillans

■ 論文題目

犬における前房閃輝性融解の1例

■ 要 旨

5歳齢、避妊雌のシェットランド・シープドッグが、閃輝性融解に伴う眼内コレステリン肉芽腫と診断された。症例の臨床経過では、前房の閃輝性融解が認められていた。犬の閃輝性融解は通常硝子体腔に認められる疾患であり、前房の閃輝性融解の報告はなされていない。また、犬の閃輝性融解は非進行性疾患として報告されており、長期におよぶ臨床経過は十分に示されていない。本論文では、前房閃輝性融解に引き続き生じた眼内コレステリン肉芽腫の形成と、それにより緑内障となった長期におよぶ犬の閃輝性融解の症例を報告する。

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

- 1) Rapid growth rate results in remarkably hardened breast in broilers in during the middle stage of rearing: a biochemical and histopathological study.

Kawasaki T, Iwasaki T, Yamada M, Yoshida T, **Watanabe, T.**
PLos One. 23: journal. pone.0193307. 2018.

- 2) Glucagon-like Peptide-1 receptor expression in pancreatic D cells of three avian species; white leghorn chickens, bobwhite quails and common ostriches.

Watanabe T., Hiramatsu K, Nishimura K, Ono T.
J. Poult. Sci. 55: 199-203. 2018.

II. その他<Others>

- 1) Sclerified Parenchyma Differentiation in Hydrangea Veins is Essential for Robust Decorative Sepals (*Hydrangea* spp.).

Kitamura Y, **Watanabe T.**, Kato Y, Teoh W, Kataoka K, Yamaguchi Y, Haruta H, Nishioka H, Kametani K.
Hort. J. 87: 549-556. 2018.

RESEARCH ARTICLE

Rapid growth rate results in remarkably hardened breast in broilers during the middle stage of rearing: A biochemical and histopathological study

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Abstract

The high incidence of meat of impaired quality poses a serious problem in the poultry industry. In recent years, the incidence of the pectoralis major muscle that appeared pale colored, remarkably hardened, and exudative, called "wooden breast" or "woody breast" has increased in slaughter houses. In the present study, 19-day-old Ross 308 broiler chickens affected ($n = 10$) and unaffected ($n = 10$) with remarkably hardened breast were selected from a commercial broiler farm, and reared to 55 days of age under a controlled environment. Among the affected birds, 5 of 10 birds appeared exhausted with markedly suppressed weight gain and 4 of 10 birds died during the rearing period. In contrast, all unaffected birds survived and most gained weight. Four of 10 unaffected birds lost the ability of back-to-back wing contact by the late stage of rearing. The biochemical analysis of blood plasma samples of 20-day-old birds revealed that creatine kinase and L-aspartate aminotransferase values in most affected birds were higher than those in unaffected birds; however, these values in unaffected birds increased rapidly with lost wing contactability and increasing age. Postmortem examinations revealed that the mean diameter of myofibers in affected birds was smaller than that in unaffected birds. Moreover, symptoms of degenerative and regenerative muscles were observed in most birds in both groups. Among them, a decrease in, or defect of, the characteristic polygonal shape of myofibers was the most common change within the pectoralis major muscles in both groups. The present study demonstrated that broilers affected with remarkably hardened breast during the middle stage of rearing would have suppressed physical status and weight gain, or would die. It was suggested that rapid growth in broilers might be a cause of remarkably hardened breast.

■ 論文題目

ブロイラーの急速な成長は飼育中期の胸肉を著しく硬化させる: 生化学的及び病理組織学的研究

■ 要 旨

家禽産業では著しく硬化した胸肉の発生が増加しており深刻な問題となっている。硬化した胸肉はその正常からWooden Breast（木の胸）と呼ばれている。本研究では19日齢から55日齢までの経時的なブロイラーの血液性状の変化と、屠殺後の胸肉の組織構造を解析することにより、発生機序を生化学的及び病理組織学的に明らかにすることを目的とした。ほとんどの発症鶏は飼育中期から血中クレアチンキナーゼとL-アスパラギン酸アミノトランスフェラーゼの上昇が始まっていた。このことから、発症鶏は飼育中期から胸肉の変性が始まり、出荷日齢では形態学的にも異常を呈することが示された。

■ 最終責任者 Takafumi Watanabe (Corresponding Author)

《Research Note》

Glucagon-like Peptide-1 Receptor Expression in the Pancreatic
D Cells of Three Avian Species; White Leghorn Chickens,
Northern Bobwhites, and Common Ostriches

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Glucagon-like peptide (GLP)-1 is released from the intestinal L cells in response to food ingestion and stimulates insulin secretion from the pancreatic B cells, by binding to its specific receptor (GLP-1R), which is expressed on the pancreatic B cells in the mammalian pancreas. Previously, we demonstrated that chicken GLP-1R was expressed on the pancreatic D cells by using a specific antibody against chicken GLP-1R. In the present study, we compared the localization of GLP-1R in the pancreases of three avian species; white leghorn chicken, northern bobwhite, and common ostrich, using the double immunofluorescence technique. We found that the types of pancreatic islets in the northern bobwhite pancreas were similar to those found in the chicken pancreas. The ostrich pancreas contained several types of pancreatic islets. GLP-1R-immunoreactive cells were found in all types of pancreatic islets in both northern bobwhite and ostrich and expressed somatostatin immunoreactivity. The present results indicate that the pancreatic D cells are the target cells of GLP-1, and GLP-1 might play a physiological role via somatostatin in the avian species.

Key words: common ostrich, glucagon-like peptide-1 receptor, immunohistochemistry, northern bobwhite, pancreatic islet, white leghorn chicken

J. Poultry Sci., 55: 199-203, 2018

■ 論文題目

3種類の鳥類（ホワイトレグホン、コリンウズラ、ダチョウ）の膵臓D細胞でのグルカゴン様ペプチド-1受容体の発現

■ 要 旨

哺乳類は食餌刺激によって消化管内分泌細胞からグルカゴン様ペプチド-1（GLP-1）を分泌し、膵臓のB細胞に発現する受容体を刺激してインスリン分泌を促す。しかしながら、我々はニワトリではGLP-1受容体はソマトスタチンを分泌するD細胞に発現することを組織学的に明らかにしている。本研究では、ホワイトレグホン、コリンウズラ、ダチョウの3種類の鳥類の膵臓でのGLP-1受容体の発現部位を解析した。ダチョウは他の2種と膵島の組織構造は異なっていたが、いずれの種でもGLP-1受容体はD細胞に発現していた。このことから、鳥類ではGLP-1はソマトスタチンを介して生理学的な作用を及ぼしていることが示唆された。

■ 最終責任者 Kohzy Hiramatsu (Corresponding Author)

Sclerified Parenchyma Differentiation in Hydrangea Veins is Essential for Robust Decorative Sepals (*Hydrangea* spp.)

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Information about mechanical reinforcement of decorative organs could help development of a novel technique that would give flowers with robust floral organs and broadly contribute to postharvest flower preservation. Hydrangeas (*Hydrangea* spp.) exhibit remarkable characteristics in terms of mechanical reinforcement of decorative sepals. Although decorative sepals at the flowering stage shrink when they are desiccated, decorative sepals after flowering maintain their shape even after desiccation. In this study, the lignifications of the vein cells in decorative sepals were analyzed using phloroglucinol/HCl-staining. The microstructure of the cell wall was analyzed using transmission electron microscopy (TEM). The three-dimensional structure of vein cells was analyzed using serial block-face scanning electron microscopy (SBF-SEM). Tubular- and spindle-shaped dead cells with a lignified pitted secondary cell wall were observed around the vessel elements in decorative sepals after flowering. These cells were observed as living cells without a secondary cell wall in the veins of decorative sepals at flowering and in fully expanded leaves. Further, 10 hydrangea cultivars were analyzed for development of mechanical reinforcement in vein cells, and some of them were compared by desiccation testing. Decorative sepals of a cultivar lacking those cells exhibited shrinkage after flowering when they were desiccated. In conclusion, dead cells with a lignified pitted secondary cell wall contribute to the reinforcement of veins in decorative sepals of hydrangeas and become sclerified parenchyma cells. Axial parenchyma sclerifying in veins after flowering is essential for robust hydrangea floral organs and represent a new type of mechanical reinforcement tissue in plant decorative floral organs.

Key Words: axial parenchyma, bordered pit, phloroglucinol/HCl-staining, secondary cell wall, serial block-face scanning electron microscopy.

■ 論文題目

アジサイの導管形質の強固な分化は丈夫な装飾花に不可欠である

■ 要 旨

アジサイの装飾花（一般的に花卉として認識されている部位）に物理的な強化を与える技術は収穫後の切り花としての価値の維持に貢献する。収穫後の装飾花の収縮には導管の細胞壁の構造が関与すると言われており、本研究では細胞壁の変化を透過型電子顕微鏡と3次元電子顕微鏡で解析した。開花後の装飾花の導管では管状および紡錘形の死細胞が2次細胞壁を形成していた。この2次細胞壁を有する導管は装飾花の物理的な強化に寄与しており、適切な形成を誘導することがアジサイの切り花としての価値を高めることが示唆された。

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- 1) Survey of tick-borne zoonotic viruses in wild deer in Hokkaido, Japan.
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J. Vet. Med. Sci. 80: 985-988. 2018

II. その他 <Others>

- 1) A Simple Mechanism Based on Amino Acid Substitutions is not a Critical Determinant of High Mortality of Japanese Encephalitis Virus Infection in Mice.
Takamatsu Y, **Uchida L**, Raekiansyah M, Luz MA, Morita K, Hayasaka D.
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Yu F, Adungo F, Konongoi SL, Inoue S, Sang R, Ashur S, Kwallah AO, **Uchida L**, Buerano CC, Mwau M, Zha Y, Nie Y, Morita K.
Virol J. 15: 178. 2018



NOTE

Public Health

Survey of tick-borne zoonotic viruses in wild deer in Hokkaido, Japan

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ABSTRACT. Tick-borne encephalitis (TBE) and severe fever with thrombocytopenia syndrome (SFTS) are both tick-borne zoonotic diseases caused by TBE virus (TBEV) and SFTS phlebovirus (SFTSV). In 2016, a second domestic TBE case was reported in Hokkaido, Japan, after an absence of 23 years. We conducted IgG ELISA for TBEV and SFTSV on 314 deer (*Cervus nippon yesoensis*) serum samples collected from 3 places in Hokkaido. There were 7 seropositive samples for TBEV but none for SFTSV by ELISA. The specificity of the 7 positive samples was confirmed by neutralization tests against TBEV, and 5 sera showed 320 to 640 of 50% focus reduction endpoint titers. Our results provide information about the infectious status of TBEV in wild deer in Hokkaido, Japan.

KEY WORDS: serosurvey, severe fever with thrombocytopenia syndrome virus, tick-borne encephalitis virus, wild deer, zoonotic disease

■ 論文題目

北海道のエゾシカにおけるマダニ媒介性ウイルス感染症のサーベイランス

■ 要 旨

ダニ媒介性脳炎（TBE）と重症熱性血小板減少症候群（SFTS）は共にマダニにより媒介されるウイルス性人獣共通感染症である。2016年、北海道において23年ぶりとなるTBEの患者が報告された。我々は北海道で捕獲されたエゾシカ、計314頭の血清を用い、TBEウイルス（TBEV）およびSFTSウイルス（SFTSV）に対するIgG ELISAを実施した。その結果、7頭でTBEVに対し高い抗体価が確認されたが、SFTSVに対し陽性抗体は認められなかった。さらに、上記7頭の内、5頭でTBEVに対し320～640倍の中和抗体価（Fifty percent focus reduction neutralization endpoint titers, FRNT₅₀）が確認された。本研究は、北海道におけるエゾシカのTBEV感染状況とウイルス分布域を明らかにするものである。

■ 最終責任者 Katsuro Hagiwara (Corresponding Author)



Article

A Simple Mechanism Based on Amino Acid Substitutions is not a Critical Determinant of High Mortality of Japanese Encephalitis Virus Infection in Mice

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Abstract: For the development of effective treatment strategies for Japanese encephalitis (JE), it is important to identify the viral factors causing severe disease during JE virus (JEV) infection. In this study, we assessed whether amino acid substitutions are critical factors for higher mortality of JaTH160 compared with JaOArS982 in mice using the technique of infectious cDNA clones. We raised the possibility that two amino acids of C₁₂₄ and NS₃₄₈₂ of JaTH160 may contribute to increased mortality in mice. However, simultaneous substitutions of these amino acids did not significantly increase the virulence of JaOArS982, suggesting that high mortality due to JaTH160 viral infection cannot be simply attributed to the specific amino acids. Multiple and complex, but not simple, mechanisms may induce the high mortality of JaTH160 infection in mice.

Keywords: Japanese encephalitis virus; pathogenicity; mouse; recombinant; amino acid substitution

論文題目

マウスにおける日本脳炎ウイルスの病原性はリバーシジェネティクスに基づくアミノ酸置換解析だけでは解明できない

要 旨

効果的な治療法開発のため、日本脳炎 (JE) においてウイルス側の病原性の解明が求められている。本研究で我々は日本脳炎ウイルス (JEV) JaTH160株 (高致死率) と JaOArS982株 (低致死率) について、感染性クローンをを用いたリバーシジェネティクス手法に基づき、両者の相違アミノ酸のマウスにおける病原性を評価した。その結果、JaTH160株におけるカプシド-124番目およびNS3-482番目のアミノ酸がマウスでの致死率に寄与することが明らかとなった。しかし、両変異を導入したJaOArS982株では致死率の増加は確認されなかった。以上の結果から、JaTH160株のマウスでの高い致死率は単純なアミノ酸のみでは決定されず、他の要因が関与することが示唆された。

■ 最終責任者 Daisuke Hayasaka (Corresponding Author)

RESEARCH

Open Access



Comparison of enzyme-linked immunosorbent assay systems using rift valley fever virus nucleocapsid protein and inactivated virus as antigens

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Abstract

Background: Rift Valley Fever (RVF) is a mosquito-borne viral zoonosis. To detect RVF virus (RVFV) infection, indirect immunoglobulin G (IgG) and immunoglobulin M (IgM) enzyme linked immunosorbent assays (ELISAs) which utilize recombinant RVFV nucleocapsid (RVFV-N) protein as assay antigen, have reportedly been used, however, there is still a need to develop more sensitive and specific methods of detection.

Methods: RVFV-N protein was expressed in *Escherichia coli* (*E. coli*) and purified by histidine-tag based affinity chromatography. This recombinant RVFV-N (rRVFV-N) protein was then used as antigen to develop an IgG sandwich ELISA and IgM capture ELISAs for human sera. Ninety six serum samples collected from healthy volunteers during the RVF surveillance programme in Kenya in 2013, and 93 serum samples collected from RVF-suspected patients during the 2006–2007 RVF outbreak in Kenya were used respectively, to evaluate the newly established rRVFV-N protein-based IgG sandwich ELISA and IgM capture ELISA systems in comparison with the inactivated virus-based ELISA systems.

Results: rRVFV-N protein-based-IgG sandwich ELISA and IgM capture ELISA for human sera were established. Both the new ELISA systems were in 100% concordance with the inactivated virus-based ELISA systems, with a sensitivity and specificity of 100%.

Conclusions: Recombinant RVFV-N is a safe and affordable antigen for RVF diagnosis. Our rRVFV-N-based ELISA systems are safe and reliable tools for diagnosis of RVFV infection in humans and especially useful in large-scale epidemiological investigation and for application in developing countries.

Keywords: RVFV, Nucleocapsid protein, Expression, IgG sandwich ELISA, IgM capture ELISA

■ 論文題目

リフトバレー熱ウイルスの組換えヌクレオカプシドと不活化ウイルスを用いた ELISA 法の比較

■ 要 旨

リフトバレー熱は蚊媒介性の人獣共通感染症である。診断には組換えヌクレオカプシド (rRVFV-N) を用いた IgG および IgM ELISA が報告されているが、より高感度、高特異性の診断手法の開発が必要とされている。本研究で我々は His タグを付加した rRVFV-N 発現システムを構築し、この抗原を用い IgG sandwich ELISA および IgM capture ELISA を実施した。2013 年、ケニアでリフトバレー熱のサーベイランスで収集した健康人血清 96 検体および 2006-07 年、ケニアでのリフトバレー熱の流行時収集されたリフトバレー熱疑い患者の血清 96 検体を供試した。既存の不活化ウイルスを用いた手法と、我々の開発した手法を比較した結果、IgG sandwich ELISA および IgM capture ELISA 共に 100% 既法の結果に一致し、感度、特異度は共に 100% であった。我々の開発した rRVFV-N を用いた診断系は既法に比べ安全かつ安価で、途上国における大規模疫学調査に有用である。

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- 1) Detection of anti-neutrophil cytoplasmic antibodies in dogs with immune-mediated inflammatory diseases.

Tamamoto T, Nagao A, Sugano M.

Vet. Immunol. Immunopathol. 203: 47-51. 2018.

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Short Communication

Detection of anti-neutrophil cytoplasmic antibodies in dogs with immune-mediated inflammatory diseases

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Anti-neutrophil cytoplasmic antibody
Dog
Immune-mediated inflammatory disease

ABSTRACT

Anti-neutrophil cytoplasmic antibody (ANCA) is a type of autoantibody against neutrophil cytoplasm. In veterinary medicine, few studies have reported the detection of ANCA in dogs, and most of these studies were performed in dogs with inflammatory bowel disease (IBD). The aim of this study was to evaluate whether ANCA is detected in dogs with immune-mediated inflammatory diseases (IMIDs) other than IBD. Serum samples were collected before treatment initiation from 40 client-owned dogs with various diseases and 22 healthy beagle dogs; the dogs were classified into two groups: IMID group ($n = 16$) and control group ($n = 46$). ANCA was detected using the indirect fluorescent antibody test. Of the 16 dogs in the IMID group, 13 (81.3%) tested positive for ANCA. In contrast, of the 46 dogs in the control group, 13 (28.03%) tested positive for ANCA. Moreover, a significant association between ANCA positivity and IMIDs was identified in the IMID group than in the control group ($P = 0.0003$). In the control group, however, dogs with bacterial infection showed a relatively high rate of ANCA positivity. Therefore, ANCA positivity was observed in dogs with both IMIDs and bacterial infection. This suggested that ANCA positivity is associated with diseases accompanied by neutrophil activation and infiltration.

■ 論文題目

免疫介在性炎症性疾患に罹患した犬における抗好中球細胞質抗体の検出

■ 要 旨

抗好中球細胞質抗体 (ANCA) は自己抗体の一種であり、産生されると好中球を活性化する作用を持つ。医学領域では膠原病などで産生が亢進することが知られており、病態との関連が示唆されているが、獣医学領域ではほとんど検討されていない。そこで免疫介在性炎症性疾患罹患犬の血清中の ANCA の検出を試みたところ、高い割合で陽性を示した。健常犬や腫瘍罹患犬と比較しても有意に陽性率は高かったが、細菌感染症罹患犬との間には有意差は認められなかった。今後は ANCA の陽性と好中球活性化の間でさらなる研究が必要と考えられる。

■ 最終責任者 Takashi Tamamoto (Corresponding Author)



FULL PAPER

Pathology

Variation of amino acid sequences of serum amyloid a (SAA) and immunohistochemical analysis of amyloid a (AA) in Japanese domestic cats

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ABSTRACT. Amyloid A (AA) amyloidosis, a fatal systemic amyloid disease, occurs secondary to chronic inflammatory conditions in humans. Although persistently elevated serum amyloid A (SAA) levels are required for its pathogenesis, not all individuals with chronic inflammation necessarily develop AA amyloidosis. Furthermore, many diseases in cats are associated with the elevated production of SAA, whereas only a small number actually develop AA amyloidosis. We hypothesized that a genetic mutation in the SAA gene may strongly contribute to the pathogenesis of feline AA amyloidosis. In the present study, genomic DNA from four Japanese domestic cats (JDCs) with AA amyloidosis and from five without amyloidosis was analyzed using polymerase chain reaction (PCR) amplification and direct sequencing. We identified the novel variation combination of 45R-51A in the deduced amino acid sequences of four JDCs with amyloidosis and five without. However, there was no relationship between amino acid variations and the distribution of AA amyloid deposits, indicating that differences in SAA sequences do not contribute to the pathogenesis of AA amyloidosis. Immunohistochemical analysis using antisera against the three different parts of the feline SAA protein—i.e., the N-terminal, central, and C-terminal regions—revealed that feline AA contained the C-terminus, unlike human AA. These results indicate that the cleavage and degradation of the C-terminus are not essential for amyloid fibril formation in JDCs.

KEY WORDS: AA amyloidosis, fibril formation, Japanese domestic cat, SAA gene

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■ 論文題目

日本の飼育猫における血清アミロイド A アミノ酸配列の多様性とアミロイド A の免疫組織化学的評価

■ 要 旨

アミロイドーシス発症には SAA の遺伝的多様性が関与しているのではないかという仮説を立て、アミロイドーシス罹患猫とそうではない猫からゲノム DNA を採取し、変異を調べたところ、これまで報告されていない新たな変異を発見したが、アミロイドーシス発症との関連性は明らかではなかった。また、免疫組織化学により沈着しているアミロイド繊維の由来を検索したところ、人とは異なり、猫では C 末端側が残っていることが明らかとなった。

■ 最終責任者 Kazuyuki Uchida (Corresponding Author)

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- 1) Duodenal infusion of fatty acids differentially affects plasma glucagon-like peptide-1 and ghrelin concentrations in sheep.

Fukumori R., Masuda Y., Takeuchi A., Yanai R., Atchalalt K., Sarentonglaga B., Ogata K., Yamaguchi M., Hara A., Satoh A., Sugino T., Nagao Y.

J. Anim. Sci. 96: 1889-189. 2018.

II. その他<Others>

- 1) Effects of pasture intake on immunoglobulin concentrations in the milk of dairy cows.

Yamaguchi M., **Fukumori R.**, Sarentonglaga B., Ogata K., Hara A., Sato A., Maeda I., Azuma N., Nagao Y.

Milk Science 67: 167-174. 2018.

- 2) Effects of insulin-like growth factor-1 on the in vitro maturation of canine oocytes.

Sato A., Sarentonglaga B., Ogata K., Yamaguchi M., Hara A., Atchalalt K., Sugane N., **Fukumori R.**, Nagao Y.

J. Reprod. Dev. 64: 83-88. 2018.

- 3) Histochemical properties of bovine and ovine mammary glands during fetal development.

Hara A., Abe T., Hirao A., Sanbe K., Ayakawa H., Sarantonglaga B., Yamaguchi M., Sato A., Khurchabilig A., Ogata K., **Fukumori R.**, Sugita S., Nagao Y.

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Duodenal infusion of fatty acids differentially affects plasma glucagon-like peptide-1 and ghrelin concentrations in sheep

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ABSTRACT: The aim of this study was to investigate how intraduodenal infusions of fatty acids (FA) affect appetite-related gut peptides such as glucagon-like peptide-1 (GLP-1) and ghrelin in sheep. We hypothesized that these peptides can be highly reactive to unsaturated long-chain FA, because they are well known to decrease dry matter intake (DMI). Four ewes were fitted with a duodenal cannula and a jugular vein catheter for a 6-h duodenal infusion of the 9 FA (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, and C18:3) and water (control). The concentration of each FA was 1.6 g per metabolic body weight (BW), approximately corresponding to the amount of supplemented fat in a standard dairy cow diet. Each infusion was separated by at least 2 d. During the infusion period, blood samples were collected periodically to determine changes in plasma GLP-1, ghrelin, and metabolite concentrations. Duodenal infusions of

C18:1, C18:2, and C18:3 led to higher plasma GLP-1 ($P < 0.05$) and lower glucose ($P < 0.05$) than control. Plasma ghrelin concentrations were greater in C18:1 and C18:3 infusions than control ($P < 0.05$). Plasma ketone bodies were higher in C8:0 and C10:0 infusions ($P < 0.05$), but plasma triglyceride concentrations were lower in C8:0, C10:0, C12:0, and C16:0 infusions ($P < 0.05$) than control. Fatty acid infusions except for C18:3 led to higher plasma NEFA concentrations than control ($P < 0.05$). These results confirmed that the hypophagic effect of dietary unsaturated long-chain FA is mediated by GLP-1 (an anorexigenic effect) secretion. However, we also observed higher plasma ghrelin (an orexigenic effect) partially by unsaturated long-chain FA. Thus, the gut peptide secretions when ruminant animals ingest FA supplements would complexly affect satiety and further studies are needed to determine their each impact on DMI.

Key words: ghrelin, GLP-1, long-chain fatty acids, medium-chain fatty acids, ruminants

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■ 論文題目

ヒツジにおける十二指腸内脂肪酸注入が GLP-1 およびグレリンの血漿濃度に及ぼす影響

■ 要 旨

エネルギー含量の向上、乳脂率低下予防、繁殖成績の向上を目的として用いられている油脂サプリメントは乾物摂取量を低下させやすい。本研究では油脂を構成する脂肪酸の種類によって、摂食関連ペプチドである GLP-1 やグレリンの分泌応答が異なることを報告した。

■ 最終責任者 Rika Fukumori (Corresponding Author)

—Original Article—

Effects of insulin-like growth factor-1 on the *in vitro* maturation of canine oocytes

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Abstract. The maturation rate of canine oocytes during *in vitro* maturation (IVM) needs to be improved. The present study was designed to evaluate the effects of insulin-like growth factor-1 (IGF-1) on the IVM of canine oocytes. Ovaries were obtained by ovariectomy and were sliced to release cumulus-oocyte complexes (COCs). In Experiment 1, the effects of different concentrations of IGF-1 on the nuclear maturation of oocytes was investigated. The COCs were cultured in a modified medium (mTCM199) with IGF-1 (0, 0.5, 5, 10, and 50 µg/ml). At the end of the 48 h culture, oocytes were fixed and stained to evaluate their nuclear stage. Supplementation with 50 µg/ml IGF-1 induced a significantly higher metaphase II (MII) rate ($P < 0.05$) compared to the 0 and 0.5 µg/ml IGF-1 groups. In Experiment 2, the expression levels of *insulin receptor* (*INSR*), *IGF-1 receptor* (*IGF-1R*), and *IGF-2 receptor* (*IGF-2R*) genes, localized to canine oocytes and cumulus cells, were investigated before and after IVM. The expression level of *IGF-1R* in cumulus cells after IVM was higher than that before IVM ($P < 0.05$). In Experiment 3, it was investigated whether an inhibitor of PTEN (phosphatase and tensin homolog), bpV, affects the nuclear maturation of oocytes. Regardless of bpV supplementation at a concentration of 0.2 to 200 µmol/l, there was no significant difference in the proportion of oocytes that reached the MII stage. These results indicated that IGF-1 has a favorable effect on the IVM of canine oocytes, possibly through the stimulation of the Ras/MAPK pathway via *IGF-1R* expressed in cumulus cells.

Key words: bpV, Canine oocyte, Insulin-like growth factor-1 (IGF-1), Insulin-like growth factor-1 receptor (IGF-1R), *In vitro* maturation

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■ 論文題目

インスリン様成長因子-1 がイヌ卵子の体外成熟に及ぼす影響

■ 要 旨

イヌ卵子の体外成熟培養技術は確立されておらず、成熟率が未だ低い。本研究ではインスリン様成長因子-1 (IGF-1) がイヌ卵子の成熟率に及ぼす影響を検討した。避妊手術によって摘出した卵巣から卵丘細胞卵子複合体 (COCs) を採取し、培養に供した。0~50 µg/mLの濃度でIGF-1を添加した培地でCOCsを培養したところ、50 µg/mL添加において卵子のMII期成熟率が増加した。卵子および卵丘細胞のIGF-1受容体遺伝子発現量は培養前と比較して培養後で増加した。IGF-1の細胞内シグナル伝達経路を阻害するPTENの阻害薬を成熟培地に添加して培養を行ったが、卵子成熟率に影響はなかった。以上の結果から、IGF-1はイヌ卵子の成熟過程において好適な効果をもたらす可能性が示された。

■ 最終責任者 Yoshikazu Nagao (Corresponding Author)



Histochemical properties of bovine and ovine mammary glands during fetal development

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ABSTRACT. In order to obtain more information on the development of bovine and ovine fetal mammary glands, a series of mammary glands from fetuses of different ages were analyzed. A total of 16 bovine fetuses with curved crown rump lengths ranging from 12 cm (80 days) to 75 cm (240 days) and 15 ovine fetuses ranging from 55 days to 131 days were examined. We used hematoxylin and eosin stain and Oil-Red-O stain to analyze the developmental and morphogenetic processes of mammary glands. In addition, we used immunohistochemical staining to determine the pattern of expression of cytokeratin 18 (CK18) during luminal epithelial differentiation, α -smooth-muscle actin (α -SMA) for myoepithelial differentiation, Ki-67 for cell proliferation, and estrogen receptor α (ER α). Our analyzes showed: (a) The primary mammary duct begin to proliferate in a lengthwise within the teat at 90 days in bovine fetuses and 63 days in ovine fetus; (b) luminal epithelial cells and myoepithelial cells appeared from 90 days in bovine fetuses and 63 days in ovine fetus; (c) proliferation of epithelial cells appeared to coincide with the development of the primary and secondary ducts; and (d) ER α was not found in the fetal mammary gland, but adipocytes showed the presence of ER α . Overall, these results indicate that the sequence of events in the prenatal development of the mammary gland of sheep is similar to that of cattle.

KEY WORDS: α -SMA, bovine, CK18, fetal mammary gland, ovine

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■ 論文題目

ウシとヒツジにおける胎子期の乳腺組織の発達過程

■ 要 旨

本研究では異なる胎齢のウシおよびヒツジ胎子から乳腺組織を採取し、切片を作製、ヘマトキシリン-エオジン染色、Oil-Red-O染色により形態観察を行うとともに、免疫染色によって細胞の分化、増殖過程を観察した。ウシでは90日齢、ヒツジでは63日齢において乳管の伸長、管腔上皮細胞および筋上皮細胞の出現が見られた。

■ 最終責任者 Yoshikazu Nagao (Corresponding Author)

Original Paper

Effects of pasture intake on immunoglobulin concentrations in the milk of dairy cows

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Abstract

It is well known that the concentrations of β -carotene and vitamin E increase in the blood and milk of grazing dairy cows and are responsible for regulating their cell-mediated immunity; however, little is known about the effects of grazing on humoral immunity. The objective of this study was to determine the effects of pasture intake on immunoglobulin concentrations in the milk of dairy cows. Lactating dairy cows were maintained in a rotational grazing system from May to October (grazing period) or in a paddock from November to March (non-grazing period). Milk samples from grazing and non-grazing cows were analyzed for the concentrations of IgG, IgA, IgM, β -carotene, and vitamin E. Pasture intake had significant effects on β -carotene, vitamin E, and IgM concentrations. There were significant positive correlations between β -carotene and IgM, IgM and IgG, IgM and IgA, and IgG and IgA concentrations in the milk from grazing dairy cows. These results suggest that grazing management is effective to improve the nutritional status of fat-soluble vitamin such as β -carotene (vitamin A) and vitamin E, resulting in the increased concentration of milk IgM and the correlated responses on milk IgG and IgA.

■ 論文題目

乳牛の放牧による乳汁中免疫グロブリン濃度への影響

■ 要 旨

放牧時の生草の摂取によって血中や乳汁中の β カロテンおよびビタミンE濃度が高まることが知られているが、免疫成分に及ぼす影響については明らかにされていない。本研究では、放牧乳牛における乳汁成分の季節変動を解析した。放牧期における乳中IgG、IgA、IgM、 β カロテン、ビタミンE濃度は非放牧期のそれらと比較して高く、 β カロテンとIgM、IgMとIgG、IgMとIgA、IgGとIgAの間に正の相関が認められた。

■ 最終責任者 Yoshikazu Nagao (Corresponding Author)

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II . その他 <Others>

- 1) Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs.

Oyama N., Sano T, Yamamori M, Tamura J, Umar MA, Endo Y, Ishikawa Y, Itoh A, **Miyoshi K**, Yamashita K.

J. Vet. Med. Sci. 80: 1450-1455. 2018.

- 2) Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves.

El-Hawari SF, Sakata H, Oyama N, Tamura J, Higuchi C, Endo Y, **Miyoshi K**, Sano T, Suzuki K, Yamashita K.

J. Vet. Med. Sci. 80: 361-367. 2018.



Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs

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■ 論文題目

犬に皮下投与したロベナコキシブの分布に対する機械的調節呼吸と静脈内輸液を実施したセボフルラン麻酔の影響

■ 要 旨

健康なビーグル犬 10 頭 (1-11 歳, 体重 9.3-14.3kg, 雄 6 頭, 雌 4 頭) に覚醒時または調節呼吸と静脈内輸液を実施したセボフルラン全身麻酔下でロベナコキシブ 2mg/kg を皮下投与し、血漿ロベナコキシブ濃度の推移を観察した。覚醒時にロベナコキシブを投与した犬における最大血漿ロベナコキシブ濃度 ($C_{\max}$) は $2.2 \mu\text{g/mL}$ [1.2-4.6] (中央値[最小-最大]) であり、投与後の $C_{\max}$ 到達時間 ($T_{\max}$) は 90 分 [60-120] であった。これに対して、セボフルラン麻酔下の犬では $C_{\max}$ は有意に低下し ($1.3 \mu\text{g/mL}$ [0.8-1.4], $P=0.008$)、 $T_{\max}$ は有意に延長した (120 分 [120-240], $P=0.018$)。調節呼吸と静脈内輸液を実施したセボフルラン麻酔は、犬におけるロベナコキシブの薬物動態に影響を及ぼした。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves

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■ 論文題目

子牛におけるキシラジン-アルファキサロン併用注射麻酔の麻酔効果と心肺機能への影響

■ 要 旨

健康な子牛 6 頭 (6-9 ヶ月齢, 体重 114-310 kg) を繰り返し用い、アルファキサロン単独静脈内投与 (IV) またはキシラジン 0.1mg/kg 筋肉内投与 15 分後にアルファキサロンを to effect IV 投与し、麻酔効果と心肺機能の変化を比較した。気管挿管可能となる麻酔導入量は、キシラジン-アルファキサロンでアルファキサロン単独投与より有意に低く (1.23 ± 0.17 vs 2.28 ± 0.65 mg/kg, $P=0.010$)、気管挿管許容時間も有意に長かった (16.8 ± 7.2 vs 7.3 ± 1.6 分間, $P=0.022$)。アルファキサロン投与 5 分後には、アルファキサロン単独投与で頻脈 (166 ± 47 回/分)、高血圧 (平均血圧 147 ± 81 mmHg)、および低酸素血症 (動脈血酸素分圧 [PaO_2] 43 ± 10 mmHg) を認めたが、キシラジン-アルファキサロンでは低酸素血症 (PaO_2 47 ± 7 mmHg) のみを認めた。キシラジンによる麻酔前投薬は、アルファキサロンの麻酔導入量を減少し、麻酔時間を延長したが、低酸素血症を防ぐために酸素吸入を考慮すべきである。

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1) Next-generation Sequencing Analysis of Bacterial Flora in Bovine
Prototheca Mastitic Milk.

Kano R, Kobayashi Y, Nishikawa A, **Murata R**, Itou T, Suzuki K, Kamata
H.

Med. Mycol. J. 59: E41-E46. 2018.

Original Article

Next-generation Sequencing Analysis of Bacterial Flora in Bovine *Prototheca* Mastitic Milk

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ABSTRACT

Prototheca zopfii is an achlorophyllic algae that causes bovine mastitis, resulting in a reduction in milk production and the secretion of thin, watery milk with white flakes. The aim of the present study was to evaluate the bacterial flora in the udder environment in protothecal mastitis. We used metagenomic next-generation sequencing (NGS) analysis to identify 16S rRNA genes from bacterial flora present in milk samples from protothecal mastitic dairy cows.

Seven clinical strains of *P. zopfii* genotype 2 were isolated from 7 milk samples from 7 cases (Holstein cow) of protothecal mastitis; another 9 milk samples were obtained from 9 normal Holstein cows. The samples were collected in 2017 from cows in one dairy located in the Kushiro region in Hokkaido, Japan, which had a history of protothecal mastitis infection.

The NGS produced 10,000 to 15,000 sequences in each DNA sample. To facilitate comparison, we grouped the sequencing results according to the culture-based protothecal mastitis diagnosis; sequences derived from the milk samples obtained from healthy cows were grouped separately.

Sequences classified as *Streptococcus* spp., *Pseudomonas* spp. and *Sphingomonas* spp., *Caulobacter segnis*, *Macroccoccus caseolyticus*, *Methylobacterium tarhaniae*, and *Sphingomonas leidyi* were the main sequences detected in the groups of samples from cows characterized by culture as having protothecal mastitis. Notably, *Calothrix desertica* (a cyanobacterium) sequences showed higher prevalence in these samples.

To our knowledge, this is the first study to report that *C. desertica* sequences, effectively absent in the samples derived from healthy cows, are detected at high prevalence in samples from protothecal mastitic animals.

Key words : bacterial flora, bovine *Prototheca* mastitis, next-generation sequencing, *Prototheca zopfii*

■ 論文題目

次世代シーケンスによるウシのプロトテカ乳房炎乳の細菌叢解析

■ 要 旨

メタゲノム次世代シーケンス (NGS) によって、ウシのプロトテカ乳房炎乳に存在する細菌叢の16S rRNA遺伝子を解析した。2017年、北海道釧路の1件の酪農家において、7つの *Prototheca zopfii* 遺伝子型2 臨床分離株を7つのプロトテカ乳房炎ホルスタインから、9株を健康なホルスタインから得た。

NGSでは各検体から10,000から15,000の配列が検出され、レンサ球菌や緑膿菌をはじめとした細菌種の存在が明らかとなった。とりわけ、*Calothrix desertica* (シアノバクテリア) の配列がプロトテカ乳房炎乳中に高率に存在することは新しい知見である。

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

- 1) Innate immune response of bovine mammary epithelial cells to
Mycoplasma bovis.

Gondaira S, Higuchi H, Iwano H, Nishi K, Nebu T, Nakajima K,
Nagahata H.

J. Vet. Sci. 19: 79-87. 2018

II. その他<Others>

Innate immune response of bovine mammary epithelial cells to *Mycoplasma bovis*

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Mycoplasma spp. are contagious bacteria, and mycoplasmal mastitis is a serious productivity problem on dairy farms. Bovine mammary epithelial cells (bMECs) have an important role in the elimination of pathogens, but the effect of *Mycoplasma bovis* on bMECs has not been fully described. To elucidate the immune response against intramammary infection by *M. bovis*, we undertook microarray analysis to examine and profile mRNA expression in bMECs after stimulation with *M. bovis*. We also compared the effects of *M. bovis*, *Staphylococcus aureus*, and *Escherichia coli* on immune-related mRNA expression in bMECs. Transcriptome analysis indicated a significant decrease in the level of mRNA-encoding lysine-specific demethylase 4D, suggesting that the immune response is suppressed by a decrease in histone demethylase activity. Interleukin (IL)-1 β , IL-6, tumor necrosis factor alpha, toll-like receptor (TLR) 2, and TLR4 mRNA expression levels were significantly increased in bMECs stimulated with heat-killed *M. bovis*, but the expression levels were lower than those following stimulation by heat-killed *S. aureus* or *E. coli*. Our results suggest that *M. bovis* weakly affects mRNA expression in bMECs compared to the effects of *E. coli* or *S. aureus*. Moreover, live *M. bovis* may induce suppression of the immune response in bMECs.

Keywords: *Mycoplasma bovis*, bovine mammary epithelial cells, cytokines, innate immunity, microarray analysis

■ 論文題目

Mycoplasma bovis に対するウシ乳腺上皮細胞の自然免疫応答

■ 要 旨

マイコプラズマ性乳房炎は酪農場において甚大な経済的損失をもたらす疾患である。ウシ乳腺上皮細胞は病原体に対する免疫応答に重要な役割を担っている。*Mycoplasma bovis* に対するウシ乳腺上皮細胞の免疫応答を明らかにするため、*M. bovis* 刺激における免疫応答に関連するmRNA発現量を定量した。また、*M. bovis* の比較対象として *Staphylococcus aureus* および *Escherichia coli* がウシ乳腺上皮細胞に及ぼす影響を検討した。*M. bovis* の生菌刺激に対するウシ乳腺上皮細胞の網羅的遺伝子発現解析結果から、ヒストン脱メチル化により免疫応答を活性化する lysine-specific demethylase 4D の発現量が無刺激対照と比較して有意に低下した。*M. bovis* の死菌刺激によるウシ乳腺上皮細胞における IL-1 β 、IL-6、TNF α 、TLR 2、TLR 4 の mRNA 発現量は有意に増加したが、*S. aureus* および *E. coli* による刺激と比較してそれらの発現量は低値であった。以上の結果から *M. bovis* は *S. aureus* および *E. coli* と比較して弱い免疫応答性を示し、*M. bovis* の生菌はウシ乳腺上皮細胞の免疫応答を抑制することが考えられた。

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1) Synchysis scintillans of the anterior chamber in a dog.

Sano Y, Okamoto M, Hayashi M, Sato T, Maehara S, Matsuda K
J. Vet. Med. Sci. 80: 1733-1736. 2018.

II . その他<Others>



NOTE

Pathology

Synchysis scintillans of the anterior chamber in a dog

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ABSTRACT. Intraocular cholesterol granuloma (CG) associated with synchysis scintillans (SS) was diagnosed in a 5-year-old spayed Shetland sheepdog. During the initial clinical examination, the patient exhibited SS in the anterior chamber. Canine SS is usually found in the vitreous cavity, and SS in the anterior chamber has not been described. Since canine SS has been reported to be a non-progressive condition, and its long-term clinical course has not been adequately documented. The present case report describes the long-term clinical course of a case of canine SS, in which SS occurred in the anterior chamber, leading to intraocular CG formation, and eventually glaucoma.

KEY WORDS: anterior chamber, dog, intraocular cholesterol granuloma, synchysis scintillans

■ 論文題目

犬における前房閃輝性融解の1例

■ 要 旨

5歳齢、避妊雌のシェットランド・シープドッグが、閃輝性融解に伴う眼内コレステリン肉芽腫と診断された。症例の臨床経過では、前房の閃輝性融解が認められていた。犬の閃輝性融解は通常硝子体腔に認められる疾患であり、前房の閃輝性融解の報告はなされていない。また、犬の閃輝性融解は非進行性疾患として報告されており、長期におよぶ臨床経過は十分に示されていない。本論文では、前房閃輝性融解に引き続き生じた眼内コレステリン肉芽腫の形成と、それにより緑内障となった長期におよぶ犬の閃輝性融解の症例を報告する。

■ 最終責任者 Yuto Sano (Corresponding Author)

動物生殖学 (Theriogenology)

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

- 1) Relationship between bovine endometrial thickness and plasma progesterone and estradiol concentrations in natural and induced estrus.
Sugiura T, Akiyoshi S, Inoue F, Yanagawa Y, Moriyoshi M, Tajima M, Katagiri S.
J. Reprod. Dev. 64: 135-143. 2018.

II. その他<Others>

—Original Article—

Relationship between bovine endometrial thickness and plasma progesterone and estradiol concentrations in natural and induced estrus

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Abstract. The objective of this study was to investigate cyclical changes in endometrial thickness in relation to progesterone (P_4) and estradiol-17 β (E_2) concentrations during natural and induced estrus in 15 cows. In the prostaglandin (PG) $F_{2\alpha}$ -induced estrus group, ultrasonography (USG) at 6-h intervals was used to determine endometrial thickness 48–24 h before the PGF_{2 α} treatment until 24 h after ovulation (ovulation = Day 0). In the natural estrus group, USG was performed every 48 h from Day 3 to Days 15–18 after the first ovulation, and then every 6 h until 24 h after ovulation. Endometrial thickness was standardized using Day 13 as a reference day. Blood was collected during every USG examination and plasma P_4 and E_2 concentrations were determined. Endometrial thickness of the induced estrus group ($n = 11$) was greater than that of the natural estrus group ($n = 9$) between 60 and 12 h before ovulation ($P < 0.05$). In the natural estrus group, prior to an increase in endometrial thickness, a decrease in P_4 and an increase in E_2 were detected. In the induced estrus group, based on the time of ovulation, an increase in endometrial thickness was detected at the same time of a decrease in P_4 before an increase in E_2 . These results suggest that decreases in P_4 concentrations may be a cue to changes in endometrial thickness, while increases in E_2 concentrations appear to sustain and/or enhance these changes.

Key words: Dairy cows, Edema, Endometrium, Estrous cycle, Ultrasonography

(J. Reprod. Dev. 64: 135–143, 2018)

■ 論文題目

自然発情ならびに誘起発情における乳牛の子宮内膜厚と血中プロジェステロンおよびエストラジオール濃度との関係

■ 要 旨

本研究の目的は、15頭の乳牛の自然発情ならびに誘起発情における子宮内膜厚の変化と血中プロジェステロン (P_4) およびエストラジオール (E_2) 濃度との関係を調査することであった。プロスタグランジン (PG) $F_{2\alpha}$ による誘起発情群では、PGF_{2 α} 投与の24～48時間前より超音波検査により子宮内膜厚を測定し、排卵後24時間まで6時間間隔で実施した。自然発情群では、排卵後3～15日まで超音波検査を48時間間隔で実施し、その後自然発情を経て排卵後24時間までは6時間間隔で実施した。子宮内膜厚は、排卵後13日目を基準に標準化して表した。超音波検査と同時に血液を採取し、血漿中の P_4 および E_2 濃度を測定した。誘起発情群の子宮内膜厚は排卵前60～12時間の間で自然発情群よりも有意に厚くなった ($P < 0.05$)。自然発情群では、 P_4 濃度の低下に次いで E_2 濃度が上昇したのち、子宮内膜厚が増加した。誘起発情群では、排卵を基準にした場合、 P_4 濃度の低下と同時に E_2 濃度の上昇と子宮内膜厚の増加が認められた。これらの結果より、 P_4 濃度の低下は子宮内膜厚増加の役割を果たし、 E_2 は子宮内膜厚増加の促進に作用すると考えられる。

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II. その他<Others>

- 1) Effects of immunosuppressive prednisolone therapy on pancreatic tissue and concentration of canine pancreatic lipase immunoreactivity in healthy dogs.

Ohta H, Kojima K, Yokoyama N, Sasaki N, Kagawa Y, **Hanazono K**, Ishizuka T, Morishita K, Nakamura K, Takagi S, Takiguchi M.
Can J Vet Res. 82: 278-286. 2018.

- 2) Mandibular vascular hamartoma in a cat.

Takagi S, Kagawa Y, **Hanazono K**, Murakami S, Deguchi T, Izumi Y, Hosoya K, Kim S, Okumura M.
J Vet Med Sci. 80: 1456-1458. 2018.

Effects of immunosuppressive prednisolone therapy on pancreatic tissue and concentration of canine pancreatic lipase immunoreactivity in healthy dogs

Hiroshi Ohta, Kazuki Kojima, Nozomu Yokoyama, Noboru Sasaki, Yumiko Kagawa, Kiwamu Hanazono, Tomohito Ishizuka, Keitaro Morishita, Kensuke Nakamura, Satoshi Takagi, Mitsuyoshi Takiguchi

Abstract

The objective of this study was to examine the effects of immunosuppressive prednisolone therapy on pancreatic tissue and the concentration of serum canine pancreatic lipase immunoreactivity (cPLI) in healthy dogs. Six healthy beagle dogs were subcutaneously administered an immunosuppressive dose of prednisolone [4 mg/kg body weight (BW)] once daily for either 2 or 3 weeks. Serum cPLI concentration was measured before and after treatment. Ultrasonographic examination of the pancreas and laparoscopic biopsy and histopathological examination of the right pancreatic lobe and the liver were also conducted before and after treatment. The expression of pancreatic lipase messenger ribonucleic acid (mRNA) in the pancreas and liver was examined by polymerase chain reaction (PCR). Although the serum cPLI concentration was significantly higher on day 14 and on the day of the second laparoscopy than before treatment, it was classified as normal ($\leq 200 \mu\text{g/L}$) in 5 dogs and as abnormal ($\geq 400 \mu\text{g/L}$) in only 1 dog. None of the 6 dogs showed clinical signs of pancreatitis during the study period. After treatment, ultrasonographic examination of the pancreas showed no changes except for a hypoechoic pancreas in 1 dog. Histopathological examination of the right pancreatic lobe in all dogs showed no evidence of pancreatitis after treatment. Pancreatic lipase mRNA expression was detected in the pancreas, but not in the liver, before and after treatment. The administration of 4 mg/kg BW per day of prednisolone for 2 or 3 weeks increased the serum cPLI concentration without clinical signs of pancreatitis, although an abnormal cPLI concentration ($\geq 400 \mu\text{g/L}$) was observed in only 1 dog. No ultrasonographic or histological evidence of pancreatitis was observed in any of the dogs.

■ 論文題目

健康犬における免疫抑制プレドニゾロン療法による膵臓組織と膵リパーゼ活性への影響

■ 要 旨

健康犬6頭にプレドニゾロン4mg/kgを2または3週間経口投与し、犬膵特異的リパーゼ、膵臓の超音波画像、腹腔鏡により採取した膵臓の病理組織検査、PCRによる肝臓、膵臓におけるリパーゼ発現への影響を評価した。プレドニゾロン投与2週間後には犬膵特異的リパーゼは投与前に比較して有意に上昇したが、超音波検査、病理組織検査において膵炎を示す像は認めなかった。またPCRにおいてリパーゼ発現は膵臓には認められるものの、肝臓には認めなかった。プレドニゾロン投与における犬膵特異的リパーゼの上昇は膵炎の存在を示すものではなかった。

■ 最終責任者 Hiroshi Ohta (First Author and Corresponding Author)



NOTE

Surgery

Mandibular vascular hamartoma in a cat

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ABSTRACT. A 10-year-old cat presented for evaluation with a 1-month history of salivation and oral bleeding. A right mandibular mass was palpated and computed tomography examination revealed entire bone proliferation. Mandibular bone biopsy was performed, and histopathological diagnosis was vascular hamartoma. The cat suddenly died on day 140.

KEY WORDS: cat, hamartoma, mandibular

■ 論文題目

下顎に発生した血管過誤腫の猫の1例

■ 要 旨

1ヶ月前より流涎と口腔出血を伴う猫が来院した。触診とCT検査により右下顎骨に骨増生を伴う腫瘤を認めた。生検を実施したところ病理組織検査の結果は血管過誤腫であった。来院から140日後に本症例は突然死した。

■ 最終責任者 Satoshi Takagi (Corresponding Author)

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

- 1) Corticosterone biosynthesis in mouse clonal myoblastic C2C12 cells.
Fujiki J., Maeda N, Sato M, Ieko T, Inoue H, Iwasaki T, Iwano H, Yokota H.
Steroids. 138: 64-71. 2018.
- 2) Single amino acid mutation in Dengue virus NS4B protein has opposing effects on viral proliferation in mammalian and mosquito cells.
Fujiki J., Nobori H, Sato A, Sasaki M, Carr M, Hall WW, Orba Y, Sawa H.
Jpn. J. Infect. Dis. 71: 448-454. 2018.
- 3) Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent Staphylococcus aureus Bacteriophage.
Fujiki J., Nakamura T, Furusawa T, Ohno H, Takahashi H, Kitana J, Usui M, Higuchi H, Tanji Y, Tamura Y, Iwano H.
Pharmaceuticals (Basel). 11(1). pii: E25. 2018.

II. その他<Others>

- 1) Bacteriophage ΦSA012 has a broad host range against Staphylococcus aureus and effective lytic capacity in a mouse mastitis model.
Iwano H, Inoue Y, Takasago T, Kobayashi H, Furusawa T, Taniguchi K,
Fujiki J., Yokota H, Usui M, Tanji Y, Hagiwara K, Higuchi H, Tamura Y.
Biology (Basel). 7(1). pii: E8 2018.

2) Biotransformation of Bisphenol A and Adverse Effects on the Next Generation.

Iwano H, Inoue H, Nishikawa M, **Fujiki J**, Yokota H.

IntechOpen, Book title: Endocrine Disruptors (Chapter 5)

doi:10.5772/intechopen.78275



Corticosterone biosynthesis in mouse clonal myoblastic C2C12 cells

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Corticosterone
Pregnenolone
Sulfate
Conjugate

ABSTRACT

Corticosterone (CORT), the major glucocorticoid in rodents, is secreted from the adrenal gland, affects various organs in the body and regulates energy metabolism as a stress response. Although local steroidogenesis of androgens and estrogens in skeletal muscles has been previously reported, local CORT synthesis in skeletal muscle remains unconfirmed. In the present study, we investigated steroidogenic activities in a clonal myoblastic cell line, C2C12 cells. Three enzymes involved in CORT synthesis, 3 β -hydroxysteroid dehydrogenase (3 β -HSD), cytochrome P450c21 and cytochrome P45011 β , were identified in C2C12 cells by detecting the enzymatic reaction products with LC-MS/MS analysis. Only one enzyme that mediates cholesterol cleavage was not detected in the cells. After the addition of pregnenolone-sulfate conjugates to the cell culture medium, pregnenolone was detected and increased according to the incubation time. In conclusion, CORT synthesis occurs in C2C12 cells, and it is suggested that the initial steroidogenesis substrate is the pregnenolone-sulfate conjugate.

■ 論文題目

マウス由来 C2C12 筋芽細胞におけるコルチコステロン生合成

■ 要 旨

げっ歯類における主要な糖質コルチコイドであるコルチコステロン (CORT) は、副腎皮質から分泌され、体内の様々な臓器に作用することでストレス応答としてエネルギー代謝を制御している。骨格筋におけるアンドロゲン、エストロゲンの局所的なステロイド合成が報告されている一方で、骨格筋局所的なCORT合成は未だ報告されていない。そこで本研究では、マウス由来C2C12筋芽細胞におけるステロイド合成能について解析を実施した。LC-MS/MSを用いた酵素活性試験において、CORT生合成に必須である3種類のステロイド酵素 (3 β -HSD, P450c21, P45011 β) の活性が認められた。一方で、ステロイド合成においてコレステロールの側鎖を切断しプレグネノロンを合成する酵素 (P450scc) の活性は認められなかった。また、C2C12筋芽細胞の培養上清にプレグネノロン硫酸抱合体を添加し培養した結果、培養上清から脱抱合を受けたプレグネノロンが経時的に検出された。以上の結果から、C2C12筋芽細胞においてCORTが合成され、その出発基質としてプレグネノロン硫酸抱合体が機能していることが示唆された。

■ 最終責任者 Hiroshi Yokota (Corresponding Author)

Original Article

Single Amino Acid Mutation in Dengue Virus NS4B Protein Has Opposing Effects on Viral Proliferation in Mammalian and Mosquito Cells

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SUMMARY: Dengue virus (DENV) has a considerable impact on the global health and is known to cause morbidity and mortality every year. By passaging DENV2 in baby hamster kidney (BHK)-21 cells, we isolated a mutant clone of DENV2 that shows rapid cytopathic effects in BHK-21 cells as compared with that showed by the parent strain. To investigate the relationship between amino acid mutations and proliferation activity of the isolated DENV2 clone, we performed full genome sequencing and identified 3 amino acid mutations in the coding region, the envelope T120K, NS4A M85T, and NS4B G124A. Genetically modified recombinant DENV2 (rDENV2) carrying the NS4A M85T and NS4B G124A mutations produced higher titers of progeny virus in BHK-21, Vero, and Huh-7 cells than in the wild-type (WT) rDENV2. rDENV2 with mutations at NS4A M85T and NS4B G124A failed to produce any plaques in C6/36 mosquito cell lines. Furthermore, rDENV2 possessing only the NS4B G124A mutation showed no plaque production in C6/36 cells but had higher viral titers in Vero and Huh-7 cells than the WT rDENV2 had. Our results clearly showed that the DENV2 NS4B G124A mutation has opposing effects on the virus proliferation in mosquito and certain mammalian cell lines.

■ 論文題目

デングウイルス非構造タンパク質 NS4B における 1 アミノ酸変異は、哺乳類由来細胞と蚊由来細胞において相反するウイルス増殖性をもたらす

■ 要 旨

デングウイルス 2 型 (DENV2) を BHK-21 細胞を用いて継代することで、親株に比べて急速な細胞変性効果を示す DENV2 変異株を単離した。単離された DENV2 変異株と変異株が有するアミノ酸変異の関係を精査するため全ゲノム解析を実施した結果、DENV2 変異株のコード領域における 3 つのアミノ酸変異 (エンベロープ T120K, NS4A M85T, NS4B G124A) を特定した。NS4A M85T および NS4B G124B を含む組換え DENV2 は、WT に比べて哺乳類由来である BHK-21, Vero, Huh-7 細胞において高い感染価を示した。一方で、蚊由来細胞である C6/36 細胞ではプラーク形成能が欠損していた。さらに、NS4B G124A のみを含む組換え DENV2 は、C6/36 においてプラークの形成を示さなかった。一方で、Vero, Huh-7 細胞では WT に比べて高い感染価を示した。以上の結果は、DENV2 NS4B における 1 アミノ酸変異 (G124A) が、哺乳類由来細胞と蚊由来細胞において相反するウイルス増殖性をもたらすことを示していると考えられる。

■ 最終責任者 Hirofumi Sawa (Corresponding Author)



Article

Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent *Staphylococcus aureus* Bacteriophage

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Abstract: Antibiotic-resistant bacteria (ARB) have spread widely and rapidly, with their increased occurrence corresponding with the increased use of antibiotics. Infections caused by *Staphylococcus aureus* have a considerable negative impact on human and livestock health. Bacteriophages and their peptidoglycan hydrolytic enzymes (endolysins) have received significant attention as novel approaches against ARB, including *S. aureus*. In the present study, we purified an endolysin, Lys-phiSA012, which harbors a cysteine/histidine-dependent amidohydrolase/peptidase (CHAP) domain, an amidase domain, and a SH3b cell wall binding domain, derived from a polyvalent *S. aureus* bacteriophage which we reported previously. We demonstrate that Lys-phiSA012 exhibits high lytic activity towards staphylococcal strains, including methicillin-resistant *S. aureus* (MRSA). Analysis of deletion mutants showed that only mutants possessing the CHAP and SH3b domains could lyse *S. aureus*, indicating that lytic activity of the CHAP domain depended on the SH3b domain. The presence of at least 1 mM Ca²⁺ and 100 μM Zn²⁺ enhanced the lytic activity of Lys-phiSA012 in a turbidity reduction assay. Furthermore, a minimum inhibitory concentration (MIC) assay showed that the addition of Lys-phiSA012 decreased the MIC of oxacillin. Our results suggest that endolysins are a promising approach for replacing current antimicrobial agents and may contribute to the proper use of antibiotics, leading to the reduction of ARB.

Keywords: antibiotic resistant; multidrug resistant; antimicrobial agent; phage therapy; bacteriophage; endolysin; staphylococci; *Staphylococcus aureus*

■ 論文題目

多価黄色ブドウ球菌ファージ由来 LysK 様エンドライシン (Lys-phiSA012) の機能解析

■ 要 旨

抗生物質の使用の増加に伴い、薬剤耐性菌は世界的に急速な広がりを見せている。特に、黄色ブドウ球菌 (*Staphylococcus aureus*: SA) は、ヒトおよび家畜の健康において甚大な被害をもたらしている。また、バクテリオファージ、およびバクテリオファージ由来溶菌酵素 (エンドライシン) は、SAを含む薬剤耐性菌に対する新たなアプローチとして大きな注目を集めている。そこで本研究では、我々がこれまでに報告した多価バクテリオファージからエンドライシン (Lys-phiSA012) を単離・精製し、その溶菌機能について解析を実施した。Lys-phiSA012は、MRSAを含む複数のSAに対して高い溶菌活性を示した。また、Lys-phiSA012が有する2つの触媒ドメイン (CHAP, Amidaseドメイン)、1つの菌体認識ドメイン (SH3bドメイン) の欠失変異体を構築し、溶菌活性を評価した結果、SH3bドメインに依存的なCHAPドメインによる溶菌活性が示唆された。また、1 mMのカルシウムイオンおよび100 μ Mの亜鉛イオンの添加は、Lys-phiSA012の溶菌活性を亢進させた。さらに、Lys-phiSA012の併用は、オキシシリンのMICを減少させた。以上の結果から、エンドライシンは薬剤耐性菌の抑制に貢献する有用なアプローチであることが示唆された。

■ 最終責任者 Hidetomo Iwano (Corresponding Author)

Article

Bacteriophage Φ SA012 Has a Broad Host Range against *Staphylococcus aureus* and Effective Lytic Capacity in a Mouse Mastitis Model

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Abstract: Bovine mastitis is an inflammation of the mammary gland caused by bacterial infection in dairy cattle. It is the most costly disease in the dairy industry because of the high use of antibiotics. *Staphylococcus aureus* is one of the major causative agents of bovine mastitis and antimicrobial resistance. Therefore, new strategies to control bacterial infection are required in the dairy industry. One potential strategy is bacteriophage (phage) therapy. In the present study, we examined the host range of previously isolated *S. aureus* phages Φ SA012 and Φ SA039 against *S. aureus* strains isolated from mastitic cows. These phages could kill all *S. aureus* (93 strains from 40 genotypes) and methicillin-resistant *S. aureus* (six strains from six genotypes) strains tested. Using a mouse mastitis model, we demonstrated that Φ SA012 reduced proliferation of *S. aureus* and inflammation in the mammary gland. Furthermore, intravenous or intraperitoneal phage administration reduced proliferation of *S. aureus* in the mammary glands. These results suggest that broad host range phages Φ SA012 is potential antibacterial agents for dairy production medicine.

Keywords: bacteriophage; mastitis; *Staphylococcus aureus*

■ 論文題目

バクテリオファージΦSA012は黄色ブドウ球菌に対し広範な溶菌スペクトルを有し、マウス乳房炎モデルにおいて効果的な溶菌活性を示す

■ 要 旨

ウシ乳房炎は細菌感染に起因する乳腺の炎症であり、その治療のために抗生物質を連用することからも酪農産業において最も大きな経済的損失である。また、黄色ブドウ球菌 (*Staphylococcus aureus*: SA) はウシ乳房炎の主な原因菌であり、薬剤耐性化も問題となっている。この様に、細菌感染症を制御する新たな抗菌戦略が求められる中、ファージセラピーはその一つとして注目されている。本研究では、我々がこれまでに分離したバクテリオファージ (ΦSA012およびΦSA039) の乳房炎由来SAに対する宿主域を精査した。これらのバクテリオファージは、40パタンの遺伝子型に分類される93株のSAを溶菌し、さらに6パタンの遺伝子型に分類される6株のMRSAも溶菌した。さらに、マウス乳房炎モデルにおいて、ΦSA012の投与は、SAの増殖と乳腺における炎症を抑制した。これらの結果は、広範な宿主域を示すΦSA012が酪農産業における有用な抗菌性物質となる可能性を示唆している。

■ 最終責任者 Hidetomo Iwano (First Author and Corresponding Author)

Biotransformation of Bisphenol A and Its Adverse Effects on the Next Generation

Hidetomo Iwano, Hiroki Inoue, Miyu Nishikawa,
Jumpei Fujiki and Hiroshi Yokota

Additional information is available at the end of the chapter

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Abstract

Although we are exposed to many chemical substances in routine daily life, the body has metabolic systems capable of detoxifying and eliminating these chemicals. Bisphenol A (BPA) is an endocrine disrupter of great concern because of its estrogenic activity, but studies have indicated no severe adverse effects in adult rodents exposed to BPA due to metabolic detoxification. BPA is metabolized by glucuronidation mediated by phase II enzymes such as UDP-glucuronosyltransferase. Numerous recent studies in rodents have indicated that maternal BPA exposure causes adverse effects in offspring. It was also shown that bisphenol analogs are efficiently absorbed via the oral route and distributed to the reproductive tract in pregnant rats, with its residue capable of crossing the placental barrier in the late stage of gestation. Both animal and human studies have demonstrated that BPA and the BPA metabolite BPA-GA are detectable in fetal and amniotic fluid, suggesting the presence of a placental transfer mechanism. In this review, we discuss the pharmacokinetics of BPA, particularly its (1) metabolism and disposition in the intestine, (2) metabolism and disposition in the liver, and (3) transfer from maternal tissues to the fetus.

Keywords: bisphenol A, UDP-glucuronosyltransferase (UGT), multidrug resistance-associated protein (MRP), organic anion-transporting polypeptide (Oatp), xenobiotic-metabolizing enzymes (XMEs), liver perfusion, β -glucuronidase

■ 論文題目

ビスフェノール A の生体内代謝と次世代への悪影響について

■ 要 旨

エストロゲン活性のあるビスフェノール A (BPA) は、内分泌かく乱物質の 1 つであるが、正常な成体においては薬物代謝酵素第 II 相の UDP グルクロン酸転移酵素により容易に解毒代謝される。一方で、妊娠期の BPA の投与は、次世代に様々な悪影響をあたえること、さらに妊娠後期には BPA 代謝物が胎盤を通過し、胎仔側へ移行していることなどが報告され、BPA の代謝動態と次世代への影響の関連性が重要視されてきている。本総説では、BPA の代謝動態を中心に、特に、①小腸での移行と代謝動態、②肝臓における代謝動態、③胎仔への移行 について解説した。

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

- 1) Structural determination, distribution, and physiological actions of ghrelin in the guinea-pig.

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- 2) Alpha_{1B}-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atrium.

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Research Article

Structural determination, distribution, and physiological actions of ghrelin in the guinea pig

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cDNA cloning
Functional characterization
Tissue distribution

ABSTRACT

We identified guinea pig ghrelin (gp-ghrelin), and examined its distribution and physiological actions in the guinea-pig. Gp-ghrelin is a 28-amino acid peptide (GASFR SPEHH SAQQR KESRK LPAKI QPR); seven amino acids are different from that of rat ghrelin at positions 2, 5, 10, 11, 19, 21, and 25, which include the conserved region known in mammals. The third serine residue is mainly modified by *n*-decanoyl acid. Both gp-ghrelin and rat ghrelin increased intracellular Ca^{2+} concentration of HEK293 cells expressing guinea pig growth hormone secretagogue receptor 1a (GHS-R1a), and the affinity of gp-ghrelin was slightly higher than that of rat ghrelin. In addition, gp-ghrelin was also effective in CHO cells expressing rat GHS-R1a with similar affinity to that of rat ghrelin. Gp-ghrelin mRNA was predominantly expressed in the stomach, whereas the expression levels in other organs was low. High levels of GHS-R1a mRNA expression were observed in the pituitary, medulla oblongata, and kidney, while medium levels were noted in the thalamus, pons, olfactory bulb, and heart. Immunohistochemistry identified gp-ghrelin-immunopositive cells in the gastric mucosa and pancreas. Intraperitoneal injection of gp-ghrelin increased food intake in the guinea pig. Gp-ghrelin did not cause any mechanical responses in isolated gastrointestinal smooth muscles *in vitro*, similar to rat ghrelin. In conclusion, the N-terminal structures that are conserved in mammals were different in gp-ghrelin. Moreover, the functional characteristics of gp-ghrelin, other than its distribution, were dissimilar from those in other Rodentia.

■ 論文題目

モルモットグレリンの同定とその分布、生理的役割に関する研究

■ 要 旨

本論文ではこれまでアミノ酸構造が同定されていなかったモルモットグレリンを分子生物学的手法と database を用いて解析した。構造は GASFR SPEHH SAQQR KESRK LPAKI QPR であり、28 個のアミノ酸からなり、3 位のセリンはデカン酸により修飾を受けていた。モルモットグレリンは他の動物種と同様に胃粘膜に最も発現が認められた。合成モルモットグレリンは、モルモットおよびラットの GHS-R1a をほぼ同じ親和性で興奮させた。摘出モルモット消化管標本ではグレリンによって著明な収縮が引き起こされなかった。その理由としては消化管の GHS-R1a 発現量が極めて低いことが原因と考えられた。グレリンによりモルモットで摂餌量と血中グルコースの増加が認められた。今回同定したモルモットグレリンは他の哺乳動物と異なる N 末端構造（特に 10 位まで）を有しており、今後モルモットにおけるグレリン研究に有用であると考えられた。

■ 最終責任者 Takio Kitazawa (Corresponding Author)



Cardiovascular pharmacology

Alpha_{1B}-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atriumShuangyi Zhang^a, Rena Takahashi^b, Natsumi Yamashita^a, Hiroki Teraoka^a, Takio Kitazawa^{a,b,*}^a Veterinary Pharmacology, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan^b Comparative Animal Pharmacology, Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

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 α_1 -adrenoceptor
 α_{1B} -adrenoceptor

ABSTRACT

Modulation of cardiac contractility by α -adrenoceptor is well known in several mammals. Mice are useful experimental animals, but α -adrenoceptor-mediated responses have been examined only in the ventricles. To determine function of α -adrenoceptors in the atrium, effects of α -adrenoceptor agonists on spontaneous contraction and electrical-field stimulation (EFS)-induced contraction were examined. In addition, expression of α_{1A} , α_{1B} , α_{1D} and β_1 -adrenoceptor mRNAs were examined. In the right atrium, noradrenaline and phenylephrine caused positive inotropic and positive chronotropic actions. However, methoxamine, clonidine and xylazine caused positive inotropic actions, but contractile frequency was decreased at high concentrations. Phenylephrine-induced positive inotropic and chronotropic actions were partially decreased by propranolol, and both actions remained in the presence of propranolol were inhibited by phentolamine or prazosin. A low concentration of silodosin (< 100 nM) did not but a high concentration (1 μ M) decreased the phenylephrine-induced chronotropic actions. Negative chronotropic actions of clonidine and xylazine were insensitive to propranolol and phentolamine. The EFS-induced contraction of the left atrium was potentiated by noradrenaline, phenylephrine and methoxamine but was not changed by clonidine or xylazine. Propranolol partially decreased the actions of phenylephrine, and prazosin caused additional inhibition. Expression of β_1 -, α_{1A} -, α_{1B} - and α_{1D} -adrenoceptor mRNAs was found in the atrium, and the expression level of β_1 -adrenoceptor was the highest. Of α_1 -adrenoceptors, the expression level of α_{1B} was higher than that of α_{1A} and α_{1D} . In conclusion, α_{1B} -adrenoceptors are expressed in the mouse atrium and mediate both positive chronotropic and inotropic actions. In contrast, the α_2 -adrenoceptor is not functional in the isolated atrium.

■ 論文題目

Alpha_{1B}-アドレナリン受容体を介したマウス心房の陽性変力および陽性変時作用

■ 要 旨

マウス心房筋収縮に影響を与える α 受容体について薬理学的な解析を行った。Alpha-1 受容体作動薬の phenylephrine は右心房標本の収縮高と心拍数を濃度依存性に増加させた。一方、 α -2 受容体作動薬の clonidine, xylazine は高濃度では収縮頻度を低下させ、収縮高を増大させた（反応は α , β いずれの遮断薬でも抑制されなかった）。Phenylephrine の作用は一部、propranolol で抑制されたが、残存する作用は prazosin で減弱した。このことは、 α -1 受容体を介した収縮高、心拍数増大機構がマウス心房筋に存在することを示している。Alpha-1 受容体の遺伝子発現をマウス心臓の各部位（右心房、左心房、心室）で比較すると $1B > 1A = 1D$ の順となった。Silodosin は、低濃度では phenylephrine の作用(propranolol 処置下)に影響を与えなかったが、高濃度(1 μ M)では phenylephrine の作用を抑制した。このことから、収縮高・心拍数の増大に関与する α -1 受容体は、1B タイプであることが示唆された。

■ 最終責任者 Takio Kitazawa (Corresponding Author)



Contamination Status of Seven Elements in Hooded Cranes Wintering in South-West Kyushu, Japan: Comparison with Red-Crowned Cranes in Hokkaido, Japan

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Abstract

The hooded crane is designated as an endangered species. The cranes breed primarily in wetlands in southeast Russia and China in summer. Most of the hooded crane population winters in the Izumi plain in Japan. It is difficult to know the contamination status of their habitat because of their vast breeding area. We determined the levels of Cd, Pb, As, (total) Hg, Se, Zn, and Cu in the liver, kidney, and muscle of hooded cranes that were found dead in Izumi in the periods 2003–2006 and 2014–2015 compared with the levels in red-crowned cranes in Hokkaido, Japan, as the only cranes in which these elements had been studied extensively. There were no notable differences between levels of the seven elements in the two periods. Overall, tissue levels of the elements examined in hooded cranes were comparable to those in red-crowned cranes except for Hg and Se. Tissue levels of Hg and Se were clearly lower in hooded cranes than in red-crowned cranes that were found dead from 2000. One lead poisoning case was confirmed. The results suggest that Hooded cranes wintering in Izumi are not extensively contaminated with the seven elements examined.

■ 論文題目

九州南西部で越冬するナベヅルにおける 7 元素の汚染状況：北海道に生息するタンチョウとの比較

■ 要 旨

ナベヅルは主にアムール川流域を含むロシア、中国、モンゴルで繁殖し、大部分が出水（鹿児島）で越冬する渡り鳥である。アムール川やその周辺は重金属を含む環境汚染が問題になっているが、ナベヅルおよびその営巣地の汚染状況に関する情報は全く得られていない。本研究では、2003–06年、2014–15年（以下2000年代、2010年代）に出水で死亡したナベヅルの肝臓、腎臓、骨格筋の代表的有害元素（Hg、Cd、Pb、As）と一部の必須元素含量（Se、Cu、Zn）を測定した。2000年代のナベヅルの肝臓で鉛中毒と思われる成鳥が1羽確認されたが、Hg、Cd、Pb、Asとも清浄値の範囲であった。タンチョウと比較すると、総じてほぼ同程度であったが、Hgについてのみナベヅルはいずれの臓器とも顕著に低かった。2000年代から2010年代にかけてナベヅル臓器でHgやCd含量の僅かな変化が認められたが他の元素とも問題と考えられるレベルではなかった。今後とも継続的な検討が望まれる。

■ 最終責任者 Hiroki Teraoka (First Author and Corresponding Author)

Limited expression of functional cytochrome p450 2c subtypes in the liver and small intestine of domestic cats

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ABSTRACT

1. Compared to information for herbivores and omnivores, knowledge on xenobiotic metabolism in carnivores is limited. The cytochrome P450 2C (CYP2C) subfamily is recognized as one of the most important CYP groups in human and dog. We identified and characterized CYP2C isoforms and variants in cat, which is an obligate carnivore.
2. Quantitative RT-PCR and immunoblot analyses were carried out to evaluate the expression of CYP2C in the liver and small intestine. A functional CYP2C isoform was heterologously expressed in yeast microsomes to determine the enzymatic activity.
3. Cat had two CYP2C genes, 21 and 41, in the genome; however, CYP2C21P was a pseudogene that had many stop codons. Three splicing variants of CYP2C41 were identified (v1-v3), but only one of them (v1) showed a complete deduced amino acid sequence as CYP2C protein. Transcripts of feline CYP2C41v1 were detected but the amounts were negligible or very small in the liver and small intestine. Immunoreactivity to an antihuman CYP2C antibody was confirmed in the recombinant feline CYP2C41v1 but not in the feline liver.
4. Recombinant feline CYP2C41v1 metabolized several substrates, including dibenzylfluorescein that is specific to human CYP2C.
5. The results suggest a limited role of functional CYP2C isoforms in xenobiotic metabolism in cat.

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■ 論文題目

酵素活性をもつシトクローム P450 2C はネコ肝臓にほとんど発現していない

■ 要 旨

シトクロームP450 (CYP) 2Cはヒトやイヌにおいて、ワルファリンやトルブタミド、NSAIDsなどの薬物代謝において重要な酵素である。本研究では、ネコのCYP2C分子種を初めて同定し、臓器における発現量と代謝酵素活性を検討した。その結果、CYP2C21PとCYP2C41の2種類を新規に同定した。CYP2C41は3種のスプライスバリエントであり、v1のみ代謝酵素活性を示した。CYP2C41v1は肝臓や小腸で発現していたが、CYP他の分子種に比べて発現量は極めて少なかった。ネコCYP2C21v1に反応するヒトCYP2C抗体を用いたりコンビナント免疫ブロットでもCYP2Cを検出できなかった。ネコの薬物代謝におけるCYP2Cの関与は少ないことが示唆された。このことはネコのNSAIDs代謝能が低いことと関係があるかもしれない。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)



Aroclor 1254 and BDE-47 inhibit dopaminergic function manifesting as changes in locomotion behaviors in zebrafish embryos

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HIGHLIGHTS

- Aroclor 1254 and BDE-47 caused hyperactivities in zebrafish embryos.
- Inhibition of tyrosine hydroxylase and VMAT2 also induced hyperactivities.
- Hyperactivities by these treatments were inhibited by precursors of dopamine synthesis.
- Organohalogen reduced dopamine contents and increased the DOPAC/dopamine ratio.

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ABSTRACT

Contamination with polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the environment is a major concern due to their persistent bioaccumulative toxicity that can disturb neurobehavioral functions including movements. Recently, it was reported that some PBDE including BDE-47 stimulates locomotor activities of zebrafish embryos by unknown mechanism. In this study, motor movements of the zebrafish embryo were used as a model system to evaluate the neuronal toxicity of a non-coplanar PCB-dominant mixture (Aroclor 1254) and BDE-47. Both organohalogen increased tail shaking and rotation of embryos in a concentration-dependent manner. Chemical inhibition and gene knock-down of tyrosine hydroxylase and vesicular monoamine transporter 2 (VMAT2) also induced hyperactivities. Hyperactivities induced by these treatments were all inhibited by supplementation of L-tyrosine and L-dopa, precursors of dopamine synthesis. Both organohalogen reduced dopamine contents and increased the 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine ratio in whole embryos. The results suggest that functional inhibition of dopaminergic neurons is involved in hyperactivities of zebrafish embryos caused by Aroclor 1254 and BDE-47.

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■ 論文題目

PCBs と臭素化難燃物はドーパミン神経機能を抑制して発達中ゼブラフィッシュの運動活性を変化させる

■ 要 旨

PCBsおよびポリ臭素化難燃物（PBDEs）などのポリハロゲン化難燃物は有毒な難分解性化学物質であることから、使用が禁止または制限されているが、その毒性機序の多くは不明である。本研究では、ポリハロゲン化難燃物がドーパミン合成を低下させることにより、発生初期のゼブラフィッシュの自発運動を促進させることを示した。

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- 1) Generation and validation of novel anti-bovine CD163 monoclonal antibodies ABM-1A9 and ABM-2D6.

Shimamoto Y, Nio-Kobayashi J, Watarai H, Nagano M, Saito N, Takahashi E, Higuchi H, Kobayashi A, Kimura T, Kitamura H.

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Generation and validation of novel anti-bovine CD163 monoclonal antibodies ABM-1A9 and ABM-2D6

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ABSTRACT

The scavenger receptor CD163 is widely used as a cell signature for alternatively active “M2” macrophages in mammals. In this study, we generated two monoclonal antibodies, ABM-1A9 and ABM-2D6, against the extracellular region of bovine CD163. Conventional Western blotting using the antibodies yielded immunoreactive bands of bovine CD163 at 130 and 150 kDa in non-reduced and reduced spleen lysates, respectively. The minimum limit of detectable concentration of both antibodies was relatively lower (5.0 ng/mL) than that of the anti-human CD163 monoclonal antibody AM-3K (> 1.0 µg/mL), which has been used previously for the detection of bovine CD163. An immunohistochemical study using formalin-fixed paraffin-embedded sections revealed that ABM-1A9 and ABM-2D6 clearly stained some Iba1⁺ macrophages in the lymph nodes of cattle with mastitis. Moreover, the CD163-stained macrophages were frequently observed engulfing leukocytes. ELISA using ABM-2D6 distinguished levels of circulating soluble CD163 in healthy cattle (less than 16.9 pmol/mL) and cattle with mastitis (more than 33.7 pmol/mL). These new monoclonal antibodies can be used in the diagnosis and evaluation of inflammatory disease prognosis in cattle with immunohistological analyses and blood test applications.

■ 論文題目

新規の抗牛CD163モノクローナル抗体ABM-1A9およびABM-2D6の作製と検証

■ 要 旨

スカベンジャー受容体CD163は、哺乳動物における選択的に活性な「M2」マクロファージに対するセルグナチャーとして広く使用されている。本研究では、ウシCD163の細胞外領域に対する2つのモノクローナル抗体、ABM-1A9とABM-2D6を作製した。抗体を用いたウエスタンブロッティングは、非還元および還元それぞれの脾臓ライセート中に130および150kDaのウシCD163免疫反応性バンドを生じた。両抗体の検出可能な最小限界濃度は、ウシのCD163検出のために以前に使用されていた抗ヒトCD163モノクローナル抗体AM-3Kの濃度 (> 1.0 µg/mL) よりも相対的に低かった (5.0ng/mL)。ホルマリン固定パラフィン包埋切片を用いた免疫組織化学的研究により、ABM-1A9とABM-2D6は、乳房炎の牛のリンパ節にあるいくつかのIba1 +マクロファージを明確に染色することが判明し、さらにCD163に染色されたマクロファージは白血球の取り込みが頻繁に観察された。ABM-2D6を用いたELISAは、健康な牛 (16.9pmol / mL未満) と乳房炎の牛 (33.7pmol / mL以上) の循環血液中の可溶性CD163のレベルを識別した。これらの新しいモノクローナル抗体は、免疫組織学的分析および血液検査において、ウシの炎症性疾患の予後の診断および評価に使用することができる。

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- 1) Effect of pasture on behavior and salivary cortisol concentrations in breeding sows

Sugano M., Yamada M, Tsuchiya A, Yoshida H and Moriyoshi M.
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Effects of pasturing on behavior and salivary cortisol concentrations in breeding sows

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ABSTRACT

After being released from confinement and given access to pasture during the daytime, behavioral changes and fluctuations in salivary cortisol concentrations were observed in seven sows. It was found that while the sows were in the confinement period (days -1 to 0), their resting behavior occupied over 84% of their activity. However, sows increased their moving activity while in the daytime pasture period (days 1 to 5). The resting behavior reached its minimum value on day 2 and gradually increased from day 4. On the other hand, salivary cortisol concentration during confinement period did not differ between morning and evening, and it remained at 0.077 to 0.086 $\mu\text{g} / \text{dl}$. It rises from the evening of the first day of release and shows the highest value (0.293 $\mu\text{g} / \text{dl}$) on the second day morning. The high value lasted until the 4th day and turned to decrease after the 5th day. The salivary cortisol concentrations in the afternoon on days 1 and 4 were significantly higher ($p < 0.05$) than the afternoon values of day 0. Salivary cortisol concentrations began to decrease on day 5. This implied that the sows adapted to the new environment. The coincidence between the increasing resting time and decreasing cortisol concentrations on day 5 may suggest a relationship between behavioral patterns and salivary cortisol concentrations. However, establishing

the exact dynamics of cortisol requires a longer period of study.

Key words: Behavior, Cortisol Concentration, Pasture, Sow, Stress

INTRODUCTION

In the modern pig farming industry in Japan, sows are confined in narrow battery cages to reduce both labor and production costs¹⁾. However, such confined environments can produce stress in the sows. Stress may reduce immune function and increase infection. Fujita et al.²⁾ reported that lymphocytic proliferation was found to increase in stressed crossbred pigs being raised in a crowded environment. Insufficient exercise through long-term confinement could weaken the musculoskeletal system and articulation of the sows, which would produce a negative impact on general health and physiological function. Heat stress was also stated to inhibit ovarian follicular development by Tania et al.³⁾. The lack of large ovarian follicles results in an increased number of anestrus sows, delays in returning to estrus, and sequentially reduced litter size and delivery rates. Such adverse effects of stress on pig rearing indicate how crucial it is to provide facilities and management with minimized stress conditions.

■ 論文題目

ストールで飼育された雌豚の行動および唾液中コルチゾール濃度に及ぼす放牧の影響

■ 要 旨

ストール飼養期間中の唾液中コルチゾール濃度は、朝および夕ともに差はなく0.077~0.086 $\mu\text{g}/\text{dl}$ で推移した。解放初日の夕方より上昇し、2日目朝に最高値(0.293 μg)を示した。高値は4日目午後まで続き5日目以降減少に転じた。ストール飼育時(0日)午後と比較して、1日目午後と4日目午後のコルチゾール濃度に有意な差が認められた($p < 0.05$)。行動割合と唾液中コルチゾールの関係について、中間放牧飼育後、5日目以降休息割合が増加した時期と唾液中コルチゾール濃度が減少する時期が一致していた。これは、雌豚が新しい環境に適合したことを意味している。しかしながら、ストレスとコルチゾール濃度の関係を正確に把握するにはさらに長い期間の測定が必要であると思われた。

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- 1) Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs.

Oyama N., **Sano T.**, Yamamori M, Tamura J, Umar MA, Endo Y, Ishikawa Y, Itoh A, Miyoshi K, Yamashita K.

J. Vet. Med. Sci. 80: 1450-1455. 2018.

- 2) Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves. El-Hawari SF, Sakata H, Oyama N, Tamura J, Higuchi C, Endo Y, Miyoshi K, **Sano T.**, Suzuki K, Yamashita K.

J. Vet. Med. Sci. 80: 361-367. 2018.



Influence of sevoflurane anesthesia with mechanical ventilation and fluid-therapy on distribution of subcutaneously administered robenacoxib in dogs

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■ 論文題目

犬に皮下投与したロベナコキシブの分布に対する機械的調節呼吸と静脈内輸液を実施したセボフルラン麻酔の影響

■ 要 旨

健康なビーグル犬 10 頭 (1-11 歳, 体重 9.3-14.3kg, 雄 6 頭, 雌 4 頭) に覚醒時または調節呼吸と静脈内輸液を実施したセボフルラン全身麻酔下でロベナコキシブ 2mg/kg を皮下投与し、血漿ロベナコキシブ濃度の推移を観察した。覚醒時にロベナコキシブを投与した犬における最大血漿ロベナコキシブ濃度 ($C_{\max}$) は $2.2 \mu\text{g/mL}$ [1.2-4.6] (中央値[最小-最大]) であり、投与後の $C_{\max}$ 到達時間 ($T_{\max}$) は 90 分 [60-120] であった。これに対して、セボフルラン麻酔下の犬では $C_{\max}$ は有意に低下し ($1.3 \mu\text{g/mL}$ [0.8-1.4], $P=0.008$)、 $T_{\max}$ は有意に延長した (120 分 [120-240], $P=0.018$)。調節呼吸と静脈内輸液を実施したセボフルラン麻酔は、犬におけるロベナコキシブの薬物動態に影響を及ぼした。

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Anesthetic and cardiorespiratory effects of single-bolus intravenous alfaxalone with or without intramuscular xylazine-premedication in calves

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■ 論文題目

子牛におけるキシラジン-アルファキサロン併用注射麻酔の麻酔効果と心肺機能への影響

■ 要 旨

健康な子牛 6 頭 (6-9 ヶ月齢, 体重 114-310 kg) を繰り返し用い、アルファキサロン単独静脈内投与 (IV) またはキシラジン 0.1mg/kg 筋肉内投与 15 分後にアルファキサロンを to effect IV 投与し、麻酔効果と心肺機能の変化を比較した。気管挿管可能となる麻酔導入量は、キシラジン-アルファキサロンでアルファキサロン単独投与より有意に低く (1.23 ± 0.17 vs 2.28 ± 0.65 mg/kg, $P=0.010$)、気管挿管許容時間も有意に長かった (16.8 ± 7.2 vs 7.3 ± 1.6 分間, $P=0.022$)。アルファキサロン投与 5 分後には、アルファキサロン単独投与で頻脈 (166 ± 47 回/分)、高血圧 (平均血圧 147 ± 81 mmHg)、および低酸素血症 (動脈血酸素分圧 [PaO₂] 43 ± 10 mmHg) を認めたが、キシラジン-アルファキサロンでは低酸素血症 (PaO₂ 47 ± 7 mmHg) のみを認めた。キシラジンによる麻酔前投薬は、アルファキサロンの麻酔導入量を減少し、麻酔時間を延長したが、低酸素血症を防ぐために酸素吸入を考慮すべきである。

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