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2017 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 年より業績集を発刊しています。

2017 年の筆頭著者（FA）及び責任著者（CA）の論文合計は 48 報でした。過去 5 年間では、2012 年：44 報、2013 年：48 報、2014 年：51 報、2015 年：44 報、2016 年：45 報となっており、論文数は横ばい状況です。これは日本の科学技術論文数のここ 10 年の推移とほぼ同じ傾向で、学群全体の研究活動状況は日本の縮図のようでもあります。過去 4 年間に FA/CA 論文のない教員が 12 名、そのうち 3 名は全く論文がありません。研究には波があるので、コンスタントに論文を出すのには難しいところもありますが、長期にわたり論文が出ない状況については、強く自己点検していただきたいと思います。研究を実施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 名でした。嘱託助手の先生方を除くと獣医学群の教員は約 60 名ですので、応募率は 60%程度となります。2019 年度は教員全員が科研費に申請するよう、今から準備していただきたいと思います。

世界に目を向けると、科学論文数のトップは中国で、ここ 10 年で 124%増加し、その基盤となる研究開発費（民間も含む）は約 5 倍になっています。それに対し、日本の研究費（民間も含む）は 1.3 倍でしかなく、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す。政府は科学技術力の低下が懸念されていることを踏まえ基礎研究強化を図るため 2018 年度は 2, 500 億円増額し、2020 年度まで 3,000 億円ずつ増額する方針です。これにより研究費を獲得する機会が増える可能性が高くなるものと期待しています。

2017 年度の獣医学専攻博士課程の在籍者数は 34 名で獣医学研究科開設以来最大となる 13 名の修了認定がなされています。獣医保健看護学専攻修士課程は在籍者数 4 名で 4 名の修了認定がなされています。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。引き続き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。

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II．その他<Others>

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

- 1) Anti-diabetic activities of traditional Chinese herbal medicine in streptozotocin-induced diabetic rats.

Nozaki T, Minaguchi J, Takehana K, Ueda H.

Okajima Folia Anat. Jpn. 93: 111-118, 2017.

II. その他<Others>

- 1) Remodeling of rat stromal-vascular cells to brite/beige adipocytes by prolyl-hydroxyproline.

Minaguchi JA, Ogata S, Takahashi N, Hirose T, Ueda H, Takehana K.

J. Vet. Med. Sci. 79: 547-553, 2017.

- 2) Natural larval *Echinococcus multilocularis* infection in a Norway rat, *Rattus norvegicus*, captured indoors in Hokkaido, Japan.

Fukumoto S, Yamada S, Fushikida M, Toyada S, Nishikawa T, Higuchi H,

Ueno H, Ueda H, Sugiyama H, Morishima Y.

J. Vet. Med. Sci. 79: 1857-1860, 2017.

- 3) Morphometric analysis of growing tenocytes in superficial digital flexor tendon of piglets.

Takahashi N, Tangkawattana P, Ootomo Y, Hirose T, Minaguchi J,

Ueda H, Yamada M, Takehana K.

J. Vet. Med. Sci. 79: 1960-1967, 2017.

(Original)

Anti-diabetic activities of traditional Chinese herbal medicine in streptozotocin-induced diabetic rats

By

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Key Words: Traditional Chinese herbal medicine, insulin-dependent diabetes, streptozotocin, liver, pancreas

Summary: Zhen Qi Hypoglycemic Capsules (ZQHC) is a traditional Chinese herbal medicine containing medical activities by ougi (*Astragalus membranaceus*) and ousei (*Polygonatum rhizome*). Although ZQHC has been traditionally utilized as an anti-diabetic medicine in China, there is no evidence. Therefore, this study investigated the beneficial effects of ZQHC against diabetes using streptozotocin (STZ)-induced diabetic rats by biochemical and morphological methods. Eight-week old male Fisher strain rats were intraperitoneally injected with STZ (50 mg/kg of B.W.) to induce diabetes and were fed ad lib feeding with normal diet containing 4% ZQHC for 30 days. Blood and urine samples were collected for biochemical analysis, and liver and pancreas samples were prepared for morphological analysis. Values of blood glucose, AST and ALT of ZQHC oral administrated diabetic rats were lower than those of diabetic rats without administration. Morphological analysis revealed that ZQHC induced sustainment of insulin secreted β cells survival and suppression of hepatocellular fat droplet accumulation. These results suggested that oral administration of ZQHC has anti-diabetic activities those were mainly associated with improvement of liver metabolism.

■ 論文題目

ストレプトゾトシン誘発糖尿病ラットにおける漢方薬の効果

■ 要 旨

ストレプトゾトシン誘発糖尿病ラットを用い、抗糖尿病作用を有するとされる漢方薬の治療効果を検証した。その結果、血液中のインスリン値には顕著な差がなかったが、TG量、AST量、ALT量は低い値を示し、肝臓組織中には脂肪滴の蓄積が見られなかった。本漢方薬は脾臓にではなく、肝臓の代謝作用の改善効果を有することが示唆された。

■ 最終責任者 Hiromi Ueda (Corresponding Author)



FULL PAPER

Anatomy

Remodeling of rat stromal-vascular cells to brite/beige adipocytes by prolyl-hydroxyproline

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ABSTRACT. The aim of this study was to determine the effects of prolyl-hydroxyproline (Pro-Hyp) on the proliferation and differentiation of rat stromal-vascular cells (SVCs) being cultured in a medium with (Pro-Hyp group) or without Pro-Hyp (control group). The results showed that there was no significant difference in proliferation rate of SVCs, lipid droplet (LD) diameter or intracellular concentration of triglycerides between two groups. However, the diameter range of LDs in the Pro-Hyp group tended to be smaller than that in the control group. Transmission electron microscopy showed a tendency for increase in the area of mitochondria and decrease in the number of mitochondria in the Pro-Hyp-treated SVCs. The mRNA expression levels of white adipose tissue differentiation markers (*Cbp*, *Fabp* and *Serpina3k*) were significantly lower, but those of the brown adipose tissue differentiation markers (*Dio2*, *Ucp1* and *Ucp3*) were significantly higher in the Pro-Hyp group than in the control group. Our results suggested that Pro-Hyp can facilitate SVCs to differentiate into "brite/beige" adipocytes.

KEY WORDS: brite/beige adipocyte, collagen derived peptide, prolyl-hydroxyproline, stromal-vascular cell

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■ 論文題目

prolyl-hydroxyproline によるラット stromal-vascular 細胞の brite/beige 脂肪細胞への分化

■ 要 旨

Prolyl-hydroxyproline の効果についてラットの SVC の分化を指標に検討した結果、脂肪滴直径の分布が小さくなり、ミトコンドリア面積の増加と数の減少が見られ、遺伝子発現のパターンからも褐色脂肪細胞様に変化することが示された。

■ 最終責任者 Jun A. Minaguchi (First Author)



NOTE

Parasitology

Natural larval *Echinococcus multilocularis* infection in a Norway rat, *Rattus norvegicus*, captured indoors in Hokkaido, Japan

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ABSTRACT. Natural infection with larval *Echinococcus multilocularis* was recognized in one of eight Norway rats, *Rattus norvegicus*, caught indoors in 2009 in Ebetsu, Hokkaido, northern Japan. Cystic lesions were found in the right median and lateral lobes of the liver, with numerous alveolar cysts in the periphery of the lesions. Protoscolices were formed within large cysts. The laminated layers of the cysts were positive for PAS staining. Nested PCR using the primers specific for *Taenia* mitochondrial 12S rDNA yielded a 250-bp product, and the sequence of the PCR product matched that of *E. multilocularis* isolates from Hokkaido and Germany. This is the third natural alveolar hydatidosis in *R. norvegicus* in Japan.

KEY WORDS: alveolar hydatidosis, *Echinococcus multilocularis*, indoor infection, Norway rat, protoscolex

■ 論文題目

北海道内で検出された建造物内で捕獲された多包条虫幼虫(多包虫)ドブネズミ *Rattus norvegicus* 自然感染例

■ 要 旨

2009年に北海道江別市の建造物内で捕獲された8頭のドブネズミ、*Rattus norvegicus*の1頭に *Echinococcus multilocularis* の幼虫による自然感染が認められた。嚢胞性病変は肝臓の右中央および側葉に見られ、病変の周辺には多数の繁胞形成が見られた。原頭節は大きな嚢胞中で形成された。嚢胞のクチクラ層はPAS染色陽性であった。*Taenia*科条虫 mitochondrial 12S rDNA に特異的なプライマーを用いたPCR反応により、250bpの産物を得、PCR産物の配列は、北海道およびドイツの *E. multilocularis* 分離株の配列と一致した。これは、日本の *R. norvegicus* における3番目にあたり、さらに世界で初めての室内感染が示唆された自然感染多包虫症例であった。

■ 最終責任者 Shin-ichiro Fukumoto (First Author)



FULL PAPER

Anatomy

Morphometric analysis of growing tenocytes in the superficial digital flexor tendon of piglets

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ABSTRACT. The fine structures of different tendons in various animals at different ages have been studied extensively to reveal their arrangement and growth patterns. However, knowledge of the microstructures of the growing tenocytes in the tendons of piglets is still lacking. Thus, we performed the first morphometric analysis to describe the characteristics of tenocytes in the metacarpal superficial digital flexor tendon of 0-, 10- and 20-day-old piglets. In the present study, hydrochloric acid/collagenase digestion was applied to remove the interstitial connective tissue to obtain clear visualization of intact tenocytes and their cytoplasmic processes (Cp). Then, the morphometry of the tenocytes was investigated by optical and electron microscopy. The mean $\pm$ SE values of the fascicle area, number of tenocytes/fascicle, cell density, number of Cp/tenocyte, length of Cp, and thickness of Cp were compared among the three age groups. Significant differences (judged at $P < 0.05$) were found in almost all morphometric aspects among the age groups, except for the number of Cp/cell ($P = 0.545$) and thickness of the Cp ($P = 0.105$). A decrease of cell density corresponded with an increase in the length of the Cp, which were extended to connect either with the Cp of the other tenocytes or the surrounding endotendineum. Moreover, an increase of the fascicle area reflected the increase in tendon diameter. The revealed morphometric characteristics are thus the outcome of tendon growth.

KEY WORDS: morphometry, piglets, superficial digital flexor tendon, tenocyte

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■ 論文題目

子豚の浅指屈筋腱の腱細胞の成長に伴う変化の形態計測的解析

■ 要 旨

腱細胞の成長変化を観察する為に、HCl とコラゲナーゼを用い結合組織を除去した浅趾屈筋腱の中足骨中央部を使用し、光学顕微鏡と透過型電子顕微鏡を用いて観察した。日齢を重ねるにつれ腱細胞の細胞質突起が長くなり、細胞密度が低下した。この細胞密度の減少は他の腱細胞や腱内膜につながる細胞質突起の伸長に対応している。これらの結果は腱の成長による横断面積の増加を支持するものであった。

■ 最終責任者 Prasarn Tangkawattana (Corresponding Author)

組織解剖学

Kazushige Takehana

Professor

教授 竹花 一成

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

II. その他<Others>

- 1) Collagen Peptide Ingestion Alters Lipid Metabolism-Related Gene Expression and the Unfolded Protein Response in Mouse Liver.
Tometsuka C, Koyama Y, Ishijima T, Toyoda T, Teranishi M, Takehana K, Abe K, Nakai Y.
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- 2) Changes in skin structure of the *Zip13*-KO mouse by Makomo (*Zizania latifolia*) feeding.
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Collagen peptide ingestion alters lipid metabolism-related gene expression and the unfolded protein response in mouse liver

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Abstract

Ingestion of collagen peptide (CP) elicits beneficial effects on the body, including improvement in blood lipid profiles, but the underlying mechanisms remain unclear. The purpose of this study was to investigate the effects of CP ingestion on the liver, which controls lipid metabolism in the body. Male BALB/c6 mice were bred with the AIN-93M diet containing 14% casein or the AIN-93M-based low-protein diet containing 10% casein or a diet containing 6% casein + 4% CP for 10 weeks (n 12/group). Total, free and esterified cholesterol levels in the blood decreased in the CP group. DNA microarray analysis of the liver revealed that expressions of genes related to lipid metabolic processes such as the PPAR signalling pathway and fatty acid metabolism increased in the CP group compared with the 10% casein group. The expressions of several genes involved in steroid metabolic process, including *Cyp17a1* and *Cyp19a1*, were decreased, despite being targets of transcriptional regulation by PPAR. These data suggest that lipid metabolism in the liver is altered by CP ingestion, and the decrease in blood cholesterol levels in the CP group is not due to enhancement of the steroid metabolic process. On the other hand, expressions of genes related to the unfolded protein response (UPR) significantly decreased at the mRNA level, suggesting that CP ingestion lowers endoplasmic reticulum stress. Indeed, protein levels of phosphorylated inositol-requiring enzyme 1 decreased after CP ingestion. Taken together, CP affects the broader pathways in the liver – not only lipid metabolism but also UPR.

Key words: Collagen peptide; Liver; Lipid metabolism; Unfolded protein response; DNA microarray

■ 論文題目

コラーゲン摂取がマウス肝臓の脂質代謝および小胞体ストレス応答にもたらす作用

■ 要 旨

コラーゲンペプチド (CP) 摂取の肝臓への作用を解明すべく、DNAマイクロアレイを用いてマウス肝臓の遺伝子発現を解析した。結果、CP摂取群ではPPAR α を含む脂質代謝関連因子の発現上昇が認められた。CP摂取群では血中コレステロールの低下や小胞体ストレス応答の変動も認められた。

■ 最終責任者 Yuji Nakai (Corresponding Author)



Changes in skin structure of the *Zip13*-KO mouse by Makomo (*Zizania latifolia*) feeding

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ABSTRACT. Ehlers-Danlos syndrome (EDS) is a group of disorders caused by abnormalities in the extracellular matrix (ECM). Transforming growth factor- β (TGF- β) plays a crucial role in formation of the ECM by the SMAD (Sma- and Mad-related protein, mothers against decapentaplegic homolog) pathway. It has been reported that loss of function of zinc transporter ZRT/IRT-like protein 13 (ZIP13) is the cause of the spondylocheiro dysplastic form of EDS (SCD-EDS: OMIM 612350). Our previous study suggested that TGF- β 1 has a relationship with the skin pathological condition in the *Zip13*-Knockout (KO) mouse, which is a model of SCD-EDS. Thus far, effective treatment based on modern medicine for this syndrome has not yet been established. According to an approach of traditional Chinese medicine, the present study investigates the medicinal effects of Makomo (*Zizania latifolia*) on certain aspects of SCD-EDS, such as skin morphology and plasma TGF- β 1, in *Zip13*-KO mice. Increases in densities of collagen fibers and fibrils without a significant change in thickness of the dermal layer were observed in the group of mice fed a Makomo-containing diet. No change in the amount of collagen suggests that Makomo feed does not elevate collagen synthesis, but changes the length of glycosaminoglycan chains and decreases the distance between collagen fibrils. In conclusion, the changes of the skin structure suggest that Makomo can increase the mechanical strength of skin.

KEY WORDS: Chinese medicine, collagen fiber, Makomo, skin structure, *Zip13*-KO mouse

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■ 論文題目

マコモの経口摂取による *Zip13*-KO マウスの皮膚の構造変化

■ 要 旨

皮膚の脆弱性を示すエーラス・ダンロス症候群のモデルマウスにマコモの経口摂取を行ったところ、膚線維芽細胞活性が変化した結果としてコラーゲン線維の密度が上昇し皮膚の脆弱性の改善が示唆された。

■ 最終責任者 Takuya Hirose (Corresponding Author)

(Original)

Anti-diabetic activities of traditional Chinese herbal medicine in streptozotocin-induced diabetic rats

By

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Key Words: Traditional Chinese herbal medicine, insulin-dependent diabetes, streptozotocin, liver, pancreas

Summary: Zhen Qi Hypoglycemic Capsules (ZQHC) is a traditional Chinese herbal medicine containing medical activities by ougi (*Astragalus membranaceus*) and ousei (*Polygonatum rhizome*). Although ZQHC has been traditionally utilized as an anti-diabetic medicine in China, there is no evidence. Therefore, this study investigated the beneficial effects of ZQHC against diabetes using streptozotocin (STZ)-induced diabetic rats by biochemical and morphological methods. Eight-week old male Fisher strain rats were intraperitoneally injected with STZ (50 mg/kg of B.W.) to induce diabetes and were fed ad lib feeding with normal diet containing 4% ZQHC for 30 days. Blood and urine samples were collected for biochemical analysis, and liver and pancreas samples were prepared for morphological analysis. Values of blood glucose, AST and ALT of ZQHC oral administrated diabetic rats were lower than those of diabetic rats without administration. Morphological analysis revealed that ZQHC induced sustainment of insulin secreted β cells survival and suppression of hepatocellular fat droplet accumulation. These results suggested that oral administration of ZQHC has anti-diabetic activities those were mainly associated with improvement of liver metabolism.

■ 論文題目

ストレプトゾトシン誘発糖尿病ラットにおける漢方薬の効果

■ 要 旨

ストレプトゾトシン誘発糖尿病ラットを用い、抗糖尿病作用を有するとされる漢方薬の治療効果を検証した。その結果、血液中のインスリン値には顕著な差がなかったが、TG量、AST量、ALT量は低い値を示し、肝臓組織中には脂肪滴の蓄積が見られなかった。本漢方薬は膵臓にではなく、肝臓の代謝作用の改善効果を有することが示唆された。

■ 最終責任者 : Hiromi Ueda (Corresponding Author)



FULL PAPER

Anatomy

Remodeling of rat stromal-vascular cells to brite/beige adipocytes by prolyl-hydroxyproline

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ABSTRACT. The aim of this study was to determine the effects of prolyl-hydroxyproline (Pro-Hyp) on the proliferation and differentiation of rat stromal-vascular cells (SVCs) being cultured in a medium with (Pro-Hyp group) or without Pro-Hyp (control group). The results showed that there was no significant difference in proliferation rate of SVCs, lipid droplet (LD) diameter or intracellular concentration of triglycerides between two groups. However, the diameter range of LDs in the Pro-Hyp group tended to be smaller than that in the control group. Transmission electron microscopy showed a tendency for increase in the area of mitochondria and decrease in the number of mitochondria in the Pro-Hyp-treated SVCs. The mRNA expression levels of white adipose tissue differentiation markers (*Cbp*, *Fabp* and *Serpina3k*) were significantly lower, but those of the brown adipose tissue differentiation markers (*Dio2*, *Ucp1* and *Ucp3*) were significantly higher in the Pro-Hyp group than in the control group. Our results suggested that Pro-Hyp can facilitate SVCs to differentiate into “brite/beige” adipocytes.

KEY WORDS: brite/beige adipocyte, collagen derived peptide, prolyl-hydroxyproline, stromal-vascular cell

■ 論文題目

prolyl-hydroxyproline によるラット stromal-vascular 細胞の brite/beige 脂肪細胞への分化

■ 要 旨

Prolyl-hydroxyproline の効果についてラットの SVC の分化を指標に検討した結果、脂肪滴直径の分布が小さくなり、ミトコンドリア面積の増加と数の減少が見られ、遺伝子発現のパターンからも褐色脂肪細胞様に変化することが示された。

■ 最終責任者：Jun A. Minaguchi (First Author)



FULL PAPER

Anatomy

Morphometric analysis of growing tenocytes in the superficial digital flexor tendon of piglets

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ABSTRACT. The fine structures of different tendons in various animals at different ages have been studied extensively to reveal their arrangement and growth patterns. However, knowledge of the microstructures of the growing tenocytes in the tendons of piglets is still lacking. Thus, we performed the first morphometric analysis to describe the characteristics of tenocytes in the metacarpal superficial digital flexor tendon of 0-, 10- and 20-day-old piglets. In the present study, hydrochloric acid/collagenase digestion was applied to remove the interstitial connective tissue to obtain clear visualization of intact tenocytes and their cytoplasmic processes (Cp). Then, the morphometry of the tenocytes was investigated by optical and electron microscopy. The mean $\pm$ SE values of the fascicle area, number of tenocytes/fascicle, cell density, number of Cp/tenocyte, length of Cp, and thickness of Cp were compared among the three age groups. Significant differences (judged at $P < 0.05$) were found in almost all morphometric aspects among the age groups, except for the number of Cp/cell ($P = 0.545$) and thickness of the Cp ($P = 0.105$). A decrease of cell density corresponded with an increase in the length of the Cp, which were extended to connect either with the Cp of the other tenocytes or the surrounding endotendineum. Moreover, an increase of the fascicle area reflected the increase in tendon diameter. The revealed morphometric characteristics are thus the outcome of tendon growth.

KEY WORDS: morphometry, piglets, superficial digital flexor tendon, tenocyte

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■ 論文題目

子豚の浅指屈筋腱の腱細胞の成長に伴う変化の形態計測的解析

■ 要 旨

腱細胞の成長変化を観察する為に、HCl とコラゲナーゼを用い結合組織を除去した浅趾屈筋腱の中足骨中央部を使用し、光学顕微鏡と透過型電子顕微鏡を用いて観察した。日齢を重ねるにつれ腱細胞の細胞質突起が長くなり、細胞密度が低下した。この細胞密度の減少は他の腱細胞や腱内膜につながる細胞質突起の伸長に対応している。これらの結果は腱の成長による横断面積の増加を支持するものであった。

■ 最終責任者：Prasarn Tangkawattana (Corresponding Author)

組織解剖学

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Lecturer

講師 美名口 順

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

1) Remodeling of rat stromal-vascular cells to brite/beige adipocytes by prolyl-hydroxyproline.

Minaguchi JA, Ogata S, Takahashi N, Hirose T, Ueda H, Takehana K.
J. Vet. Med. Sci. 79: 547-553. 2017.

II. その他<Others>



FULL PAPER

Anatomy

Remodeling of rat stromal-vascular cells to brite/beige adipocytes by prolyl-hydroxyproline

Jun A. MINAGUCHI^{1)*}, Sakino OGATA¹⁾, Naoki TAKAHASHI¹⁾, Takuya HIROSE¹⁾, Hiromi UEDA²⁾ and Kazushige TAKEHANA¹⁾

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ABSTRACT. The aim of this study was to determine the effects of prolyl-hydroxyproline (Pro-Hyp) on the proliferation and differentiation of rat stromal-vascular cells (SVCs) being cultured in a medium with (Pro-Hyp group) or without Pro-Hyp (control group). The results showed that there was no significant difference in proliferation rate of SVCs, lipid droplet (LD) diameter or intracellular concentration of triglycerides between two groups. However, the diameter range of LDs in the Pro-Hyp group tended to be smaller than that in the control group. Transmission electron microscopy showed a tendency for increase in the area of mitochondria and decrease in the number of mitochondria in the Pro-Hyp-treated SVCs. The mRNA expression levels of white adipose tissue differentiation markers (*Cbp*, *Fabp* and *Serpina3k*) were significantly lower, but those of the brown adipose tissue differentiation markers (*Dio2*, *Ucp1* and *Ucp3*) were significantly higher in the Pro-Hyp group than in the control group. Our results suggested that Pro-Hyp can facilitate SVCs to differentiate into "brite/beige" adipocytes.

KEY WORDS: brite/beige adipocyte, collagen derived peptide, prolyl-hydroxyproline, stromal-vascular cell

■ 論文題目

prolyl-hydroxyproline はラット stromal-vascular cells の brite/beige 脂肪細胞へのリモデリングを誘導する

■ 要 旨

コラーゲン分解産物の1つであるprolyl-hydroxyproline (Pro-Hyp)を白色脂肪細胞の前駆細胞であるラットstromal-vascular cells (SVCs)に添加したところ脂肪滴の形態および遺伝子発現パターンが褐色脂肪細胞様に変化した. このことからPro-HypはSVCsを白色脂肪細胞ではなく褐色脂肪細胞様に分化させるようにはたらくことが示唆された.

■ 最終責任者: Jun MINAGUCHI (Corresponding Author)

獣医生理学

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Professor

教授 北村 浩

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

- 1) Ubiquitin-specific protease 2 modulates lipopolysaccharide-elicited expression of proinflammatory cytokines in macrophage-like HL-60 cells.

Kitamura H, Ishino T, Shimamoto Y, Okabe J, Miyamoto T, Takahashi E, Miyoshi I.

Mediat. Inflamm. 2017:6909415. 2017.

- 2) Macrophage ubiquitin-specific protease 2 modifies insulin sensitivity in obese mice

Saito N, Kimura S, Miyamoto T, Fukushima S, Amagasa M, Shimamoto Y, Nishioka C, Okamoto S, Toda C, Washio K, Asano A, Miyoshi I, Takahashi E, Kitamura H

Biochem. Biophys. Rep. 9: 322-329, 2017.

II . その他<Others>

Research Article

Ubiquitin-Specific Protease 2 Modulates the Lipopolysaccharide-Elicited Expression of Proinflammatory Cytokines in Macrophage-like HL-60 Cells

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We investigated the regulatory roles of USP2 in mRNA accumulation of proinflammatory cytokines in macrophage-like cells after stimulation with a toll-like receptor (TLR) 4 ligand, lipopolysaccharide (LPS). Human macrophage-like HL-60 cells, mouse macrophage-like I774.1 cells, and mouse peritoneal macrophages demonstrated negative feedback to USP2 mRNA levels after LPS stimulation, suggesting that USP2 plays a significant role in LPS-stimulated macrophages. USP2 knockdown (KD) by short hairpin RNA in HL-60 cells promoted the accumulation of transcripts for 25 of 104 cytokines after LPS stimulation. In contrast, limited induction of cytokines was observed in cells forcibly expressing the longer splice variant of USP2 (USP2A), or in peritoneal macrophages isolated from *Usp2a* transgenic mice. An ubiquitin isopeptidase-deficient USP2A mutant failed to suppress LPS-induced cytokine expression, suggesting that protein ubiquitination contributes to USP2-mediated cytokine repression. Although USP2 deficiency did not accelerate TNF receptor-associated factor (TRAF) 6-nuclear factor- κ B (NF- κ B) signaling, it increased the DNA binding ratio of the octamer binding transcription factor (Oct)-1 to Oct-2 in *TNF*, *CXCL8*, *CCL4*, and *IL6* promoters. USP2 decreased nuclear Oct-2 protein levels in addition to decreasing the polyubiquitination of Oct-1. In summary, USP2 modulates proinflammatory cytokine induction, possibly through modification of Oct proteins, in macrophages following TLR4 activation.

■ 論文題目

ユビキチン選択的プロテアーゼ 2 はリポ多糖体によるマクロファージ様細胞株 HL-60 での炎症性サイトカインの誘導を制御する。

■ 要 旨

培養マクロファージをリポ多糖体 (LPS) で刺激すると、ユビキチン選択的プロテアーゼ (USP) 2 の発現が低下した。USP2 遺伝子をノックダウンした HL-60 細胞では、LPS 刺激後、104 のサイトカイン中 25 の発現が有意に高まった。この上昇は USP2 のバリエーションのうち主に USP2A に依ることと、そのイソペプチダーゼ活性に依ることを明らかにした。USP2A による炎症性サイトカインの発現抑制は *Usp2a* トランスジェニックマウスからの分離マクロファージでも認められた。HL-60 細胞では、USP2 は、TRAF6-NF- κ B 経路を直接的な標的とせず、OCT-2 の減少と OCT-1 のポリユビキチン化を変化させることにより、サイトカインプロモーター領域の OCT-1/OCT-2 結合比を変えることで、転写制御することを明らかにした。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)



Macrophage ubiquitin-specific protease 2 modifies insulin sensitivity in obese mice



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ABSTRACT

We previously reported that ubiquitin-specific protease (USP) 2 in macrophages down-regulates genes associated with metabolic diseases, suggesting a putative anti-diabetic role for USP2 in macrophages. In this study, we evaluate this role at both cellular and individual levels. Isolated macrophages forcibly expressing *Usp2a*, a longer splicing variant of USP2, failed to modulate the insulin sensitivity of 3T3-L1 adipocytes. Similarly, macrophage-selective overexpression of *Usp2a* in mice (*Usp2a* transgenic mice) had a negligible effect on insulin sensitivity relative to wild type littermates following a three-month high-fat diet. However, *Usp2a* transgenic mice exhibited fewer M1 macrophages in their mesenteric adipose tissue. Following a six-month high-fat diet, *Usp2a* transgenic mice exhibited a retarded progression of insulin resistance in their skeletal muscle and liver, and an improvement in insulin sensitivity at an individual level. Although conditioned media from *Usp2a*-overexpressing macrophages did not directly affect the insulin sensitivity of C2C12 myotubes compared to media from control macrophages, they did increase the insulin sensitivity of C2C12 cells after subsequent conditioning with 3T3-L1 cells. These results indicate that macrophage USP2A hampers obesity-elicited insulin resistance via an adipocyte-dependent mechanism.

■ 論文題目

マクロファージのユビキチン選択的プロテアーゼ 2 は肥満マウスのインスリン感受性を制御する。

■ 要 旨

ユビキチン選択的プロテアーゼ (USP) 2Aのマクロファージ選択的なトランスジェニック (*Usp2aTg*) マウスに高脂肪餌を3ヵ月間給餌すると、コントロールマウスと比べ、内臓脂肪組織へのM1マクロファージの集簇が抑えられた。給餌期間を6ヵ月まで延長すると、*Usp2aTg* マウスの骨格筋および肝臓でのインスリン感受性が改善し、全身レベルでもインスリン抵抗性が軽減された。*Usp2aTg* から分離したマクロファージの培養液はC2C12筋細胞のインスリン応答性を変化させなかったが、このマクロファージの培養液で3T3-L1脂肪細胞を一定期間培養した培養液はC2C12筋細胞のインスリン応答性を高めた。以上より、マクロファージのUSP2はマクロファージ-脂肪細胞の相互作用を介して、全身のインスリン感受性を維持することを見出した。

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- 1) Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease.

Sotohira Y, Suzuki K, Sano T, Arai C, Asakawa M, Hayashi H.
J. Vet. Med. Sci. 79: 852-854. 2017.

II. その他<Others>



NOTE

Wildlife Science

Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease

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ABSTRACT. The aim of this study was to objectively assess stress of kangaroos affected by lumpy jaw disease (LJD) using plasma and hair cortisol concentrations. The plasma and hair samples were collected from kangaroos with LJD and healthy controls. Collected hair samples were extracted with methanol after washing with isopropanol, following which they were processed with the cortisol enzyme immunoassay kit. The plasma cortisol concentration of LJD animals tended to be higher than that of the control. Ventral hair cortisol, but not dorsal hair, of LJD animals was significantly higher than that of the control. In conclusion, stress in kangaroos infected with LJD could be assessed by measuring ventral hair cortisol.

KEY WORDS: hair cortisol, kangaroo, lumpy jaw disease, objectively assessment, stress

■ 論文題目

lumpy jaw disease (LJD)罹患カンガルーの被毛中のコルチゾールを用いたストレス評価

■ 要 旨

本研究の目的は、血漿および被毛中コルチゾールを用いて lumpy jaw disease (LJD)罹患カンガルーのストレスを客観的に評価することである。LJD 罹患カンガルーおよび健常個体より血液および被毛を採取し、血漿および被毛から抽出した溶液中のコルチゾール濃度を ELISA キットにより測定した。LJD 罹患カンガルーの血漿中コルチゾール濃度はコントロールよりも高い傾向があった。LJD 罹患カンガルーの腹側被毛中コルチゾール含有量はコントロールよりも有意に高い値を示した。以上のことより、LJD に罹患したカンガルーのストレスは腹側被毛中コルチゾール含有量を測定することにより評価することができることが示唆される。

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II. その他<Others>

1) Characterization of feline cytochrome P450 2B6.

Okamatsu G, Komatsu T, Ono Y, Inoue H, Uchide T, Onaga T, Endoh D,
Kitazawa T, Hiraga T, Uno Y, Teraoka H.

Xenobiotica 47: 93-102. 2017.

RESEARCH ARTICLE

Characterization of feline cytochrome P450 2B6

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Abstract

1. Little is known about drug metabolism in carnivores. Although the domestic cat (*Felis catus*) is an obligate carnivore and is the most common companion animal, usage and dosage of many drugs are determined according to information obtained from humans and dogs. We determined the complete cDNA sequence of CYP2B6 from the feline lung.
2. Feline CYP2B6 consists of 494 deduced amino acids, showing highest identity with the dog CYP2B8 ortholog, followed by those of horse, pig, primate and human.
3. Feline CYP2B6 transcripts were expressed predominantly in the lung and slightly in the small intestine but not in the liver without significant sex-dependent differences. Western blot analysis with an anti-human CYP2B6 antibody confirmed the presence of CYP2B6 protein in the lung but not in the liver.
4. Feline CYP2B6 proteins heterologously expressed in *Escherichia coli* metabolized several substrates specific to human CYP2B6, including 7-ethoxy-4-(trifluoromethyl) coumarin (EFC). The metabolic activity was strongly inhibited by medetomidine and atipamezole, potent inhibitors of canine CYP2B11 (now officially CYP2B6) as well as by ticlopidine and sertraline, inhibitors selective to human CYP2B6.
5. The results suggest that feline CYP2B6 is a functional CYP2B ortholog that plays a role in the local defense mechanism in the cat respiratory system and intestine.

Keywords

Carnivore, cytochrome P450 2B, domestic cats, felid, xenobiotics

History

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■ 論文題目

ネコのコトクローム P450 2B6 の特徴

■ 要 旨

ネコの肺より得られた CYP 2B6 の cDNA の完全配列を解析し、それから推測するとネコ CYP 2B6 が 494 個のアミノ酸残基から構成され、ウマ、ブタ、霊長類およびヒトの CYP 2B6 相同遺伝子と高い相同性をもつことが明らかとなった。ネコ CYP 2B6 遺伝子転写物は肺に優勢に発現し、肝臓を除く小腸に僅かに発現しており、そのことがウェスタンブロットでも証明された。大腸菌に発現させたネコ CYP 2B6 の代謝活性は CYP 2B11 の強力な阻害薬であるメデトミジンとアチパメゾール、およびヒト CYP 2B6 の選択的阻害薬であるチクロピジンとセトラリンによって強力に抑制された。以上の結果よりネコの CYP 2B6 は機能的な CYP 2B6 相同遺伝子であり、ネコの呼吸器および腸管における局所防御機構において機能することが示唆された。

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- 1) Adverse effects of maternal exposure to bisphenol F on the anxiety- and depression-like behavior of offspring.

Ohtani N, Iwano H, Suda K, Tsuji E, Tanemura K, Inoue H, Yokota H.
J. Vet. Med. Sci. 79: 432-439. 2017.

II. その他<Others>

- 1) *Mycoplasma bovis* escapes bovine neutrophil extracellular traps.

Gondaira S, Higuchi H, Nishi K, Iwano H, Nagahata H.
Vet. Microbiol. 199: 68-73. 2017.



FULL PAPER

Toxicology

The Journal of
Veterinary
Medical
Science

Adverse effects of maternal exposure to bisphenol F on the anxiety- and depression-like behavior of offspring

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ABSTRACT. Bisphenol A (BPA), a well-known endocrine disruptor, is metabolized and eliminated rapidly from the body in adult animals. However, many authors have reported that perinatal BPA exposure alters development of the brain, reproductive system and behavior in the next generation. Recently, BPA substitutes, especially bisphenol F (BPF), have been used because of concerns about the influence of BPA on children, although the actual effects on the next generation are unknown. In this study, we observed behavioral adverse effects of the offspring of mice exposed to BPA or BPF in fetal period. Female C57BL/6 mice were given oral BPA or BPF (0 or 10 mg/kg body weight) daily from gestational day 11.5 to 18.5. The open field test, the elevated plus maze test and the forced swim test were performed at postnatal week 10. BPF exposure altered offspring behavior significantly, resulting in increases in anxiety and depressive state. The influence of BPF was stronger than that of BPA. We demonstrated novel evidence that BPF influences the behavior of offspring.

KEY WORDS: anxiety behavior, bisphenol A, bisphenol F, depression, endocrine disruptors

■ 論文題目

妊娠期の bisphenol F の母親への投与は子供のうつ様行動を引き起こす

■ 要 旨

Bisphenol Aは、内分泌かく乱化学物質として知られており、胎児期の曝露は、脳や生殖器の発達、ならびに成長後の行動に悪影響を与えることが知られている。近年、BPAの代替物質として Bisphenol Fの使用が多くなっているが、その影響は精査されていない。そこで妊娠期母親マウスに BPAとBPFを投与し、子供が成長してからの行動に影響がないかを調べた。その結果、BPFを胎児期に曝露された子供は、行動が臆病になり、うつ様の行動を示すことが明らかとなった。

■ 最終責任者: Hidetomo Iwano (Corresponding Author)



Mycoplasma bovis escapes bovine neutrophil extracellular traps



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ABSTRACT

Mycoplasma bovis is a significant pathogen in bovine infections including mastitis, pneumonia, arthritis and otitis media, and is the cause of large economic losses in beef and dairy farms. During infection with *M. bovis*, recruited neutrophils are not sufficient to eradicate *M. bovis* from the infection site. The release of neutrophil extracellular traps (NETs) is one of the innate immune responses of neutrophils but the effect of *M. bovis* on NET formation by bovine neutrophils has not yet been clarified. The objective of our research was to examine the effect of *M. bovis* on NET formation and the killing activity of bovine neutrophils. We showed that NETs were not detected following stimulation of neutrophils by *M. bovis* alone or with Phorbol 12-myristate 13-acetate (PMA). Reactive oxygen species production is essential for NET formation but the levels in neutrophils stimulated with *M. bovis* at multiplicity of infections of 10, 100, and 1000 were similar to those of unstimulated cells. NET formation induced by PMA stimulated neutrophils disappeared following the addition of *M. bovis* but this phenomenon was not observed when ethylenediaminetetraacetic acid (EDTA) was added. *M. bovis* colony forming units were significantly decreased by the addition of EDTA in the presence of NETs. Our results suggested that *M. bovis* infection alone did not induce NETs and that *M. bovis* nucleases, as hypothesis-based, contributed to resistance against the killing activity of NETs.

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■ 論文題目

マイコプラズマボビスが、ウシ好中球の細胞外トラップを回避する。

■ 要 旨

マイコプラズマボビスは、ウシの乳房炎や肺炎の原因菌であり、多大な経済的損失をもたらす。マイコプラズマ感染において、好中球は、マイコプラズマを感染部位から完全に排除することが出来ない。そこで、好中球による細菌排除の反応の1つ neutrophil extracellular traps (NETs) への影響を調べた。マイコプラズマボビスは、好中球の NETs 形成に影響を阻害することが分かった。

■ 最終責任者: Hidetoshi Higuchi (Corresponding Author)

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1) Dexamethasone-induced hepatomegaly and steatosis in larval zebrafish.

Yin G, Cao L, Du J, Jia R, Kitazawa T, Kubota A, Teraoka H.

J. Toxicol. Sci. 42: 455-459. 2017.

2) Functional expression and comparative characterization of four feline P450 cytochromes using fluorescent substrates.

Okamatsu G, Kawakami K, Komatsu T, Kitazawa T, Uno Y, Teraoka H.

Xenobiotica 47: 951-961. 2017.

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Okamatsu G, Komatsu T, Ono Y, Inoue H, Uchide T, Onaga T, Endoh D, Kitazawa T, Hiraga T, Uno Y, Teraoka H.

Xenobiotica 47: 93-102. 2017.

II. その他<Others>

1) Protective effect of *Ganoderma lucidum* polysaccharide against carbon tetrachloride-induced hepatic damage in precision-cut carp liver slices.

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Letter

Dexamethasone-induced hepatomegaly and steatosis in larval zebrafish

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[Recommended by Chiharu Tohyama]

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ABSTRACT — Fish hepatobiliary syndrome, characterized by hepatomegaly and fatty liver, has been frequently reported in many cultured fish species and has caused a dramatic economic loss in China. Glucocorticoids are thought to be important non-nutritional factors for hepatomegaly and fatty liver development. In the present study, a dexamethasone-induced zebrafish model of fatty liver and hepatomegaly was established, and the role of glucocorticoid receptor (GR) in the development of hepatomegaly and fatty liver was investigated using developing zebrafish. Exposure of larval zebrafish at 5 days post fertilization (dpf) to dexamethasone for 24 hr caused significant increases of liver size and number of fish with hepatic steatosis at 6 dpf. The increase of liver size caused by dexamethasone was significantly reversed by treatment with RU486, a GR antagonist, and by gene knock-down with a morpholino against the GR. The dexamethasone-induced hepatic steatosis was also inhibited by treatment with RU486. Overall, the results highlight larval zebrafish as a useful model for stress-induced liver failure.

Key words: Glucocorticoid, Hepatomegaly, Liver, Steatosis, Zebrafish

■ 論文題目

ゼブラフィッシュ稚魚におけるデキサメサゾン誘発性肝肥大と線維症

■ 要 旨

肝肥大と脂肪肝を特徴とする肝・胆道疾患は養殖魚における疾患として頻繁に発生し、経済的に大きな打撃をあたえる。ストレスホルモンである副腎皮質ホルモンは非栄養性の肝肥大や脂肪肝の原因として重要である。魚類モデルとしても有用なゼブラフィッシュ稚魚においてデキサメサゾンは肝肥大と脂肪蓄積を起こし、その作用はグルココルチコイド受容体のノックダウンで抑制された。ゼブラフィッシュ稚魚は魚類の肝肥大、脂肪肝モデルとして有用であることが示唆された。

■ 最終責任者： Hiroki Teraoka (Corresponding Author)



RESEARCH ARTICLE

Functional expression and comparative characterization of four feline P450 cytochromes using fluorescent substrates

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Abstract

1. Cytochrome P450s (CYP) are a major group of metabolizing enzymes for xenobiotics in humans and other mammals. The properties of CYP isoforms in the domestic cat, an obligate carnivore, are largely unknown at present. In this study, we studied relative expression in tissues and enzymatic properties of nine significant feline CYP isoforms.
2. CYP2E2 transcript was most abundant in the feline liver, followed by CYP2A13 and 2E1. Transcripts of CYP3A131, 1A2 and 1A1 were also present in the liver, while CYP2D6 and 3A132 were only slightly expressed. CYP3A131 was a major transcript in the small intestine.
3. Four major CYP isoforms in the feline liver and small intestine (CYP1A2, CYP2A13, CYP2E2 and CYP3A131) were heterologously expressed in *Escherichia coli* to generate functional monooxygenase systems. We carried out screenings of 17 test compounds known to be inhibitors of CYP isoforms in other mammals as well as two anticancer drugs to assess the activity modulation of feline CYP isoforms using fluorogenic substrates. These CYP isoforms showed similar selectivity to counterparts in other mammals against inhibitors as a whole but with many exceptions.
4. The present study suggests the usefulness of the feline CYP recombinant system to obtain chemical affinity information and possible drug interactions in CYP metabolism of domestic cats.

Keywords

Carnivore, cytochrome P450, domestic cats, drug interaction, felid, xenobiotics

History

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■ 論文題目

4種のネコシトクローム P450 サブタイプの発現系の構築と機能の検討

■ 要 旨

ネコは主要なコンパニオンアニマルであるにもかかわらず、その代謝についての知識は不足している。本研究ではネコ肝臓におけるシトクローム P450 サブタイプの発現量を明らかにした上で、主要な CYP1A2、2A13、2E2、3A131 の大腸菌発現系を構築し、蛍光基質特異性とこれを用いた種々の薬物による阻害について検討した。

■ 最終責任者： Hiroki Teraoka (Corresponding Author)



RESEARCH ARTICLE

Characterization of feline cytochrome P450 2B6

Gaku Okamatsu¹, Tetsuya Komatsu¹, Yuka Ono¹, Hiroki Inoue¹, Tsuyoshi Uchide¹, Takenori Onaga¹, Daiji Endoh¹, Takio Kitazawa¹, Takeo Hiraga¹, Yasuhiro Uno², and Hiroki Teraoka¹

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Abstract

1. Little is known about drug metabolism in carnivores. Although the domestic cat (*Felis catus*) is an obligate carnivore and is the most common companion animal, usage and dosage of many drugs are determined according to information obtained from humans and dogs. We determined the complete cDNA sequence of CYP2B6 from the feline lung.
2. Feline CYP2B6 consists of 494 deduced amino acids, showing highest identity with the dog CYP2B ortholog, followed by those of horse, pig, primate and human.
3. Feline CYP2B6 transcripts were expressed predominantly in the lung and slightly in the small intestine but not in the liver without significant sex-dependent differences. Western blot analysis with an anti-human CYP2B6 antibody confirmed the presence of CYP2B protein in the lung but not in the liver.
4. Feline CYP2B6 proteins heterologously expressed in *Escherichia coli* metabolized several substrates specific to human CYP2B6, including 7-ethoxy-4-(trifluoromethyl) coumarin (EFC). The metabolic activity was strongly inhibited by medetomidine and atipamezole, potent inhibitors of canine CYP2B11 (now officially CYP2B6) as well as by ticlopidine and sertraline, inhibitors selective to human CYP2B6.
5. The results suggest that feline CYP2B6 is a functional CYP2B ortholog that plays a role in the local defense mechanism in the cat respiratory system and intestine.

Keywords

Carnivore, cytochrome P450 2B, domestic cats, felid, xenobiotics

History

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■ 論文題目

ネコのシトクローム P450 2B6 に関する検討

■ 要 旨

ネコは主要なコンパニオンアニマルであるにもかかわらず、その代謝についての知識は不足している。本研究ではネコの CYP2B6 を新たにクローニングし、その組織発現量や大腸菌発現系を用いて代謝活性を明らかにした。ネコ肝臓は CYP2B サブタイプを全くあるいはほとんど発現していない顕著な特性を示すことがわかった。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)

Protective effect of *Ganoderma lucidum* polysaccharide against carbon tetrachloride-induced hepatic damage in precision-cut carp liver slices

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Pao Xu · Guojun Yin

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Abstract The aim of the present study was to investigate the protective effects of *Ganoderma lucidum* polysaccharide (GLPS) against carbon tetrachloride (CCl₄)-induced hepatotoxicity in vitro in common carp. Precision-cut liver slices (PCLSs), which closely resemble the organ from which they are derived, were employed as an in vitro model system. GLPS (0.1, 0.3, and 0.6 mg/ml) was added to PCLS culture system before the exposure to 12 mM CCl₄. The supernatants

and slices were collected to detect molecular and biochemical responses to CCl₄ and PCLS treatments. The levels of CYP1A, CYP3A, and CYP2E1 were measured by ELISA; the mRNA expressions of TNF- α , IL-1 β , IL-6, and iNOS were determined by RT-PCR; and the relative protein expressions of c-Rel and p65 were analyzed by western blotting. Results showed that GLPS inhibited the elevations of the marker enzymes (GOT, GPT, LDH) and MDA induced by CCl₄; it also enhanced the suppressed activity of antioxidant enzymes (SOD, CAT, GSH-Px, T-AOC). The treatment with GLPS resulted in significant downregulation of NF- κ B and inflammatory cytokine mRNA levels and significant decreases in the hepatic protein levels of CYP1A, CYP3A, and CYP2E1. These results suggest that GLPS can protect CCl₄-induced PCLS injury through inhibiting lipid peroxidation, elevating antioxidant enzyme activity, and suppressing immune inflammatory response.

Yingjuan Liu and Chunyun Zhang contributed equally to this study.

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■ 論文題目

ソウギョ肝スライスにおける四塩化炭素誘発性肝障害に与するレイシポリ多糖の防御効果

■ 要 旨

霊芝（レイシ *Ganoderma lucidum*）は抗腫瘍、抗酸化作用および免疫調整作用を示すとして漢方の原料として永らく使用されてきた。最近、糖尿病モデルラットの肝障害を霊芝の有効成分（リポ多糖、GLPS）が顕著に抑制することが報告された。一方、肝障害は養殖魚で最も問題とされる疾患である。今回、ソウギョ（grass carp *Ctenopharyngodon idella*）の肝臓スライス培養系を用いて、四塩化炭素による肝障害を GLPS が抑制することを観察した。GLPS は抗酸化作用の他、NF- κ B と炎症性サイトカインおよびいくつかの CYP 誘導を抑制した。

■ 最終責任者 Yin Guojun (Corresponding Author)



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Research paper

Does motilin peptide regulate gastrointestinal motility of zebrafish? An *in vitro* study using isolated intestinal stripsTakio Kitazawa^{a,*}, Maria Yoshida^a, Hiroki Teraoka^a, Hiroyuki Kaiya^b^a School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan^b Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka 565-8565, Japan

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ABSTRACT

Motilin (MOT), a 22-amino-acid peptide hormone produced in the duodenal mucosa, stimulates gastrointestinal motility in mammals and birds, and it is a mediator of interdigestive motor complexes. Recently, expression of MOT-like peptide (MOTLP) and its receptor mRNAs was identified in zebrafish. The aim of the present study was to determine whether the zebrafish MOTLP (zfMOTLP, HIAFSPKEMRELEKE) affects zebrafish gastrointestinal motility, with comparison to the effect of human MOT, in which five amino acids are identical to zfMOTLP at positions 5, 9, 15, 16, and 17. zfMOTLP caused small contractions of the rabbit duodenum and chicken ileum but, the sensitivity was about 3000-times lower than that of human MOT. zfMOTLP-induced contraction in the rabbit duodenum was decreased by pretreatment of the MOT receptor antagonist GM109, indicating that zfMOTLP could bind to the MOT receptor. zfMOTLP (3–100 nM) increased the intracellular Ca^{2+} concentration in zfMOT receptor-expressing HEK293 cells, but human MOT did not cause responses even at 100 nM. In *in vitro* study using isolated zebrafish gastrointestinal strips, zfMOTLP caused only small contractions even at high doses (1–10 μ M). zfMOT receptor mRNA is detected in the gastrointestinal tract and brain to almost the same extent, and the expression level (40–70 copies/100 ng total RNA) is much lower than that in the chicken gastrointestinal tract. These results suggest that the MOTLP/MOT receptor system is present in zebrafish, but its physiological role for regulation of gastrointestinal motility might be not significant due to the weak contractile activity and low expression level of the receptor.

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■ 論文題目

モチリンはゼブラフィッシュ消化管の運動を調節しているか？摘出標本を用いての検討

■ 要 旨

ゼブラフィッシュで遺伝子レベルで存在が認められているモチリン様物質（17 アミノ酸ペプチド）の作用を種々の脊椎動物の消化管で検討した。モチリン様物質はモチリンが活性を示すウサギ十二指腸、ニワトリ回腸を収縮させたが、モチリンが収縮を誘起しないマウス腸管では収縮作用は認められなかった。ゼブラフィッシュ腸管はモチリン様物質の高濃度(1-10 μ M)で収縮を誘起した。この時、同様な濃度のモチリンでも収縮が誘起されたが、いずれの物質による収縮も極めて低かった。ゼブラフィッシュ腸管にはモチリン受容体 mRNA が存在していたが発現レベルは低かった。モチリン受容体発現細胞においてモチリン様物質は 3-100nM で興奮を誘起した。結論として、ゼブラフィッシュにはモチリンシステムが存在するものの受容体発現量が低値であることからその生理的意義は低いと考えられた。

■ 最終責任者 Takio Kitazawa (First and Corresponding Author)

Neuropeptide Y (NPY) inhibits spontaneous contraction of the mouse atrium by possible activation of the NPY1 receptor

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Email: tko-kita@rakuno.ac.jp**Summary**

1. Neuropeptide Y (NPY) causes various central and peripheral actions through activation of G-protein-coupled NPY receptors. Although a species-dependent difference in cardiac actions of NPY has been reported, the responses to NPY have not been examined in mice, widely used experimental animals. This study aimed to clarify the responses to NPY and the receptor subtype involved in the responses in mouse atrium.
2. Neuropeptide Y caused negative inotropic and negative chronotropic actions in spontaneous beating right atria. Negative inotropic actions were more marked than negative chronotropic actions. Therefore, negative inotropic actions were studied in detail for evaluation of the NPY-induced cardiac actions in mouse atrium.
3. Neuropeptide Y-induced negative inotropic actions were not affected by atropine but were abolished in the atria from pertussis toxin-treated mice.
4. In isolated atrial preparations from reserpine-treated mice, NPY-induced negative inotropic actions were significantly attenuated.
5. [Leu31, Pro34]-NPY, but not peptide YY, was effective in decreasing spontaneous contraction in atrial preparations.
6. Although Y_1 , Y_2 , Y_4 and Y_5 receptor mRNAs were expressed almost equally in the brain, NPY₁ receptor mRNA was dominantly expressed in the atrium.
7. In conclusion, NPY caused negative inotropic and chronotropic actions through activation of the Y_1 receptor in the mouse atrium. A high expression level of Y_1 mRNA in the atrium suggests a functional role of NPY in the regulation of mouse cardiac contraction.

KEYWORDSmouse atrium, negative inotropic action, neuropeptide Y, Y_1 receptor**論文題目**

NPY1 受容体介した neuropeptide Y(NPY)のマウス心房筋収縮抑制作用

要 旨

NPY の心筋収縮に与える影響には動物種差が認められる。これまでマウス心房における NPY の作用は検討されていないので今回検討を行った。NPY はマウス心房筋の自発収縮（収縮力、頻度）を抑制した。この抑制は心収縮力の抑制（変力作用）の方で著明であった。陰性変力作用は、アトロピンでは抑制されず、百日咳毒素処理で消失したことから、 G_i たんぱく質が関与する反応であることがわかった。NPY1 受容体の作動薬により NPY と同様な抑制作用が認められた。また、心房筋では NPY1 mRNA の発現レベルが最も高かった。以上のことから、マウス心房で NPY は NPY1 受容体を興奮させ、陰性の変力作用および変時作用を起こすことが明らかになった。

最終責任者 Takio Kitazawa (Corresponding Author)

The chicken is an interesting animal for study of the functional role of ghrelin in the gastrointestinal tract

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Abstract. Ghrelin has been identified in vertebrates from fish to mammals, and it has multiple biological activities including gastrointestinal (GI) motor-stimulating action. In some non-mammalian vertebrates, we examined the effects of ghrelin on contractility of the isolated GI tract as well as the mRNA expression of growth hormone secretagogue-receptor 1a (GHS-R1a) to determine whether the motor-stimulating action of ghrelin is common in vertebrates. The expression level of GHS-R1a mRNA differed depending on the species and on the GI region (stomach, small intestine, and colon). GI region-dependent expression of GHS-R1a mRNA was remarkable in chickens, and the expression levels changed depending on age. In a functional study, ghrelin did not cause contraction of unstimulated GI strips in fish (goldfish and rainbow trout) or amphibians (bullfrog and Japanese fire belly newts) even using their homologous ghrelin. In avian species, ghrelin caused contraction of the unstimulated GI tract of the chicken but not of the Japanese quail, and the responses to ghrelin in the chicken GI tract decreased with aging. Our *in vitro* studies show that the motor-stimulating action of ghrelin is not conserved across vertebrates and that the chicken is a unique animal species for evaluation of the GI-stimulating action of ghrelin of different age.

Key words: Ghrelin, Gastrointestinal contraction, GHS-R1a, Non-mammals, Chicken

■ 論文題目

ニワトリはグレリンの消化管機能（運動）に対する影響を調べる上でユニークな動物種である。

■ 要 旨

グレリンは多くの脊椎動物の胃粘膜から分離精製されるペプチドホルモンで3位のアミノ酸（一般的にはセリン）が脂肪酸修飾を受けているのが特徴である。この物質の消化管収縮に与える影響には動物種差が著明である。すなわち、魚類や両生類では胃腸管収縮はグレリンによって全く影響を受けないが、鳥類と哺乳類では電気刺激誘発性収縮がグレリンにより増強される。加えてニワトリでは非刺激胃腸管標本がグレリンにより濃度依存性の収縮を誘起し、この反応には平滑筋性のグレリン受容体の関与が知られている。このような非刺激標本でのグレリン誘発性収縮はこれまでの報告ではニワトリでしか認められていないので、このことはニワトリがグレリンの消化管収縮機能を検討するうえで極めて興味ある動物であることを示している。

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Toishi Y, Tsunoda N, Kirisawa R.
J. Vet. Med. Sci. 79: 632-635. 2017.

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- 1) Evaluation of calcium hydrogen carbonate mesoscopic crystals as a disinfectant for influenza A viruses.
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J. Vet. Med. Sci. 79: 206-212. 2017



Isolation of equine herpesvirus 3 (EHV-3) from equine coital exanthema of two stallions and sero-epidemiology of EHV-3 infection in Japan

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ABSTRACT. In the spring of 2015, two stallions reared in Farms A and B in Hokkaido in Japan showed symptoms of equine coital exanthema. Equine herpesvirus 3 (EHV-3) was isolated from penis swab samples of both stallions, and the isolates from each stallion in Farms A and B were designated as SS-1 and YS-1 strains, respectively. *Bam*HI restriction profiles of SS-1 and Japanese reference strain Iwate-1 were indistinguishable, but the *Bam*HI-A fragment of YS-1 was larger than those of SS-1 and Iwate-1 by 1.9 kbp because of the lack of two *Bam*HI sites. Nucleotide sequence analyses of glycoprotein G (gG), gB, gC and VP13/14 coding regions revealed that SS-1 and YS-1 had 99.77% to 100% identities to each other. These results suggested that the origins of SS-1 and YS-1 were different. For a sero-epidemiological survey, serum neutralizing tests using SS-1 against 319 sera of horses from eight farms in Hokkaido were conducted. Six of the eight farms were EHV-3 antibody-positive, and positive rates ranged from 2.6% to 17.6%. To determine the infection time of four EHV-3 antibody-positive horses, a retrospective study was conducted. Infection time of the four horses was in the breeding season, and re-infection or reactivation of latently infected EHV-3 might have occurred in one horse. However, these four horses had never shown any clinical symptoms. The results suggested that several EHV-3 strains are distributed in Japan and that infection is maintained widely in horses without clinical symptoms.

KEY WORDS: ECE, EHV-3, isolation, sero-epidemiology, stallion

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■ 論文題目

馬媾疹を発症した2頭のスタリオンからのウマヘルペスウイルス3型 (EHV-3) の分離と日本における EHV-3 の血清疫学

■ 要 旨

2015年の春に、2カ所の牧場で各1頭のスタリオンが馬媾疹の症状を示した。両方のスタリオンからウマヘルペスウイルス3型 (EHV-3) が分離された。糖タンパク g (gG)、gB、gCとVP13/14の塩基配列を決定した結果、両ウイルスの由来は異なっていることが示唆された。北海道の8カ所の牧場に由来する319頭の馬血清を用いて中和試験を行った結果、6カ所の牧場が抗体陽性を示した (陽性率: 2.6%から17.6%)。日本には幾つかのEHV-3株が分布し、EHV-3感染が広く維持されていることが示唆された。

■ 最終責任者 Rikio Kirisawa (First Author and Corresponding Author)



NOTE

Theriogenology

Occurrence of equine coital exanthema (ECE) in stallions in Japan and effectiveness of treatment with valacyclovir for ECE

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ABSTRACT. Equine coital exanthema (ECE) has been reported in many countries, but equine herpesvirus 3 (EHV-3) has been isolated only once in Japan. In 2015, symptoms of ECE were found, and EHV-3 was isolated in two stallions. Valacyclovir, an anti-herpesvirus agent, was administered orally. The stallions rested from mating for more than two weeks, causing enormous financial losses because of their high fees. This is the first study in which valacyclovir was administered for ECE. Though valacyclovir treatment did not shorten the duration of healing, the affected area did not expand after administration of valacyclovir. Valacyclovir therefore seems to be effective for suppression of EHV-3 infection. Further investigation about the administration protocol might be required.

KEY WORDS: acyclovir, ECE, EHV-3, stallion, valacyclovir

■ 論文題目

日本のスタリオンでの馬媾疹の発症とバラシクロビルを用いた馬媾疹の治療効果

■ 要 旨

2015年に2頭のスタリオンで馬媾疹の発症例が認められた。抗ヘルペスウイルス薬のバラシクロビルを経口投与した。発症したスタリオンは2週間以上の交配中止のため莫大な経済損失が起きた。バラシクロビル投与で治療期間の短縮は認められなかったが、病巣の拡大は阻止された。このことから、バラシクロビルはEHV-3の複製を抑制することが示唆されたが、今後、投与プロトコルの研究が必要であると思われた。

■ 最終責任者 Rikio Kirisawa (Corresponding Author)



NOTE

Virology

Evaluation of calcium hydrogen carbonate mesoscopic crystals as a disinfectant for influenza A viruses

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ABSTRACT. In this study, the virucidal effect of a novel electrically charged disinfectant CAC-717 was investigated. CAC-717 is produced by applying an electric field to mineral water containing calcium hydrogen carbonate to generate mesoscopic crystals. Virus titration analysis showed a >3 log reduction of influenza A viruses after treatment with CAC-717 for 1 min in room temperature, while infectivity was undetectable after 15 min treatment. Adding bovine serum albumin to CAC-717 solution did not affect the disinfectant effect. Although CAC-717 is an alkaline solution (pH=12.39), upon contact with human tissue, its pH becomes almost physiological (pH 8.84) after accelerated electric discharge, which enables its use against influenza viruses. Therefore, CAC-717 may be used as a preventative measure against influenza A viruses and for biosecurity in the environment.

KEY WORDS: antisepsis, biosecurity, calcium hydrogen carbonate, influenza virus, public health

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■ 論文題目

炭酸水素カルシウムのメゾスコピック構造の結晶のA型インフルエンザウイルスの消毒資材としての評価

■ 要 旨

新規の電荷を持つ消毒資材 CAC-717 のウイルス不活化効果を調べた。CAC-717 は炭酸水素カルシウムのメゾスコピック構造の結晶を含むミネラル水で自己起電力により強アルカリ (pH 12) を示す。CAC-717 を室温で1分間、A型インフルエンザウイルスに作用させると 10^3 以上のウイルス力価の低下が認められ、15分の作用では感染性が検出されなくなった。CAC-717 はヒトの皮膚に接触するとほぼ中性になった。以上の結果より、CAC-717 はA型インフルエンザウイルスの予防法に使用することが可能で、環境のバイオセキュリティに有用であることが示唆された。

■ 最終責任者 Takashi Onodera (Corresponding Author)



The full genome sequences of 8 equine herpesvirus type 4 isolates from horses in Japan

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ABSTRACT. Equine herpesvirus type 4 (EHV-4) is one of the most important pathogens in horses. To clarify the key genes of the EHV-4 genome that cause abortion in female horses, we determined the whole genome sequences of a laboratory strain and 7 Japanese EHV-4 isolates that were isolated from 2 aborted fetuses and nasal swabs of 5 horses with respiratory disease. The full genome sequences and predicted amino acid sequences of each gene of these isolates were compared with of the reference EHV-4 strain NS80567 and Australian isolates that were reported in 2015. The EHV-4 isolates clustered in 2 groups which did not reflect their pathogenicity. A comparison of the predicted amino acid sequences of the genes did not reveal any genes that were associated with EHV-4-induced abortion.

KEY WORDS: abortion, genome sequence, Japanese equine herpesvirus type 4

■ 論文題目

日本の馬から分離された8株のウマヘルペスウイルス4型の全ゲノム塩基配列

■ 要 旨

ウマヘルペスウイルス4型の流産に関連する遺伝子を調べるため、日本の実験室株、2株の流産由来株と5株の呼吸器病由来株のDNAの全塩基配列を決定し、参照株のNS80567とオーストラリア分離株の配列と比較した。その結果、日本分離株は2つのクラスターに分類されたが、EHV-4の流産に関連する遺伝子は明らかにすることはできなかった。

■ 最終責任者 Hideto Fukushi (Corresponding Author)

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Biologics. 11:23-30. 2017.



ORIGINAL PAPER

Induction of antitumor response to fibrosarcoma by Newcastle disease virus-infected tumor vaccine

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Abstract Fibrosarcoma is a locally aggressive malignant tumor with a high recurrence rate, so that wide excisional surgery is necessary for treatment. However, it is often difficult to resect with a sufficient margin of excision at the site of tumor infiltration. Recombinant tumor vaccine therapy is a useful method to induce specific immunity. In this study, we have shown its utility as a candidate for therapy by applying a recombinant Newcastle disease virus (rNDV) tumor vaccine (rNDV-TV). Although the therapeutic effect of similar viruses has been examined in several tumors, the vaccination efficacy against fibrosarcoma has not been demonstrated until now. In this study, we showed the induction of an antitumor response by rNDV-TV against murine fibrosarcoma and investigated the role of lymphocytes in tumor elimination. Intraperitoneal inoculation of murine fibrosarcoma (WEHI164) cells showed increased lethality in C.B.17scid/scid (scid) mice within 2 weeks of inoculation. The survival rate increased to 80% when the mice were transfused with CD3⁺ cells from BALB/c mice previously immunized with rNDV-TV. However, all mice died from tumor growth after inoculation with non-immunized CD3⁺ cells. Although the survival rate was around 50% in mice receiving only immunized CD4⁺ and CD8⁺ cells, the survival rate was not decreased in mice receiving CD3⁺CD4⁺CD8⁺ (natural

killer T; NKT) cells together with immunized CD4⁺ and CD8⁺ cells. This study showed rNDV-TV induced an antitumor T cell response to WEHI164 cells, and major subsets of cells involved in tumor exclusion were CD4⁺ and CD8⁺ cells, together with NKT cells.

Keywords Fibrosarcoma · Immunotherapy · Newcastle disease virus · Tumor vaccine

Introduction

Soft tissue sarcomas are rare neoplasms accounting for around 1% of all tumors [1]. Fibrosarcoma is one of more than 50 histologic subtypes of soft tissue sarcoma, as classified by the World Health Organization (WHO). In a recent evaluation, it was shown that 50% of patients with fibrosarcoma died of locally aggressive and/or metastatic disease (median follow-up 1 year; range <1–8 years) [2]. Although local control after surgery is important for reducing the high rate of local recurrence [3], radiotherapy is not sufficiently effective at present [4], and the prognosis of patients with soft tissue sarcoma has not improved during the last 20 years [5]. These facts indicate that currently used treatments are not effective for tumor elimination; therefore, alternative treatments for the prevention of postoperative recurrence are required. Currently, immunotherapy is being investigated as a fourth therapeutic option, in addition to surgical therapy, radiotherapy, and chemotherapy, for the treatment of tumors. One such immunotherapeutic approach employs oncolytic viruses to target tumors.

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- 論文題目 ニューカッスル病ウイルスに感染した繊維肉腫による抗腫瘍免疫誘導
- 要 旨 ニューカッスル病ウイルス (NDV) を繊維肉腫に感染させ、それをワクチンとしてマウスに免疫すると細胞性免疫を誘導することができ、効果的な抗腫瘍免疫を誘導することを示した。この論文では、NDV ウイルスを腫瘍免疫治療に応用できる事をマウスモデルで示した。
- 最終責任者 Katsuro Hagiwara (Corresponding Author)



NOTE

Bacteriology

Postnatal changes in the relative abundance of intestinal *Lactobacillus* spp. in newborn calves

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ABSTRACT. The intestinal microbiota of newborn calves changes during the early postnatal period and influences their health and immune function. We studied the compositional changes in the intestinal microbiome of newborn calves during the first week after birth by metagenomic analysis. In feces from newborn calves, we identified 4 bacterial phyla, namely, Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria. The relative abundance of *Lactobacillaceae* significantly increased from day 1 to day 7. We evaluated *Lactobacillus* spp. colony numbers using selective agar plates and confirmed that the abundance of *Lactobacillus* spp. significantly increased during the first 7 days after birth. In conclusion, *Lactobacillus* spp. colonized the intestinal tract of calves during the first 7 days after birth.

KEY WORDS: bacterial culture, metagenomics analysis, microbiome

■ 論文題目

新生子ウシにおける生後の消化管 *Lactobacillus* spp. の変化

■ 要 旨

生後 1 週間に子牛の消化管内の微生物叢の変化を Ion PGM を用いたメタゲノム解析により明らかにした。その結果、生後 1 週間で Bacteroides から Firmicutes への大きな細菌叢の変化が起こり、*Lactobacillus* spp. が定着する事を明らかにした。

■ 最終責任者 Katsuro Hagiwara (Corresponding Author)

Problems in the Protection of Reintroduced Przewalski's Horses (*Equus ferus przewalskii*) Caused by Piroplasmosis

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ABSTRACT: The objectives of our research on equine piroplasmosis were to clarify the prevalence of the piroplasms *Babesia caballi* and *Theileria equi* among reintroduced Przewalski's horses (takhi, *Equus ferus przewalskii*) in comparison with horses (*Equus caballus*) of nomads from around Hustai National Park in Mongolia and to determine the mortality among takhi during 2012 to 2015. The prevalence of *T. equi* was high in 2014 at 80% among nomadic horses and 84% among takhi. We found no evidence of *B. caballi* infection among takhi. A homology comparison of the 18S rRNA base sequence of *T. equi* showed the same sequence in both domestic horses and takhi. According to the national park records, piroplasmosis is a cause of high mortality in young takhi (19%). The results suggested that takhi were infected with *T. equi* in this park, and the infection is an important issue when reintroducing the takhi into the wild and increasing the numbers of the animals in nature. Continuous monitoring and vector control were noted as important issues in future conservation programs.

Key words: Babesiosis, epidemiology, Mongolia, Przewalski's horse, takhi, theileriosis.

one of the reintroduction sites in a 50,600 ha protected area; geographically, it consists of steppe, mountain steppe, mountain zones, and forest (47°35'N to 47°52'N, 105°23'E to 105°37'E) in Tov Province, Mongolia, at an altitude of 1,100–1,840 m. The average temperature in the summer is 25 °C; average temperature in the winter is –25 °C; and the annual precipitation ranges from 130 to 220 mm. The national park is 95 km west of the Mongolian capital city, Ulaanbaatar, Mongolia, Central Asia. The park has been managed and protected by a nongovernmental organization and the Mongolian government. In five reintroductions from 1992 to 2000, a total of 84 takhi were transported to this park and have since increased in number to 336 takhi in the wild.

Theileria equi (formerly *Babesia equi*) and *Babesia caballi* are hemoprotozoan parasites in equines and are transmitted by ticks (Wise et al. 2013, 2014; Scoles and Ueti 2015). During reintroduction at sites in Hustai

■ 論文題目

ピロプラズマ症により引き起こされる再導入野生馬（タヒ）の保護問題

■ 要 旨

モンゴルで絶滅したタヒを海外の動物園から引取り野生化の取り組みを進めています。その際にモンゴルの土着馬に蔓延しているピロプラズマ症がタヒの幼駒に感染して死亡する事例が必発し、全体の死亡率のヤック 20%を占めることから、この感染症を地域全体で対処する必要があることを論文で指摘しました。

■ 最終責任者 Katsuro Hagiwara (Corresponding Author)

Neutralizing activities against seasonal influenza viruses in human intravenous immunoglobulin

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[Number of times this article has been viewed](#)

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Abstract: Influenza viruses A/H1N1, A/H3N2, and B are known seasonal viruses that undergo annual mutation. Intravenous immunoglobulin (IVIG) contains anti-seasonal influenza virus globulins. Although the virus-neutralizing (VN) titer is an indicator of protective antibodies, changes in this titer over extended time periods have yet to be examined. In this study, variations in hemagglutination inhibition (HI) and VN titers against seasonal influenza viruses in IVIG lots over extended time periods were examined. In addition, the importance of monitoring the reactivity of IVIG against seasonal influenza viruses with varying antigenicity was evaluated. A/H1N1, A/H3N2, and B influenza virus strains and IVIG lots manufactured from 1999 to 2014 were examined. The HI titer was measured by standard methods. The VN titer was measured using a micro-focus method. IVIG exhibited significant HI and VN titers against all investigated strains. Our results suggest that the donor population maintains both specific and cross-reactive antibodies against seasonal influenza viruses, except in cases of pandemic viruses, despite major antigen changes. The titers against seasonal influenza vaccine strains, including past strains, were stable over short time periods but increased slowly over time.

Keywords: IVIG, influenza, seasonal, neutralization, vaccine

Introduction

Influenza viruses A/H1N1, A/H3N2, and B are known seasonal viruses that undergo annual mutation. Therefore, to control/prevent influenza, vaccine programs against virus strains are determined every season by the World Health Organization and implemented by the health authorities of various countries. Intravenous immunoglobulin (IVIG) possesses significant neutralizing activities against influenza viruses.¹⁻⁴ IVIG is manufactured from pooled plasma that is derived from >10,000 healthy donors. Therefore, IVIG contains various antibodies against numerous human pathogens. The

■ 論文題目

ヒト静注用グロブリン製剤における季節性インフルエンザウイルスに対する中和活性

■ 要 旨

インフルエンザウイルス株 A/H1N1, A/H3N2 と B について 1999 年から 2014 年に製造されたグロブリン製剤の中和活性を調べた。その結果、いずれのサンプルも対象ウイルスに対して十分な HI 抗体価ならびに中和活性が確認された。一方で、パンデミックウイルスに対しては、主要抗原の変異によりその反応性が低下していた。

■ 最終責任者 Mikihiro Yunoki (Corresponding Author)

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- 1) Characterization of pertussis-like toxin from *Salmonella* spp. that catalyzes ADP-ribosylation of G proteins

Tamamura Y, Tanaka K, Uchida I.

Scientific Report 7:2653. 2017

II. その他<Others>

- 1) Control of *Pseudomonas* mastitis on a large dairy farm by using slightly acidic electrolyzed water

Kawai K, Shinozuka Y, Uchida I, Hirose K, Mitamura T, Watanabe A, Kuruwara K, Yuasa R, Sato R, Onda K, Nagahata H.

Anim. Sci. J. 88: 1601-1605. 2017.

SCIENTIFIC REPORTS

OPEN

Characterization of pertussis-like toxin from *Salmonella* spp. that catalyzes ADP-ribosylation of G proteins

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Salmonella Typhimurium definitive phage type (DT) 104 produces a pertussis-like toxin (ArtAB-DT104), which catalyzes ADP-ribosylation of pertussis toxin sensitive G proteins. However, the prevalence of ArtAB and its toxicity have not been established. We report here that, in addition to DT104, *S. Worthington*, and *S. bongori*, produce ArtAB homologs, designated ArtAB-SW and ArtAB-Sb, respectively. We purified and characterized these ArtAB toxins, which comprise a 27-kDa A subunit (ArtA) and 13.8-kDa pentameric B subunits (ArtB). While the sequence of the A subunit, which is ADP-ribosyltransferase, is similar to the A subunit sequences of other ArtABs, the B subunit of ArtAB-Sb is divergent compared to the B subunit sequences of other ArtABs. Intraperitoneal injection of purified ArtABs was fatal in mice; the 50% lethal doses of ArtAB-DT104 and ArtAB-SW were lower than that of ArtAB-Sb, suggesting that ArtB plays an influential role in the toxicity of ArtABs. ArtABs catalyzed ADP-ribosylation of G proteins in RAW 264.7 murine macrophage-like cells, and increased intracellular cyclic AMP levels. ArtAB-DT104 and ArtAB-SW, but not ArtAB-Sb, stimulated insulin secretion in mice; however, unlike Ptx, ArtABs did not induce leukocytosis. This disparity in biological activity may be explained by differences in ADP-ribosylation of target G proteins.

Salmonella spp. cause infectious diseases, such as gastroenteritis, which is associated with diarrhea and inflam-

■ 論文題目

サルモネラ属菌が産生する百日咳毒素様 ADP-リボシル化毒素の性状解析

■ 要 旨

Salmonella Typhimurium ファージタイプ DT104、*S. Worthington*、*S. bongori* は百日咳毒素感受性 G 蛋白質を ADP-リボシル化する毒素を産生する。これらをそれぞれ、ArtAB-DT104、ArtAB-SW および ArtAB-Sb とした。3 種の毒素を精製し解析したところ、ArtAB は 27-kDa の ADP-リボシル化活性を有する A ユニットと 5 つの B ユニット (13.8 kDa) から成る複合体毒素であることを明らかにした。A ユニット間の相同性は高いものの、ArtAB-Sb の B ユニットは他の 2 つと比較して相同性が低い。ArtAB はマウスの腹腔内接種により致死活性を示し、ArtAB-Sb の LD₅₀ 値は ArtAB-DT104 および ArtAB-SW よりも高い値であることから、B ユニットの違いが毒性の差を反映していることが示唆された。3 種の ArtAB はいずれもマクロファージ由来 RAW 細胞の G 蛋白質を ADP-リボシル化し、細胞内の cAMP の濃度を上昇させる活性を示した。ArtAB は百日咳毒素と同様にマウスに対してインスリン分泌亢進活性を示すが、百日咳毒素とは異なり白血球増多活性を示さない。この違いは ArtAB と百日咳毒素における標的 G 蛋白質における亜型の違いによるものである可能性が示唆された。

■ 最終責任者 Ikuo Uchida (Corresponding Author)

ORIGINAL ARTICLE

Control of *Pseudomonas* mastitis on a large dairy farm by using slightly acidic electrolyzed water

Kazuhiro KAWAI,¹ Yasunori SHINOZUKA,¹ Ikuo UCHIDA,² Kazuhiko HIROSE,³ Takashi MITAMURA,⁴ Aiko WATANABE,¹ Kana KURUHARA,¹ Reiko YUASA,¹ Reiichiro SATO,¹ Ken ONDA¹ and Hajime NAGAHATA⁵

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ABSTRACT

The disinfection effect of slightly acidic electrolyzed water (SAEW) use in a farm where *Pseudomonas* mastitis has spread was evaluated. Despite the application of antibiotic therapy and complete cessation of milking infected quarters, numerous new and recurrent *Pseudomonas aeruginosa* clinical mastitis infections (5.8–7.1% of clinical mastitis cases) occurred on the farm from 2003 to 2005. Procedural changes and equipment modifications did not improve environmental contamination or the incidence of *Pseudomonas* mastitis. To more thoroughly decontaminate the milking parlor, an SAEW system was installed in 2006. All milking equipment and the parlor environment were sterilized with SAEW (pH 5–6.5, available chlorine 12 parts per million) before and during milking time. After adopting the SAEW system, the incidence of clinical and subclinical *Pseudomonas* mastitis cases decreased significantly ($P < 0.0001$) and disappeared. These findings suggest that SAEW effectively reduced the incidence of mastitis in a herd contaminated by *Pseudomonas* species. This is the first report to demonstrate the effectiveness of disinfection by SAEW against mastitis pathogens in the environment.

■ 論文題目

緑膿菌乳房炎多発牛群における微酸性電解質を利用した防除対策

■ 要 旨

微酸性電解水 (SAEW) を用いて緑膿菌により牛舎環境が広く汚染された牛群において乳房炎対策を実施した。2003 年から 2005 年間に、乳房炎が多発し (5.8% から 7.1% の罹患率) 抗生物質投与や搾乳の休止等による対策にもかかわらず、一定の効果が認められない牛群に対して対策を講じた。2006 年から当該フリーストール・パーラー牛舎を消毒するために SAEW システムを導入した。SAEW (pH 5–6, 有効塩素濃度 12 ppm) を用いて、搾乳用器具および環境を消毒した。本システムを導入後、緑膿菌による乳房炎が有意に減少し、発生がなくなった ($P < 0.0001$)。これらのことから、SAEW は緑膿菌に汚染された牛群における乳房炎対策に有効であることが明らかとなった。本報告は SAEW が牛舎環境中における乳房炎起因菌の消毒に有効であることを証明した最初の報告である。

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- 1) An adult cystidicolid nematode (Nematoda: Cystidicolidae) from the subcutaneous tissue around the eye of percupinefish, *Diodon nichthemerus* Cuvier.
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Research Note

OPEN ACCESS

An adult cystidicolid nematode (Nematoda: Cystidicolidae) from the subcutaneous tissue around the eye of percupinefish, *Diodon nichthemerus* Cuvier

Kozue Sasaki¹, Yoshimi Miyagawa², Itsuki Kiyatake², Kiyoko Onda³, Takaomi Ito² and Mitsuhiro Asakawa¹

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Abstract: Three adult nematode specimens, all ovigerous females belonging to the family Cystidicolidae Skryabin, 1946, were found for the first time in the subcutaneous tissue around the eye of the captive percupinefish *Diodon nichthemerus* Cuvier at a public aquarium in Osaka, Japan. Because no male was available, these could not be identified to the genus and species. This case highlights the risk of parasitism in aquaculture puffer fish, as these may ingest small shrimp, which probably act as intermediate hosts for the nematode.

Keywords: Habronematoidea, parasite, marine fish, Diodontidae, Japan

■ 論文題目

水族館飼育ポーキュパインフィッシュ *Diodon nichthemerus* (フグ目ハリセンボン科)の眼球周囲組織に寄生した線虫摘出症例と得られた Cystidicolidae 科線虫の分類学的検討

■ 要 旨

2015 年 5 月、南オーストラリアから輸入され、大阪・海遊館で飼育されていたポーキュパインフィッシュ *Diodon nichthemerus* (フグ目ハリセンボン科) 1 個体の左眼上部皮下観察により、乳白色の屈曲した細紐状のものが認められた。2-phenoxyethanol により麻酔し、外科的にその物体を摘出したところ、線虫 3 虫体であった。体長 9.0cm から 10.9cm で頭部先端は鈍、口腔周辺に 4 つの乳頭が認められた。寄生状況と頭部・尾部形態は Cystidicolidae 科の *Metabronema* 属に類似したが、頭部形態は *Neoscarophis* 属/*Cristitectus* 属に近かった。新属の可能性もあり、現段階では Cystidicolidae gen. sp. に留めた。なお、*Metabronema* 属線虫はカリビアンポーキュパインフィッシュ *Diodon hystrix* で、眼球突出症の原因虫として知られるが、この報告も線虫の形態分類学的な検討はされていなかった。この属線虫の生活史は不明であるが、海産魚寄生の Cystidicolidae 科の別属線虫種ではサクラエビ科やクルマエビ科 (中間宿主) などを摂食して感染するとされる。もし、今回見つかった種が同様な経路をとるのであれば、系統・生態的に近い養殖や愛玩用フグ類にも寄生し、視力障害や風評被害に繋がる危険性もあるので注意したい。

■ 最終責任者 Mitsuhiro Asakawa (Corresponding Author)

[Research Note]

***Soboliphyme baturini* (Nematoda: Dioctophymatidae) infection in a domestic cat suffering severe diarrhea**

Mitsuhiko Asakawa^{1,*}, Moeka Takeuchi¹
and Yoshikazu Torii²

The genus *Soboliphyme* is a group of parasitic nematodes found in the families Soricidae and Mustelidae in the Holarctic Region, including Japan. We isolated a member of the genus, *S. baturini*, from a domestic cat (*Felis catus*), which had suffered from severe diarrhea. This is the first local record in a cat in Japan, and the second one from a cat in the world. Nematol. Res. 47(1), 21–22 (2017).

Key words: diarrhea, domestic cat, *Soboliphyme*

However, because the eggs were still present in the feces, Broadline[®] (Nippon Zenyaku Kogyo Co., Ltd, Koriyama, Japan; 0.9 ml, s.c., including 83.0 mg/kg fipronil, 100 mg/kg (S)-methoprene, 83.0 mg/kg praziquantel, 4.0 mg/kg eprinomectin), was administered; soon after, six nematodes were extracted from the patient. No eggs were observed at this time.

The parasitic nematodes were fixed and preserved in 70% ethanol, and examined microscopically in lactophenol solution. Measurements and drawings of the nematodes were made with the aid of a microscope drawing attachment (Olympus Model BH2-DA). The specimens were deposited in the Wild Animal Medical Center, Rakuno Gakuen University, Hokkaido, Japan (WAMC/AS no. 15890).

All the nematodes collected were female. In the five nematodes measured, the body length was 2.5–3.9 cm (mean 3.36 ± 0.56 cm) and the width 1.2–1.6 mm (mean 1.44 ± 0.13 mm). A well-developed sucker was observed in the anterior part of the body, with a diameter of 1.1–3.3 mm (mean 2.47 ± 0.74 mm, Fig. 1). Based on its peculiar

■ 論文題目 消化器症状を呈したイエネコにおける線虫 *Soboliphyme baturini* の寄生事例

■ 要 旨

腎虫類 *Soboliphyme* 属はおもにトガリネズミ科とイタチ科に寄生する線虫であるが、今回、消化器症状を呈したイエネコを駆虫したところ、この属線虫が排出された。2015 年秋、長野県阿智村にて野外で生活をしていた個体が救護され、一般家庭に飼育された（雄・雑種月齢、来院時推定約 5 ヶ月齢、体重 1.25kg）。同年 11 月下旬、扁平な蠕虫をともなった水様下痢便が認められたことから動物病院で受診された。その下痢から猫回虫卵と鞭虫様虫卵（透明卵殻、サイズ $84 \mu\text{m} \times 38 \mu\text{m}$ ）が認められたのでミルベマイシンを経口的投与した。その結果、多数の猫回虫成虫が排泄されたが、鞭虫様虫卵は消失しなかった。そこで、ブロードライン 0.9ml を皮下投与したところ、体長約 3.3cm、体幅約 1.4mm、直径約 2.5mm の頭胞を有した雌線虫 6 虫体が糞便とともに排出された。70%エタノール液で固定後、ラクトフェノール液で透徹し、形態を観察したところ *S. baturini* と同定された。本種終宿主はイタチ科であるが、国外ではネコも知られる。ネコは国内初であったが、同県ではアメリカミンクからこの線虫は見出されていた。吸盤状を呈した口腔で胃壁に吸着し、胃粘膜損傷や胃潰瘍が生ずることが知られるので、下痢や血便などを呈した個体では、この線虫による寄生中病も類症鑑別の対象として念頭に置く必要がある。この種は淡水産ミミズ類を中間宿主とし、時に、トガリネズミ類などを待機宿主としているので、特に、このような動物を摂食する傾向のあるネコは注意したい。今回の症例もしたものと想像されたが、飼育前の状況に関しての検証は不可能であった。

■ 最終責任者 Mitsuhiko Asakawa (Corresponding Author)

Molecular survey of gastropods as intermediate hosts of protostrongylid nematodes in Uzbekistan

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ABSTRACT

Protostrongylid species are causative agents of pulmonary protostrongyliasis in caprine species in Uzbekistan. These nematodes typically require one intermediate host, terrestrial mollusks, to complete their life cycle. In this study, eight species of gastropods were found to be positive for protostrongylid larvae. Haplotypes of larvae corresponding to sequences of *Protostrongylus rufescens* and *Muellerius capillaris* were detected. Morphological identification of gastropods, based on shell characteristics, revealed six different morphotypes. Anatomic-morphological and molecular results confirmed the membership of these gastropods to the Buliminidae, Hygromiidae, Agriolimacidae and Parmacellidae and revealed eight different species: *Pseudonapaeus maydanika*, *P. sogdiana*, *P. albiplicatus*, *Pseudonapaeus* sp., *Angiomphallia regeliana*, *Xeropicta candacharica*, *Candaharia levanderi* and *Deroceras reticulatum*. This study displays the first report of *P. maydanika*, *C. levanderi* and *D. reticulatum* as natural intermediate hosts of *M. capillaris*. The infection rate of snails with protostrongylids was 27.9% (616/2,207) and the infection rate of slugs was 6.5% (18/279).

Key words : Protostrongyliasis, Caprine, Gastropod, Molecular identification, Epidemiology.

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Jpn. J. Vet. Parasitol. Vol. 16, No. 1・2

■ 論文題目 ウズベク共和国におけるProtostrongylidae 科線虫の中間宿主—腹足類

■ 要 旨

ウズベク共和国における羊の肺虫症の原因寄生虫プロストロンギルス科線虫は陸棲腹足類を中間宿主としているので、この線虫症の疫学では腹足類の調査が必須であった。そこで、同国において貝殻の形態で6種に分類された軟体類を対象にプロストロンギルス科の感染幼虫の保有状況を調査した。その結果、分子生物学的に *Protostrongylus rufescens* および *Muellerius capillaris* のハプロタイプに一致した感染幼虫を得た。また、今回、幼虫が得られた腹足類について解剖学および分子生物学的な検討によりこれら4科Buliminidae, Hygromiidae, Agriolimacidae あるいは Parmacellidae の次の8種に同定された； *Pseudonapaeus maydanika*, *P. sogdiana*, *P. albiplicatus*, *Pseudonapaeus* sp., *Angiomphallia regeliana*, *Xeropicta candacharica*, *Candaharia levanderi*, *Deroceras reticulatum*。本研究により *P. maydanika*, *Candaharia levanderi* および *D. reticulatum* が *M. capillaris* の自然中間宿主であることが証明された。全体的なプロストロンギルス科線虫の感染幼虫の保有率はカタツムリ類で27.9% (616/2,207)、一方、ナメクジ類では6.5% (18/279)と低かった。本研究は筆頭著者が2016年度の公益財団法人・松前国際友好財団助成を受け本学獣医学研究科で実施された研究の一部である。

■ 最終責任者 Mitsuhiro Asakawa (Corresponding Author)

Prevalence of liver flukes in cattle at an abattoir in Wakiso District, Uganda

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ABSTRACT

Bovine fasciolosis is one of the most important parasitic diseases of cattle causing mortality and production losses in various parts of Uganda. However, little information is available about its prevalence in Uganda. A cross sectional study aimed at determining the prevalence of liver flukes in cattle was conducted between June 2015 and May 2016 at an abattoir in Wakiso District, Uganda. The study was based on post-mortem inspection of livers of slaughtered animals at Wakiso abattoir. Out of 287 livers inspected, 44 (15.0%) were positive for liver flukes. Risk factors such as season, breed and sex did not have significant effect on the prevalence of infections ($P > 0.05$). However, age and body condition showed significant association with the prevalence of infections ($P < 0.05$). That implied cattle of older age and poor body condition increased the risk of infection. Of the total livers examined, 75% had between 1 to 20 flukes, 20% had between 21 to 100 and only 5% had more than 100 flukes. In the present study, the prevalence of liver flukes was considerably lower compared to other previous studies in Uganda. We could not obtain detailed cattle and farm information. Further studies are necessary in order to investigate the difference of these results and to design effective control and treatment of infected cattle.

Key words: cattle, fasciolosis, post-mortem inspection, risk factors, Uganda.

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■ 論文題目 ウガンダ・ワキソ県の一と畜場における牛での肝蛭寄生率実態調査

■ 要 旨

2015年6月から2016年5月、ウガンダのワキソ県にある一と畜場で牛での肝蛭寄生率の実態調査を実施した。本研究は、と体牛の肝臓を細切し、肉眼的に肝蛭の有無及び数を検査することにより行われた。合計287検体の肝臓が検査され、そのうち44検体から、肝蛭が検出された(寄生率15%)。また、季節性(雨季と乾季)、種、年齢、性差及びボディコンディションスコア(BCS)といったリスク因子と寄生率との相関関係を調べた。季節性、種、性差と寄生率には、相関関係が認められなかった($P > 0.05$)。しかし、年齢及びBCSと寄生率には、相関関係が認められた($P < 0.05$)。つまり、年齢の高い牛及びBCSが低い牛の方が、寄生率が高いことを示していた。肝蛭の寄生数に関しては、75%が1から20虫体の間であったが、100虫体を超える肝臓も認められた。本研究の結果は、先にウガンダのカンパラで行われた研究に比較して、肝蛭寄生率は低いものであったが、放牧地汚染及び感染個体を減らすためには、戦略的な予防治療策が必要であると思われた。

■ 最終責任者 Mitsuhiko Asakawa (Corresponding Author)

***Nippostrongylus smalesae* sp. nov. (Nematoda: Heligmonellidae) collected from *Maxomys whiteheadi* (Rodentia: Muridae) of Sumatra, Indonesia**

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Abstract

Nippostrongylus smalesae sp. nov. (Nematoda: Heligmonellidae) is described from a murid *Maxomys whiteheadi* collected in Riau, Sumatra, Indonesia. It is readily distinguished from all of the known congeners by having the ray 3 widely diverged distally from the ray 4 in the left lobe of bursa copulatrix. This is the tenth named species of *Nippostrongylus*. The same species but with slight morphological difference was also found from *M. whiteheadi* and *Niviventer cremoriventer* in Kalimantan (Borneo Island), suggesting wide geographical distribution on the Sundashelf islands.

■ 論文題目

インドネシア・スマトラ島産齧歯類 *Maxomys whiteheadi* から得られた Heligmonellidae 科線虫の新種 *Nippostrongylus smalesae* sp. nov. 記載

■ 要 旨

学振 RONPAKU プロジェクトの一環として、インドネシア・スマトラ島に生息する齧歯類 *Maxomys whiteheadi* の小腸から得られた Heligmonellidae 科線虫が交接嚢形態がボルネオ島に産する近縁種と異なることから新種 *Nippostrongylus smalesae* sp. nov. として記載された。この種は、筆頭著者の PhD 主論文の課題である生物地理学的な解析の重要な種の一つとなった。

■ 最終責任者 Mitsuhiko Asakawa (Corresponding Author)

Nematodes in the digestive tracts of domestic ruminants in Uzbekistan

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ABSTRACT

Thirty-four species of nematodes were recorded in the digestive tracts of domestic ruminants in Uzbek including 32 from sheep, 30 from goats, and 28 from cattle. Twenty-nine species of nematodes were found in the plain areas of Uzbekistan and 32 in the foothills and mountains. The vast majority of the detected (31) were nematodes which develop directly, and only 3 species were ones which develop indirectly. The dominant species with the highest prevalence, which also showed the greatest intensity, were *Ostertagia circumcincta*, *Marshallagia marshalli*, *Teladorsagia circumcincta*, and *Parabronema skrjabini*.

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■論文題目 ウズベク共和国産反芻家畜の消化管に寄生する線虫類

■要 旨

ウズベク共和国産反芻家畜の消化管に寄生する線虫類は34種が認められ、それぞれヤギからは32種、ウシからは30種であった。地形的には平野部で29種、山岳地帯麓部で32種が認められた。線虫類の生活史の区分けとしては直接発育するものが31種、間接発育するものが3種であった。もっとも高率に寄生し、かつ、宿主1個体あたりの寄生数が多い種は *Ostertagia ostertagi*, *Marshallagia marshalli*, *Teladorsagia circumcincta* および *Parabronema skrjabini* であった。これら線虫感染の防疫を行う場合、本論文で分析した2あるいは3つの要素に分けて対応する必要性が指摘された。なお、本研究は筆頭著者が2016年度の公益財団法人・松前国際友好財団助成を受け、本学獣医学研究科にて実施された内容の一部である。

■最終責任者 Mitsuhiro Asakawa (Corresponding Author)



Brachylaima ezohelicis sp. nov. (Trematoda: Brachylaimidae) found from the land snail *Ezohelix gainesi*, with a note of an unidentified *Brachylaima* species in Hokkaido, Japan



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ABSTRACT

In the Japanese Archipelago, *Ezohelix gainesi*, a member of bradybaenid land snails, is endemic mainly to the island of Hokkaido. During July to August of 2016, a survey to detect trematode infections from *E. gainesi* was carried out at a forest city park in Asahikawa, Hokkaido. Systemic infections of the snails with sporocysts containing short-tailed cercariae were found in 5.3% of 94 individuals examined. Furthermore, most of them (90.4%) harbored non-encysted metacercariae within their kidneys. A DNA sequence identification revealed that both of the sporocyst and the metacercaria belong to an unknown species of the family Brachylaimidae. The metacercariae showed a genetic diversity with 6 haplotypes of mitochondrial DNA (mtDNA) even in the limited sampling area. A definitive host of the unknown species could not be determined, although 34 field mice (*Apodemus speciosus*) and 21 voles (*Myodes ruficanus*) from the city park were examined for intestinal parasites. To examine the adult stage, the metacercariae were perorally administered to mice, together with anti-inflammatory treatment with methylprednisolone. Fully matured adult worms were recovered from the intestinal ileum 8 and 14 days postinfection. The gravid adults showed typical features of the genus *Brachylaima*. An morphological and biogeographical evaluation prompted us to propose *Brachylaima ezohelicis* sp. nov. for the parasite from *E. gainesi*. The autochthony of the first intermediate host and the spatial heterogeneity of mtDNA suggest that the new species found in the city park is not a recently expanded population of immigrant origin.

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■ 論文題目

北海道産カタツムリ類 *Ezohelix gainesi* から得られた Brachylaimidae 科吸虫の新種 *Brachylaima ezohelicis* sp. nov. 記載とその近縁未知種の分類的なノート

■ 要 旨

旭川医科大学および目黒寄生虫館との共同で国内の Brachylaimidae 科吸虫の生物地理学的研究が実施されているが、その一環で北海道産カタツムリ類 *Ezohelix gainesi* から得られた同科吸虫の新種 *Brachylaima ezohelicis* sp. nov. とその近縁未知種が得られ、その分類学的な論考を行った。

■ 最終責任者 Minoru Nakao (Corresponding Author)



Characterizing the temporal patterns of avian influenza virus introduction into Japan by migratory birds

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ABSTRACT. The objectives of the present study were to observe the temporal pattern of avian influenza virus (AIV) introduction into Japan and to determine which migratory birds play an important role in introducing AIV. In total, 19,407 fecal samples from migratory birds were collected at 52 sites between October 2008 and May 2015. Total nucleic acids extracted from the fecal samples were subjected to reverse transcription loop-mediated isothermal amplification to detect viral RNA. Species identification of host migratory birds was conducted by DNA barcoding for positive fecal samples. The total number of positive samples was 352 (prevalence, 1.8%). The highest prevalence was observed in autumn migration, and a decrease in prevalence was observed. During autumn migration, central to southern Japan showed a prevalence higher than the overall prevalence. Thus, the main AIV entry routes may involve crossing the Sea of Japan and entry through the Korean Peninsula. Species identification was successful in 221 of the 352 positive samples. Two major species sequences were identified: the Mallard/Eastern Spot-billed duck group (115 samples; 52.0%) and the Northern pintail (61 samples; 27.6%). To gain a better understanding of the ecology of AIV in Japan and the introduction pattern of highly pathogenic avian influenza viruses, information regarding AIV prevalence by species, the prevalence of hatch-year migratory birds, migration patterns and viral subtypes in fecal samples using egg inoculation and molecular-based methods in combination is required.

KEY WORDS: avian influenza, DNA barcoding, Eastern spot-billed duck, Mallard, Northern pintail

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■ 論文題目 渡り鳥による鳥インフルエンザウイルス導入に関する時空間的特性

■ 要 旨

2008年から2012年にかけて、10月から翌年5月まで、日本国内52か所にて野生カモ類の糞便を採集し、LAMP法により鳥インフルエンザウイルスAIVの調査を行った。また、糞便から鳥種のDNAを得、宿主の同定を行った。その結果、陽性割合が高い鳥類種がマガモ/カルガモ群であったこと、北陸から東海にかけての地域で陽性率が高かったこと、10～12月に陽性率が高くなり、その後、徐々に低減したことが判明した。このことから、日本海を陽性率の高い幼鳥が最初に渡って来ていることが判明した。この情報は、集中的なAIに対する防疫対策を施行する上で有益な情報となった。

■ 最終責任者 Manabu Onuma (Corresponding Author)



NOTE

Wildlife Science

Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease

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ABSTRACT. The aim of this study was to objectively assess stress of kangaroos affected by lumpy jaw disease (LJD) using plasma and hair cortisol concentrations. The plasma and hair samples were collected from kangaroos with LJD and healthy controls. Collected hair samples were extracted with methanol after washing with isopropanol, following which they were processed with the cortisol enzyme immunoassay kit. The plasma cortisol concentration of LJD animals tended to be higher than that of the control. Ventral hair cortisol, but not dorsal hair, of LJD animals was significantly higher than that of the control. In conclusion, stress in kangaroos infected with LJD could be assessed by measuring ventral hair cortisol.

KEY WORDS: hair cortisol, kangaroo, lumpy jaw disease, objectively assessment, stress

■ 論文題目

lumpy jaw disease (LJD)罹患カンガルーの被毛中のコルチゾールを用いたストレス評価

■ 要 旨

本研究の目的は、血漿および被毛中コルチゾールを用いて lumpy jaw disease (LJD) 罹患カンガルーのストレスを客観的に評価することである。LJD 罹患カンガルーおよび健常個体より血液および被毛を採取し、血漿および被毛から抽出した溶液中のコルチゾール濃度を ELISA キットにより測定した。LJD 罹患カンガルーの血漿中コルチゾール濃度はコントロールよりも高い傾向があった。LJD 罹患カンガルーの腹側被毛中コルチゾール含有量はコントロールよりも有意に高い値を示した。以上のことより、LJD に罹患したカンガルーのストレスは腹側被毛中コルチゾール含有量を測定することにより評価することができることが示唆される。

■ 最終責任者 Hideaki Hayashi (Corresponding Author)



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USE OF DIAZEPAM-KETAMINE IN PREVENTION OF CAPTURE MYOPATHY IN THE OSTRICH (*STRUTHIO CAMELUS*): A CASE REPORT

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ABSTRACT

Capture or exertional myopathy is an attendant complication of manual restraint in ratites, besides physical injuries that handlers may suffer. This work was carried out to restrain and immobilize two ostriches (*Struthio camelus*) in a bid to facilitate their clinical examination and transportation from one location to another, without subjecting the birds to capture myopathy that arises from the stress and exertion associated with physical restraint and capture. Two ostriches, male and female, weighing 120kg and 105kg respectively, were requested to be immobilized for relocation from Onireke to Jericho area in Oyo State, Nigeria. The birds were fasted overnight, administered with diazepam (3mg/kg) orally and ketamine (10mg/kg) intramuscularly. Mild sedation was achieved with diazepam after one hour. The birds were successfully transported. Complete recovery was achieved at about 8 hours post-administration of ketamine. Anaesthetic agents did not alter the haematologic parameters. We conclude that the diazepam-ketamine combination is generally safe for ratites and that oral administration of diazepam accompanied by intramuscular administration of ketamine provides restraint in ratites while also avoiding the risk of capture myopathy. It is suggested that the current dose of diazepam might need to be increased if the oral route is to be employed so as to shorten the onset of sedation and increase the depth of sedation.

Keywords: Capture myopathy, chemical restraint, ostrich.

■ 論文題目

飼育下カンガルーにおけるカンガルー病（LJD）発症と各種ストレスマーカーの関連性

■ 要 旨

到津の森公園ならびにひびき動物ワールドで飼育されているカンガルー38頭（健常群；13頭、LJD群；25頭）を対象に血清中コルチゾール濃度、酸化ストレス値（d-ROMs）および抗酸化力（BAP）の比較を行った。さらに唾液中コルチゾール濃度の測定も併せて行った。酸化ストレス値ならびに血清中コルチゾール濃度はLJD群で優位に高値を示したが、その他の値は両群間に有意な差は認められなかった。LJDに罹患することでストレス関連マーカーの値に変化は認められるものの、比較的大きな個体差が存在することを明らかにすることができた。

■ 最終責任者 Tadashi Sano (Corresponding Author)



NOTE

Wildlife Science

Plasma endotoxin activity in Eastern grey kangaroos (*Macropus giganteus*) with lumpy jaw disease

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ABSTRACT. Progressive pyogranulomatous osteomyelitis involving the mandible or maxilla of captive macropods, referred to as "Lumpy jaw disease (LJD)", is one of the most significant causes of illness and death in captive macropods. The aim of the present study was to evaluate the relationship between the severity of LJD and plasma endotoxin activity in kangaroos. Plasma samples obtained from moderate (n=24) and severe LJD (n=12), and healthy kangaroos (n=46), were diluted 1:20 in endotoxin-free water and heated to 80°C for 10 min. Plasma endotoxin activity was measured using the Limulus amoebocyte lysate (LAL)-kinetic turbidimetric (KT) assay. Plasma endotoxin activity was higher in kangaroos with severe LJD (0.199 ± 0.157 EU/ml) than in those with moderate LJD (0.051 ± 0.012 EU/ml, $P < 0.001$) and healthy controls (0.057 ± 0.028 EU/ml, $P < 0.001$). Our results suggest that the severity of LJD in captive macropods may be related to the plasma endotoxin activity.

KEY WORDS: captive, endotoxin, lumpy jaw, macropod, severity

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■ 論文題目 Lumpy Jaw Disease 罹患オオカンガルーの血漿中エンドトキシン活性値

■ 要 旨

下顎骨または上顎骨を含む進行性骨髄炎 (Lumpy jaw disease、LJD) は有袋類において重要な疾患である。本研究の目的は、カンガルーにおける LJD の重篤度と血漿エンドトキシン活性値との関係性を評価することであった。中等度 (n=24)、重度の LJD 罹患 (n=12) および健常カンガルー (n=46) から得た血漿をエンドトキシンの汚染のない注射用水で 20 倍希釈し、80°C で 10 分間加熱した。血漿エンドトキシン活性値は、リムルス (LAL) 比濁時間法 (KT) を用いて測定した。血漿エンドトキシン活性は、中等度 LJD (0.051 ± 0.012 EU/ml、 $P < 0.001$) および健常 (0.057 ± 0.028 EU/ml、 $P < 0.001$) よりも重度 LJD カンガルーで有意に高値を示した (0.199 ± 0.157 EU/ml)。動物園で飼育されている有袋類の LJD の重篤度と血漿中エンドトキシン活性値は関連性があることが示唆された。

■ 最終責任者 Kazuyuki Suzuki (Corresponding Author)

**Specific Identity of *Kalicephalus* Molin, 1861
(Nematoda: Diaphanocephalidae)
Specimens Collected from the Albino Japanese Ratsnake,
Elaphe climacophora (Boie, 1826) (Serpentes: Colubridae)**

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Abstract: Nine male and eight female adult nematodes in the genus *Kalicephalus* reported from albino Japanese ratsnakes, *Elaphe climacophora* from Iwakuni City, Yamaguchi Prefecture, Japan, were re-examined to confirm their taxonomic placement. Previously, these nematodes were identified as *K. natricis* Yamaguti, 1935, despite the fact that the name had been regarded by some earlier authors as a *species inquirenda*. Our close re-examination of the morphological characters of both sexes suggests that those worms actually belong to *K. sinensis* Hsü, 1934, which has been recorded from several Japanese snakes, including *E. climacophora*.

■ 論文題目

山口県岩国産シロヘビから報告された Diaphanocephalidae 科線 *Kalicephalus* 属の分類学的な再検討

■ 要 旨

国の天然記念物山口県岩国産シロヘビ（アオダイショウのアルビノ個体）では、Diaphanocephalidae 科線 *Kalicephalus* 属の多数寄生により、致死性腸炎に罹患する個体が多かったため、本学野生動物医学センターにその疫学調査が委託された。その際に得られた同属線虫の種レベルの分類学的な再検討を行い、種名を修正した。

■ 最終責任者 Hideo Hasegawa (Corresponding Author)



NOTE

Wildlife Science

Plasma lead, silicon and titanium concentrations are considerably higher in green sea turtle from the suburban coast than in those from the rural coast in Okinawa, Japan

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ABSTRACT. The purpose of this study was to compare the concentration of trace elements in the plasma of sea turtles that inhabited the suburban (Okinawa Main Island, n=8) and the rural coast (Yaeyama Island, n=57) in Okinawa, Japan. Particle induced X-ray emission allowed detection of 20 trace and major elements. The wild sea turtles in the suburban coast in Okinawa were found to have high concentrations of Pb, Si and Ti in the plasma when compared to the rural area but there were no significant changes in the Al, As and Hg concentrations. These results may help to suggest the status of some elements in a marine environment. Further, monitoring the plasma trace and major element status in sea turtles can be used as a bio-monitoring approach by which specific types of elements found here could indicate effects that are related to human activities.

KEY WORDS: marine pollution, Okinawa Main Island, particle induced X-ray emission, sea turtle, Yaeyama Island

■ 論文題目

沖縄郊外の沿岸に生息するアオウミガメの血漿中 Pb, ケイ素およびチタン濃度は地方の沿岸に生息するウミガメよりも高値である

■ 要 旨

本研究の目的は、沖縄の郊外(沖縄本島、n = 8)と地方(八重山島、n = 57)に生息したウミガメの血漿中の微量元素の濃度を比較することである。粒子誘起 X 線分析法により 20 種類の微量および主要元素の検出が可能であった。沖縄郊外に生息している野生のウミガメは地方のそれに比べて血漿中 Al、As、Hg 濃度には大きな差は認められなかったものの、Pb、Si、Ti 濃度が有意に高値であった。これらの結果は、海洋環境の状態を評価するのに役立つものと思われる。さらに、ウミガメの血漿中微量元素および主要元素の状態をモニタリングすることは、本知見によって差が認められた Pb、Si、Ti が人間の活動に関連するものであり、人間活動による影響に対するバイオモニタリングアプローチとして応用できる。

■ 最終責任者 Kazuyuki Suzuki (Corresponding Author)

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

- 1) Natural larval *Echinococcus multilocularis* infection in a Norway rat,
Rattus norvegicus, captured indoors in Hokkaido, Japan.
Fukumoto S, Yamada S, Fushikida M, Toyoda S, Nishikawa T,
Higuchi H, Ueno H, Ueda H, Sugiyama H, Morishima H.
J. Vet. Med. Sci. 79: 1857-1860. 2017



Natural larval *Echinococcus multilocularis* infection in a Norway rat, *Rattus norvegicus*, captured indoors in Hokkaido, Japan

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ABSTRACT. Natural infection with larval *Echinococcus multilocularis* was recognized in one of eight Norway rats, *Rattus norvegicus*, caught indoors in 2009 in Ebetsu, Hokkaido, northern Japan. Cystic lesions were found in the right median and lateral lobes of the liver, with numerous alveolar cysts in the periphery of the lesions. Protoscolices were formed within large cysts. The laminated layers of the cysts were positive for PAS staining. Nested PCR using the primers specific for *Taenia* mitochondrial 12S rDNA yielded a 250-bp product, and the sequence of the PCR product matched that of *E. multilocularis* isolates from Hokkaido and Germany. This is the third natural alveolar hydatidosis in *R. norvegicus* in Japan.

KEY WORDS: alveolar hydatidosis, *Echinococcus multilocularis*, indoor infection, Norway rat, protoscolex

■ 論文題目

北海道内で検出された建造物内で捕獲された多包条虫幼虫(多包虫)ドブネズミ *Rattus norvegicus* 自然感染例

■ 要 旨

2009年に北海道江別市の建造物内で捕獲された8頭のドブネズミ、*Rattus norvegicus*の1頭に*Echinococcus multilocularis*の幼虫による自然感染が認められた。嚢胞性病変は肝臓の右中央および側葉に見られ、病変の周辺には多数の繁胞形成が見られた。原頭節は大きな嚢胞中で形成された。嚢胞のクチクラ層はPAS染色陽性であった。Taenia科条虫 mitochondrial 12S rDNAに特異的なプライマーを用いたPCR反応により、250bpの産物を得、PCR産物の配列は、北海道およびドイツの*E. multilocularis*分離株の配列と一致した。これは、日本の*R. norvegicus*における3番目にあたり、さらに世界で初めての室内感染が示唆された自然感染多包虫症例であった。

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

- 1) Effects of expressing human T-cell leukemia virus type 1 (HTLV-I) oncoprotein Tax on DOK1, DOK2 and DOK3 gene expression in mice.
Ohsugi. T._
J. Vet. Med. Sci., 79: 935-938. 2017.

II . その他<Others>



NOTE

Laboratory Animal Science

Effects of expressing human T-cell leukemia virus type 1 (HTLV-I) oncoprotein Tax on *DOK1*, *DOK2* and *DOK3* gene expression in mice

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ABSTRACT. Transgenic mice expressing the *tax* gene from human T-cell leukemia virus type 1 (HTLV-I) genome developed T-cell leukemia or histiocytic sarcoma after at least 12 months. The transgenic mice showed low expression of the downstream of tyrosine kinase (DOK) family members, *DOK1*, *DOK2* and *DOK3*, which were recently reported to be tumor suppressor genes. Mice showed low *DOK2* expression at 5–6 months of age, before disease onset. The expression of *DOK1* and *DOK3* was not significantly reduced at any age tested. These results suggest that downregulation of *DOK2* by the expression of the viral *tax* gene is the first step in the development of T-cell leukemia or histiocytic sarcoma.

KEY WORDS: adult T-cell leukemia/lymphoma (ATLL), downstream of tyrosine kinase (DOK), human T-cell leukemia virus type 1 (HTLV-1)

■ 論文題目

マウスにおける HTLV-I Tax タンパク発現が *DOK1*, 2 および 3 遺伝子発現に与える影響

■ 要 旨

ヒト T 細胞白血病ウイルス 1 型 (HTLV-1) *tax* 遺伝子を発現するマウスは、12 か月齢以降に T 細胞白血病あるいは組織球肉腫を発症する。発症したマウスは、最近がん抑制遺伝子として報告された蛋白質チロシンキナーゼのアダプター分子である *DOK1*, 2 および 3 の発現が低下している。そこで発症前マウスの *DOK* 遺伝子ファミリーを解析したところ、生後 5 から 6 か月齢ですでに *DOK2* 遺伝子の発現低下がみられた。一方、*DOK1* および 3 の減少は観察されなかった。これらの結果は、T 細胞白血病あるいは組織球肉腫を発症する最初の段階として *DOK2* 遺伝子の発現低下が重要であることを示唆している。

■ 最終責任者 Takeo Ohsugi (Corresponding Author)

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II. その他<Others>

- 1) Morphometric changes in the aortic arch with advancing age in fetal to mature thoroughbred horses.
Endoh C, Matsuda K, Okamoto M, Tsunoda N, Taniyama H.
J. Vet. Med. Sci. 79: 661-669. 2017.
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Nagamine E, Hirayama K, Matsuda K, Okamoto M, Ohmachi T, Uchida K, Kadosawa T, Taniyama H.
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Vet. Pathol. 54: 218-221. 2017.



FULL PAPER

Pathology

Morphometric changes in the aortic arch with advancing age in fetal to mature thoroughbred horses

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ABSTRACT. Aortic rupture is a well recognized cause of sudden death in thoroughbred horses. Some microscopic lesions, such as those caused by cystic medial necrosis and medionecrosis, can lead to aortic rupture. However, these microscopic lesions are also observed in normal horses. On the other hand, a previous study of aortic rupture suggested that underlying elastin and collagen deposition disorders might be associated with aortic rupture. Therefore, the purpose of this study was to compare the structural components of the tunica media of the aortic arch, which is composed of elastin, collagen, smooth muscle cells and mucopolysaccharides (MPS), in fetal to mature thoroughbred horses. The percentage area of elastin was greatest in the young horses and subsequently decreased with aging. The percentage area of collagen increased with aging, and the elderly horses (aged ≥ 20) exhibited significantly higher percentage areas of collagen than the young horses. The percentage area of smooth muscle cells did not change with age. The percentage area of MPS was inversely proportional to the percentage area of elastin. The fetuses exhibited a markedly larger percentage area of MPS than the mature horses. We concluded that the medial changes seen in the aortic arch, which included a reduction in the amount of elastin and increases in the amounts of collagen and MPS, were age-related variations.

KEY WORDS: aorta, aortic arch, equine, histopathology, morphometry

■ 論文題目

サラブレッド馬の加齢に伴う大動脈弓の形態学的変化

■ 要 旨

動脈破裂はサラブレッド種馬の急死の原因として知られており、嚢胞性中膜壊死や層状中膜壊死などの顕微鏡的病変、エラスチンやコラーゲンの沈着が関与すると考えられている。そのため、本研究では大動脈弓中膜の組織構成（エラスチン、コラーゲン、平滑筋細胞、MPS）を胎子から成馬まで比較することを目的とした。エラスチンは若齢馬で最も割合が多く、加齢とともに減少した。コラーゲンは加齢とともに増加し、高齢馬では若齢馬と比較して有意に多かった。平滑筋細胞の加齢性変化はなかった。MPS の割合はエラスチンに反比例し、胎子では成馬に比べて多かった。大動脈中膜においてエラスチンの減少とコラーゲン、MPS の増加は加齢性変化であると考えられた。

■ 最終責任者 Hiroyuki Taniyama (Corresponding Author)

Invasive Front Grading and Epithelial-Mesenchymal Transition in Canine Oral and Cutaneous Squamous Cell Carcinomas

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T. Ohmachi³, K. Uchida⁴, T. Kadosawa⁵, and H. Taniyama²

Abstract

Oral and cutaneous tissues are the most frequent origin in canine squamous cell carcinoma (SCC). In SCC, changes in adhesion molecule expression and transition from epithelial to mesenchymal phenotype are thought to be important in development of invasive behavior of neoplastic cells at the leading front of the tumor. We therefore investigated histological invasive front grading and epithelial-mesenchymal transition (EMT) in both oral SCCs and cutaneous SCCs. EMT was assessed by evaluating immunohistochemical expression of E-cadherin, β -catenin, desmoglein, vimentin, and N-cadherin. Regardless of the anatomic location, invasive front grading resulted in higher histological grades than grading of the surface. Most oral SCCs were of significantly higher histologic grade than cutaneous SCCs ($P < .01$). Expression of E-cadherin, β -catenin, and desmoglein was significantly lower in oral SCC compared with cutaneous SCC ($P < .01$). A significant association was found between invasive front grading and loss of E-cadherin, β -catenin, and desmoglein ($P < .01$). Also, vimentin-positive neoplastic cells had low immunoreactivity of these adhesion molecules, and a few of these neoplastic cells were positive for N-cadherin. These results suggest not only E-cadherin and β -catenin but also desmoglein as markers for predicting biological behavior of canine SCC. Depending on their primary sites, EMT correlates with biological behavior and therefore histological grade of canine SCC. We suggest that combining invasive front grading with assessment of immunohistochemical expression of E-cadherin, β -catenin, and desmoglein may allow more accurate prediction of biological behavior of canine SCCs.

■ 論文題目

犬口腔内および皮膚扁平上皮癌における invasive front グレーディングと上皮間葉転換

■ 要 旨

犬の扁平上皮癌（SCC）は口腔及び皮膚に好発する。SCC では細胞間接着分子発現の変化と上皮間葉転換（EMT）が invasive front（IF）における腫瘍細胞の浸潤性に重要と考えられている。本研究では組織学的 IF グレーディングと EMT について口腔内および皮膚 SCC において調査した。EMT は E-cadherin、 β -catenin、desmoglein、vimentin、N-cadherin の免疫組織化学的発現により評価した。多くの口腔内 SCC は皮膚 SCC より高グレードであり、E-cadherin、 β -catenin、desmoglein の発現は有意に低かった。vimentin 陽性細胞は接着分子の発現が低く、少数は N-cadherin 陽性であった。以上から、IF グレーディングと E-cadherin、 β -catenin、desmoglein の発現評価の併用は犬の SCC の生物学的挙動の評価により有用であると考えられた。

■ 最終責任者 Hirovuki Taniyama (Corresponding Author)



NOTE

Pathology

Blindness associated with nasal/paranasal lymphoma in a stallion

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ABSTRACT. A 29-year-old stallion presented with bilateral blindness following the chronic purulent nasal drainage. The mass occupied the right caudal nasal cavity and right paranasal sinuses including maxillary, palatine and sphenoidal sinuses, and the right-side turbinal and paranasal septal bones, and cribriform plate of ethmoid bone were destructively replaced by the mass growth. The right optic nerve was invaded and involved by the mass, and the left optic nerve and optic chiasm were compressed by the mass which was extended and invaded the skull base. Histologically, the optic nerves and optic chiasm were degenerated, and the mass was diagnosed as lymphoma which was morphologically and immunohistochemically classified as a diffuse large B-cell lymphoma. Based on these findings, the cause of the blindness in the stallion was concluded to be due to the degeneration of the optic nerves and chiasm associated with lymphoma occurring in the nasal and paranasal cavities. To the best of our knowledge, this is the first report of the equine blindness with optic nerve degeneration accompanied by lymphoma.

KEY WORDS: blindness, horse, lymphoma, nasal and paranasal cavities

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■ 論文題目

種牡馬における鼻腔/副鼻腔リンパ腫に伴った盲目の1例

■ 要 旨

29歳の種牡馬が慢性的な鼻排膿に引き続き盲目を呈した。剖検では、右側の鼻腔尾側と上顎洞、口蓋洞、蝶形骨洞を含む右副鼻腔の腫瘤による占拠が認められ、鼻甲介、副鼻腔中隔、篩骨篩板は腫瘤の増殖により置換され不明瞭となっていた。右視神経は腫瘤に巻き込まれ不明瞭となり、さらに左視神経と視交叉は頭蓋底に浸潤した腫瘤により圧排されていた。組織学的に、視神経ならび視交叉は変性しており、腫瘤についてはリンパ腫と診断された。リンパ腫は、形態学的ならび免疫組織化学的に、び慢性大型B細胞性リンパ腫と分類された。これらの所見より、本症例における盲目の原因は、鼻腔/副鼻腔に発生したリンパ腫の発生に伴った視神経ならび視交叉の障害によるものと結論付けられた。本症例は、馬におけるリンパ腫に伴った視神経障害による盲目の初めての報告である。

■ 最終責任者 Hiroyuki Taniyama (Corresponding Author)

Amyloid-Producing Odontogenic Tumors of the Facial Skin in Three Cats

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Abstract

Amyloid-producing odontogenic tumors (APOTs) of the facial skin were diagnosed in 3 domestic cats. The neoplasms had the histopathological characteristics of the odontogenic tumor. The neoplastic cells were present in irregular islands, strands, and sheets. The peripheral neoplastic cells of the islands and strands were arranged in a palisading fashion, while the central cells were polyhedral to stellate and randomly arranged. Multiple spherules of homogeneous eosinophilic material were closely apposed to the neoplastic epithelial cells. The spherules stained with Congo red and produced an apple green birefringence under polarization microscopy. Indicative of amyloid, immunohistochemically, amyloid materials of the neoplasms reacted with polyclonal antibodies for ameloblastin, amelogenin, and sheathlin antibodies. Neoplastic epithelial cells also reacted with anti-ameloblastin, amelogenin, and sheathlin antibodies, with varied intensity. The histopathological and immunohistochemical characteristics of dermal neoplasms of the 3 cats were analogous to those of APOTs reported in the dog and the cat.

■ 論文題目

3頭の猫における顔面皮膚におけるアミロイド産生性歯原性腫瘍

■ 要 旨

3頭の猫の顔面皮膚における腫瘍をアミロイド産生性歯原性腫瘍（APOT）と診断した。腫瘍は歯原性腫瘍の特徴をもち、腫瘍細胞は不規則島状、索状、シート状に配列し、辺縁の細胞は柵状配列、中央の細胞は多角形から星状でランダムに配列した。多数の好酸性均質物質の沈着巣が腫瘍細胞に近接して存在し、この物質はコンゴ赤陽性で偏光顕微鏡下で青緑色複屈折性を示した。物質と腫瘍細胞は免疫組織化学的に ameloblastin、amelogenin、sheathlin に陽性を示した。以上の特徴は犬猫で報告のある APOT と一致した。

■ 最終責任者 Hiroyuki Taniyama (Corresponding Author)

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

1) Blindness associated with nasal/paranasal lymphoma in a stallion.

Sano Y, Okamoto M, Ootsuka Y, Matsuda K, Yusa S, Taniyama H.

J. Vet. Med. Sci. 79: 579-583. 2017.

II. その他<Others>



NOTE

Pathology

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Blindness associated with nasal/paranasal lymphoma in a stallion

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ABSTRACT. A 29-year-old stallion presented with bilateral blindness following the chronic purulent nasal drainage. The mass occupied the right caudal nasal cavity and right paranasal sinuses including maxillary, palatine and sphenoidal sinuses, and the right-side turbinal and paranasal septal bones, and cribriform plate of ethmoid bone were destructively replaced by the mass growth. The right optic nerve was invaded and involved by the mass, and the left optic nerve and optic chiasm were compressed by the mass which was extended and invaded the skull base. Histologically, the optic nerves and optic chiasm were degenerated, and the mass was diagnosed as lymphoma which was morphologically and immunohistochemically classified as a diffuse large B-cell lymphoma. Based on these findings, the cause of the blindness in the stallion was concluded to be due to the degeneration of the optic nerves and chiasm associated with lymphoma occurring in the nasal and paranasal cavities. To the best of our knowledge, this is the first report of the equine blindness with optic nerve degeneration accompanied by lymphoma.

KEY WORDS: blindness, horse, lymphoma, nasal and paranasal cavities

■ 論文題目

種馬における鼻腔/副鼻腔リンパ腫に伴った盲目の1例

■ 要 旨

29歳の種馬が慢性的な鼻排膿に引き続き盲目を呈した。剖検では、右側の鼻腔尾側と上顎洞、口蓋洞、蝶形骨洞を含む右副鼻腔の腫瘍による占拠が認められ、鼻甲介、副鼻腔中隔、篩骨篩板は腫瘍の増殖により置換され不明瞭となっていた。右視神経は腫瘍に巻き込まれ不明瞭となり、さらに左視神経と視交叉は頭蓋底に浸潤した腫瘍により圧排されていた。組織学的に、視神経ならび視交叉は変性しており、腫瘍についてはリンパ腫と診断された。リンパ腫は、形態学的ならび免疫組織化学的に、び慢性大型B細胞性リンパ腫と分類された。これらの所見より、本症例における盲目の原因は、鼻腔/副鼻腔に発生したリンパ腫の発生に伴った視神経ならび視交叉の障害によるものと結論付けられた。本症例は、馬におけるリンパ腫に伴った視神経障害による盲目の初めての報告である。

■ 最終責任者 Hiroyuki Taniyama (Corresponding Author)

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- 1) Survival and prevalence of *Clostridium difficile* in manure compost derived from pigs.

Usui M, Kawakura M, Yoshizawa N, San LL, Nakajima C, Suzuki Y, Tamura Y.

Anaerobe 43: 15-20. 2017

- 2) Closely related methicillin-resistant *Staphylococcus aureus* isolated from retail meat, cows with mastitis, and humans in Japan.

Sato T, Usui M, Kai A, Konishi N, Hanaki H, Matsui H, Tamura Y.

PLOS ONE 12: e0187319. 2017.

II. その他<Others>

- 1) Molecular epidemiological analysis of human- and chicken-derived isolates of *Campylobacter jejuni* in Japan using next-generation sequencing.

Ohishi T, Aoki K, Ishii Y, Usui M, Tamura Y, Kawanishi M, Onishi K, Tateda K.

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Yukawa S, Tsuyuki Y, Sato T, Fukuda A, Usui M, Tamura Y.

Jpn. J. Infect. Dis. 70: 461-463. 2017.

- 3) Phenotypic and molecular characterization of antimicrobial resistance in *Enterobacter* spp. isolates from companion animals in Japan

Harada K, Shimizu T, Mukai Y, Kuwajima K, Sato T, Kajino A, Usui M, Tamura Y, Kimura Y, Miyamoto T, Tsuyuki Y, Ohki A, Kataoka Y.

PLOS ONE. 12: e0174178. 2017.



Clostridium difficile

Survival and prevalence of *Clostridium difficile* in manure compost derived from pigs

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ABSTRACT

Pigs, particularly piglets, have been identified as reservoir hosts of *Clostridium difficile*. To examine the survival ability of this pathogen in pig feces-based manure compost, *C. difficile* spores, which were prepared to contain as few vegetative cells as possible, were artificially inoculated into pig feces and incubated at different temperatures. While *C. difficile* survived in the feces incubated at temperatures below 37 °C for over 30 days, cell numbers gradually decreased at thermophilic temperatures (over 55 °C; $p < 0.05$). Next, to clarify the prevalence of *C. difficile* in field manure compost, we isolated and characterized *C. difficile* from the final products of manure compost products of 14 pig farms. A total of 11 *C. difficile* strains were isolated from 5 of 14 (36% positive rate) samples tested. Of these 11 strains, 82% were toxigenic, with ribotype 078 being the most prevalent. Thus, the application of composted manure to land therefore poses a possible risk of *C. difficile* transfer to the food chain.

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■ 論文題目

豚農場の堆肥における *Clostridium difficile* の分布と生存

■ 要 旨

通常、堆肥中の食中毒細菌は堆肥化に伴う熱により死滅すると考えられている。しかし、*C. difficile* が芽胞形成菌であり、堆肥化によっても生存する可能性がある。そこで、*in vitro* において熱による生存性を調べたところ堆肥化条件の熱による処理を行っても一部の *C. difficile* は残存することが示された。これまでの研究で、子豚から高率に *C. difficile* が分離できたことから、豚農場の堆肥から *C. difficile* を分離したところ 14 農場中 5 農場の堆肥から *C. difficile* が分離された。以上のことから、家畜からヒトへの *C. difficile* の伝播経路として堆肥を介した経路にも注意をすべきであることが示された。

■ 最終責任者 Yutaka Tamura (Corresponding Author)

RESEARCH ARTICLE

Closely related methicillin-resistant *Staphylococcus aureus* isolates from retail meat, cows with mastitis, and humans in Japan

Tomomi Sato^{1*}, Masaru Usui^{1*}, Nonko Konishi^{2‡}, Akemi Kai^{2‡}, Hidehito Matsui^{4‡}, Hideaki Hanaki^{4‡}, Yutaka Tamura^{1*}

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pervasive healthcare-acquired (HA) pathogen with recent emergence as a community-acquired (CA) pathogen. To elucidate whether meat mediates MRSA transmission between animals and humans in Japan, this study examined MRSA isolates from retail meat ($n = 8$), cows with mastitis ($n = 7$), and humans (HA-MRSA = 46 and CA-MRSA = 54) by molecular typing, virulence gene analyses, and antimicrobial susceptibility testing. MRSA isolates from retail meat were classified into sequence type (ST) 8/*spa* type t1767 ($n = 4$), ST8/t4133 ($n = 1$), ST59/t3385 ($n = 1$), ST88/t375 ($n = 1$), and ST509/t375 ($n = 1$). All seven MRSA isolates from cows with mastitis were ST8/t1767. 46 HA-MRSA were clonal complex (CC) 5, divided into t002 ($n = 30$), t045 ($n = 12$), and t7455 ($n = 4$). 54 CA-MRSA were classified into 6 different CCs: CC1 ($n = 14$), CC5 ($n = 7$), CC8 ($n = 29$), CC45 ($n = 1$), CC89 ($n = 1$), CC509 ($n = 1$), and into 16 different *spa* types including newly identified t17177, t17193, and t17194. The majority were CC8/t1767 ($n = 16$). CC of one CA-MRSA isolate (*spa* type t1767) was not classified. Among 41 CC8 MRSA (five from meat, seven from cows with mastitis, and 29 CA-MRSA), 14 ST8/SCC*mec*IV isolates (three from meat, one from a cow with mastitis, and 10 CA-MRSA) had identical pulsed-field gel electrophoresis patterns and similar *spa* type (t1767, t4133, and t17177), and were typed as CA-MRSA/J (ST8/SCC*mec*IV), positive for *sec*+ *sel*+ *tsf* but negative for Panton–Valentine leukocidin and the arginine catabolic mobile element). These results suggest that there is a transmission cycle of CA-MRSA/J among meat, cows, and humans in Japan, although it is unclear whether the origin is cow.

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論文題目

市販肉、乳房炎、ヒト由来 MRSA の関連

要 旨

市販肉、乳房炎、及びヒトの臨床検体から分離された MRSA について分子疫学解析を実施した。結果、市中感染型の MRSA が共通して認められた。このことから、特定の MRSA がそれぞれの由来で分布・拡散していることが示唆された。

最終責任者 Yutaka Tamura (Corresponding Author)



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Original Article

Molecular epidemiological analysis of human- and chicken-derived isolates of *Campylobacter jejuni* in Japan using next-generation sequencing



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ABSTRACT

In this research, we analyzed the main sequence types (ST) and ST complexes of human- and chicken-derived isolates of *Campylobacter jejuni* in Japan by using multilocus sequence typing (MLST). We also analyzed lipooligosaccharide biosynthesis locus classes (LOS locus classes) and the numbers of isolates carrying genes coding resistance factors against various antibiotics, and observed their relationships. ST-21 complex was the main ST complex in isolates from humans ($n = 38$) and chickens ($n = 25$). None of the isolates showed resistance to imipenem, chloramphenicol, or erythromycin. Few isolates were resistant to ampicillin and streptomycin (1.3%–15%), whereas many showed resistance to tetracycline, ciprofloxacin, and nalidixic acid (38%–48%). Among the ST-21 complex isolates, ST4526 was detected at a very high rate. Those isolates showed resistance to tetracycline and ciprofloxacin, and were susceptible to ampicillin. Among the chicken-derived isolates, 37 of the 38 isolates that showed resistance to ciprofloxacin and nalidixic acid had threonine to isoleucine amino acid substitution in GyrA at codon 85 (T86I). Among the human-derived isolates, 17 of the 47 isolates that showed resistance to ciprofloxacin and 16 of the 48 isolates that showed resistance to nalidixic acid did not have T86I amino acid mutation in GyrA. The human-derived ST-21 complex isolates were classified into LOS locus classes A, B, C, D, and E. The chicken-derived ST-21 complex isolates, with the exception of one isolate, were all classified into LOS locus classes C and D. Among chicken-derived isolates, the most prevalent was ST51 (ST-443 complex) (10 isolates) and all of those were LOS locus class E.

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■ 論文題目

日本のヒト及び鶏から分離された *Campylobacter* の次世代シーケンサーによる解析

■ 要 旨

ヒト臨床由来及び鶏由来カンピロバクターについて次世代シーケンサーによる解析を行った。結果、ST-21 がヒト由来で ST-28 が鶏由来で主要な MLST 型であった。フルオロキノロン耐性鶏由来株の多くが、DNA gyrase A に T86I の変異があったが、フルオロキノロン耐性ヒト由来株においては、T86I に変異を持っていなかった。

■ 最終責任者 Yoshikazu Ishii (Corresponding Author)

Short Communication

Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolated from Dogs and Cats in Primary Veterinary Hospitals in Japan

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SUMMARY: We collected 200 *Pseudomonas aeruginosa* isolates from dogs and cats in primary veterinary hospitals in Japan to investigate their antimicrobial resistance. Resistance rates against ciprofloxacin, cefotaxime, gentamicin, amikacin, and fosfomycin were 9%, 12.5%, 4.5%, 2.5%, and 35.5%, respectively. One strain displayed resistance (0.5%) to ceftazidime. We did not detect any imipenem-resistant or multidrug-resistant *P. aeruginosa* strains as defined by the Japanese Ministry of Health, Labour, and Welfare Law Concerning the Prevention of Infections and Medical Care for Patients with Infections. In addition, we did not find any *P. aeruginosa* isolates that produced metallo- β -lactamase, the aminoglycoside 6'-N-acetyltransferase AAC(6')-Iae, or the aminoglycoside acetyltransferase AAC(6')-Ib.

■ 論文題目 動物病院で分離された緑膿菌の薬剤感受性

■ 要 旨

動物病院で分離された緑膿菌について薬剤感受性試験を実施した。結果、シプロフロキサシン、セフトアキシム、ゲンタマイシン、アミカシン、フォスフォマイシンに対してそれぞれ 9, 12.5, 4.5, 2.5, 35.5% 耐性を示した。しかし、メタロ β ラクタマーゼ、アミノグリコシド耐性遺伝子である *aac*(6')-Iae, *aac*(6')-Ib は認められなかった。

■ 最終責任者 Shoichiro Yukawa (Corresponding Author)

RESEARCH ARTICLE

Phenotypic and molecular characterization of antimicrobial resistance in *Enterobacter* spp. isolates from companion animals in JapanKazuki Harada^{1*}, Takae Shimizu¹, Yujiro Mukai², Ken Kuwajima², Tomomi Sato², Akari Kajino³, Masaru Usui³, Yutaka Tamura³, Yui Kimura⁴, Tadashi Miyamoto⁴, Yuzo Tsuyuki⁵, Asami Ohki⁶, Yasushi Kataoka²¹ Department of Veterinary Internal Medicine, Tottori University, Tottori, Japan, ² Laboratory of Veterinary Microbiology, Nippon Veterinary and Life Science University, Tokyo, Japan, ³ Laboratory of Food Microbiology and Food Safety, Rakuno Gakuen University, Hokkaido, Japan, ⁴ Miyamoto Animal Hospital, Yamaguchi, Japan, ⁵ Sanritsu Zeikova Veterinary Laboratory, Kanagawa, Japan, ⁶ Fujifilm Monoris Co., Ltd., Tokyo, Japan* k.harada@muses.tottori-u.ac.jp

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

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Abstract

The emergence of antimicrobial resistance among *Enterobacter* spp., including resistance to extended-spectrum cephalosporins (ESC), is of great concern in both human and veterinary medicine. In this study, we investigated the prevalence of antimicrobial resistance among 80 isolates of *Enterobacter* spp., including *E. cloacae* (n = 44), *E. aerogenes* (n = 10), and *E. asburiae* (n = 6), from clinical specimens of dogs and cats from 15 prefectures in Japan. Furthermore, we characterized the resistance mechanisms harbored by these isolates, including extended-spectrum β -lactamases (ESBLs) and plasmid-mediated quinolone resistance (PMQR); and assessed the genetic relatedness of ESC-resistant *Enterobacter* spp. strains by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). Antimicrobial susceptibility testing demonstrated the resistance rates to ampicillin (93.3%), amoxicillin-clavulanic acid (93.3%), ceftazidime (93.3%), chloramphenicol (46.7%), ciprofloxacin (43.3%), tetracycline (40.0%), ceftazidime (33.3%), cefotaxime (33.3%), trimethoprim-sulfamethoxazole (28.3%), gentamicin (23.3%), and meropenem (0%). Phenotypic testing detected ESBLs in 16 of 18 ESC-resistant *E. cloacae* isolates but not in the other species. The most frequent ESBL was CTX-M-15 (n = 8), followed by SHV-12 (n = 7), and CTX-M-3 (n = 1). As for AmpC β -lactamases, CMY-2 (n = 2) and DHA-1 (n = 2) were identified in ESC-resistant *E. cloacae* strains with or without ESBLs. All of the ESC-resistant *E. cloacae* strains also harbored one or two PMQRs, including *qnrB* (n = 15), *aac* (6')-Ib-cr (n = 8), and *qnrS* (n = 2). Based on MLST and PFGE analysis, *E. cloacae* clones of ST591-SHV-12, ST171-CTX-M-15, and ST121-CTX-M-15 were detected in one or several hospitals. These results suggested intra- and inter-hospital dissemination of *E. cloacae* clones co-harboring ESBLs and PMQRs among companion animals. This is the first report on the large-scale monitoring of antimicrobial-resistant isolates of *Enterobacter* spp. from companion animals in Japan.

■ 論文題目 伴侶動物由来 *Enterobacter* 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 *Enterobacter* について薬剤感受性を調べ、分離株の分子疫学解析を実施した。結果、医療上、問題となる ESBL が多数の株で認められ、分子疫学的な関連が認められた。

■ 最終責任者 Kazuki Harada (Corresponding Author)

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- 1) Particle-Induced X-ray Emission Analysis of Serum Trace and Major Elements in Cattle with Acute Coliform Mastitis.
Shimamori T, Noda J. Tsukano K, Sera K, Yokota, H., Koiwa M, Suzuki T, Suzuki, K.
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- 1) Determining the Frequency of Dry Lake Bed Formation in Semi-Arid Mongolia from Satellite Data
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Land 6: 1-9. 2017.

Particle-Induced X-ray Emission Analysis of Serum Trace and Major Elements in Cattle with Acute Coliform Mastitis

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Abstract

The aim of the present study was to examine the applicability of the direct determination of trace and major element concentrations in serum samples collected from Holstein dairy cattle with acute coliform mastitis ($n = 53$) compared with a healthy control group ($n = 39$). Twenty-eight elements (Na, Mg, Al, Si, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ce, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, and Pb) were detected by particle-induced X-ray emission (PIXE). Significant differences were observed in serum K, Fe, Zn, and Br concentrations, but not in those of the remaining twenty-four elements. Furthermore, serum Fe concentrations ($0.751 \pm 0.583 \mu\text{g/ml}$, $n = 18$) were significantly lower in dairy cattle with a poor prognosis than in those with a good prognosis ($0.945 \pm 0.393 \mu\text{g/ml}$, $n = 35$, $P < 0.05$) and healthy controls ($1.458 \pm 0.391 \mu\text{g/ml}$, $n = 39$, $P < 0.01$). We proposed a diagnostic cut-off point for serum Fe concentrations of $< 0.82 \mu\text{g/ml}$ based on receiver operating characteristic (ROC) curves in order to identify cattle with a poor prognosis. The results of the present study indicated that assessing the elemental composition of serum, particularly iron, is a promising prognostic tool for determining the outcomes of cattle with severe acute coliform mastitis.

Key Words: Acute coliform mastitis, Dairy cattle, Iron, Particle-induced X-ray emission, Prognosis

■ 論文題目

P I X Eによる血清中微量元素濃度と牛の大腸菌性乳房炎の関連性と応用

■ 要 旨

大腸菌性乳房炎はエンドトキシン由来の全身性ショックにより致死かつ経済損失が高い疾患である。そこで、乳房炎に罹患している牛個体 ($n=53$) と、罹患していない牛個体 ($n=39$) の血清中における微量元素濃度を PIXE 法にて網羅的に測定した。その結果、血清中 K、Fe、Zn、Br において著しい違いが認められた。特に Fe においては顕著な有意差が認められたことから、Fe を活用した乳房炎治療の了後状況の判定に向けた応用ができると考えた。そこで、ROC 曲線による解析を行い、血清中 Fe 濃度の診断カットオフ値を提案した。血清中の元素、特に鉄の濃度を活用することで、大腸菌性乳房炎に罹患した乳牛の了後状況の判定ができることが示唆された。

■ 最終責任者 Jun Noda (Corresponding Author)

Determining the Frequency of Dry Lake Bed Formation in Semi-Arid Mongolia From Satellite Data

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Abstract: In the Mongolian Plateau, the desert steppe, mountains, and dry lake bed surfaces may affect the process of dust storm emissions. Among these three surface types, dry lake beds are considered to contribute a substantial amount of global dust emissions and to be responsible for “hot spots” of dust outbreaks. The land cover types in the study area were broadly divided into three types, namely desert steppe, mountains, and dry lake beds, by a classification based on Normalized Difference Water Index (NDWI) calculated from MODIS Terra satellite images, and Digital Elevation Model (DEM). This dry lake beds extracting method using remote sensing offers a new technique for identifying dust hot spots and potential untapped groundwater in the dry lands of the Gobi region. In the study area, frequencies of dry lake bed formation were calculated during the period of 2001 to 2014. The potential dry lake area corresponded well with the length of the river network based on hydrogeological characterization ($R^2 = 0.59$, $p < 0.001$). We suggest that the threshold between dry lake bed areas and the formation of ephemeral lakes in semi-arid regions is eight days of total precipitation.

■ 論文題目

人工衛星データによるモンゴル国半乾燥地帯における乾燥湖底形成頻度の判定

■ 要 旨

モンゴル高原に存在する、砂漠ステップ、山岳、乾燥湖底 - 表面の形態はそれぞれが砂嵐の発生に寄与している。これら三つの地表形のうち、乾燥湖底は砂嵐によるダストの大きな発生源として全球レベルでのダスト発生のホットスポット的な存在であると考えられている。地表面タイプは正規化水指数(NDWI)、MODIS Terra 衛星画像、デジタル標高モデル(DEM)にて砂漠ステップ、山岳、乾燥湖底に分けられている。リモートセンシングによる本乾燥湖底抽出方法は新たな手法にてダスト発生のホットスポットや、未利用の地下水源をゴビ地域の乾燥地帯の特定に寄与した。本研究対象地域で、2001–2014年の乾燥湖底の発生地モデルを計算により求めた。このモデル推測乾燥湖底地は、水理学的特性と合致した ($R^2=0.59$, $p<0.001$)。これらから、乾燥湖底と半乾燥地帯における一時的な湖の形成に必要な数日降水日数は8日間であると推測した。

■ 最終責任者 Buho Hoshino (Corresponding Author)

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Risk Assessment of Campylobacteriosis Due to Consumption of Roast Beef Served in Beer Bars in Arusha, Tanzania

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Summary

The objective of the study was to assess the risk of campylobacteriosis due to consumption of cross-contaminated nyama-choma (roast beef) sold in beer bars in Arusha Municipality, Tanzania.

In 2010, thirty butchers and thirty beer bars serving roast beef, selected using stratified random sampling, were visited to estimate the prevalence and the most probable number (MPN) of thermophilic *Campylobacter* in raw and roast beef. Ten purposively selected bars serving roast chicken were also surveyed, to assess cross-contamination after cooking, as *Campylobacter* spp. are generally more prevalent in poultry. One meat sample was collected in each butcher and each bar, and sales and hygiene were assessed through questionnaires and direct observation. A risk model was developed in statistical software R, and Monte Carlo simulation was performed to estimate disease incidence among customers and the adult male population in Arusha.

In the field survey, *Campylobacter coli* was recovered only from one chicken sample, of which bacterial concentration was 0.37 MPN/g (95% CI : 0.12–1.08). The daily incidence of campylobacteriosis among customers in Arusha was estimated to be 0.15 (95% CI : 0.02–0.95). Annual incidence rates among customers and in the adult male population in Arusha were 12.4 (95% CI : 1.2–83.6) per 1,000 and 0.6 (95% CI : 0.06–4.0) per 1,000, respectively. The most influential factor was the prevalence of thermophilic *Campylobacter* in roast beef, followed by MPN. Most (26/40, 65%) bar owners sold meat from different species of animals, and cross-contamination could easily occur between poultry and beef. Nearly half (18/39, 46%) the bar owners used the same knife for raw and roast meat. Although half (20/40, 50%) had received hygiene training there was no statistical association between training and separate use of knives for raw and roast meats, considered to be the major source of contamination ($\chi^2=0.22$, $df=1$, $p=0.64$).

The study concluded campylobacteriosis from roast beef was a low public health issue in Arusha. However, considering the risks from other types of bacteria, improvement of cooking hygiene training was recommended to further reduce the risk of food-borne diseases from food consumed in beer bars.

Keywords : *Campylobacter*, Cross contamination, Risk assessment, Roast beef, Tanzania

■ 論文題目

タンザニア国アルーシャのバーで提供される牛焼肉由来カンピロバクター症のリスク評価

■ 要 旨

題目について推計学的リスク評価を実施した。成人男性における年間発生率は1000人当たり12.4人 (95%CI: 1.2–83.6人) と低かった。一方半数の店で生肉と焼肉で包丁の使い分けがない等、衛生改善の必要性も見られた。

■ 最終責任者 Kohei Makita (Corresponding Author)



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Time series analysis based on two-part models for excessive zero count data to detect farm-level outbreaks of swine echinococcosis during meat inspections

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Swine
Echinococcosis
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Two-part model
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ABSTRACT

Echinococcus multilocularis is a parasite that causes highly pathogenic zoonoses and is maintained in foxes and rodents on Hokkaido Island, Japan. Detection of *E. multilocularis* infections in swine is epidemiologically important. In Hokkaido, administrative information is provided to swine producers based on the results of meat inspections. However, as the current criteria for providing administrative information often results in delays in providing information to producers, novel criteria are needed. Time series models were developed to monitor autocorrelations between data and lags using data collected from 84 producers at the Higashi-Mokoto Meat Inspection Center between April 2003 and November 2015. The two criteria were quantitatively compared using the sign test for the ability to rapidly detect farm-level outbreaks. Overall, the time series models based on an autoexponentially regressed zero-inflated negative binomial distribution with 60th percentile cumulative distribution function of the model detected outbreaks earlier more frequently than the current criteria (90.5%, 276/305, $p < 0.001$). Our results show that a two-part model with autoexponential regression can adequately deal with data involving an excessive number of zeros and that the novel criteria overcome disadvantages of the current criteria to provide an earlier indication of increases in the rate of echinococcosis.

■ 論文題目

食肉検査時に農場レベルの豚エキノコックス症を検出するためのゼロ過剰カウントデータ二分割モデルに基づく時系列分析

■ 要 旨

東藻琴食肉検査所では、エキノコックス感染による肝臓廃棄が多発する養豚生産者に衛生指導文書を送っているが、その判断には数学的根拠が確立されていない。本研究では概して発生が少ない本病に適した時系列モデルを確立することにより、行政上の欠点を克服した。

■ 最終責任者 Kohei Makita (Corresponding Author)

RESEARCH ARTICLE

The rise and fall of rabies in Japan: A quantitative history of rabies epidemics in Osaka Prefecture, 1914–1933

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Data Availability Statement: All relevant data are within the paper.

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Abstract

Japan has been free from rabies since the 1950s. However, during the early 1900s several large-scale epidemics spread throughout the country. Here we investigate the dynamics of these epidemics between 1914 and 1933 in Osaka Prefecture, using archival data including newspapers. The association between dog rabies cases and human population density was investigated using Mixed-effects models and epidemiological parameters such as the basic reproduction number (R_0), the incubation and infectious period and the serial interval were estimated. A total of 4,632 animal rabies cases were reported, mainly in dogs (99.0%, 4,584 cases) during two epidemics from 1914 to 1921, and 1922 to 1933 respectively. The second epidemic was larger (3,705 cases) than the first (879 cases), but had a lower R_0 (1.50 versus 2.42). The first epidemic was controlled through capture of stray dogs and tethering of pet dogs. Dog mass vaccination began in 1923, with campaigns to capture stray dogs. Rabies in Osaka Prefecture was finally eliminated in 1933. A total of 3,805 rabid dog-bite injuries, and 75 human deaths were reported. The relatively low incidence of human rabies, high ratio of post-exposure vaccines (PEP) and bite injuries by rabid dogs (minimum 6.2 to maximum 73.6, between 1924 and 1928), and a decline in the proportion of bite victims that developed hydrophobia over time (slope = -0.29, se = 3, $p < 0.001$), indicated that increased awareness and use of PEP might have prevented disease. Although significantly more dog rabies cases were detected at higher human population densities (slope = 0.66, se = 0.03, $p < 0.01$), there were fewer dog rabies cases detected per capita (slope = -0.34, se = 0.03, $p < 0.01$). We suggest that the combination of mass vaccination and restriction of dog movement enabled by strong legislation was key to eliminate rabies. Moreover, the prominent role of the media in both reporting rabies cases and efforts to control the disease likely contributed to promoting the successful participation required to achieve rabies elimination.

■ 論文題目

日本の狂犬病の衰勢：1914–1933年大阪における狂犬病流行の定量的歴史解析

■ 要 旨

大阪における犬・人狂犬病の解析により、感染力の強さを解析するとともに、変遷した狂犬病対策の効果を検証した。

■ 最終責任者 Kohei Makita (Corresponding Author)



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Association of farmers' socio-economics with bovine brucellosis epidemiology in the dry zone of Sri Lanka



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Risk factors
Farmers' ethnicity
Poverty
Free grazing

ABSTRACT

The aim of the study was to investigate the farmers' socio-economic factors and their association with *Brucella* prevalence in the dry zone of Sri Lanka. A cross-sectional survey was planned and a total of 1,153 blood samples were collected from milking and dry animals of 155 farms from three selected veterinary ranges of Kalmunai, Navithanveli, and Mahaoya in the Ampara district, which is a multi-ethnic area. The Rose Bengal Test (RBT) and competitive enzyme-linked immunosorbent assay (c-ELISA) were used for the *Brucella* screening and confirmation, respectively. Socio-economic attributes such as family income, poverty, education, main job, ethnicity, parent farmer, farming experience, and training in animal husbandry were determined as potential farmer-level risk factors. Meanwhile, herd size, grazing practice, breeding method, animal brought-in to the farm, and abortions were considered as herd factors. The results revealed that the overall animal level sero-prevalence of brucellosis was 2.7% (35/1153; 95% confidence interval [CI]: 1.7, 3.7%) and the herd prevalence was 9.6% (15/155; 95% CI: 5.7, 15.7%) in the area of study. Brucellosis prevalence varies significantly ($p < 0.001$) among the selected veterinary ranges with the highest herd prevalence in Kalmunai (20.0%) followed by Navithanveli (11.9%) and Mahaoya (2.7%). Disease prevalence showed variability ($p < 0.001$) among ethnicities with the highest in Muslims (27.3%) followed by Tamils (8.1%) and Sinhalese (2.7%). Poverty was highly associated (OR = 3.75; 95% CI: 1.43–10.00) with the disease. Free movement grazing practices ($p < 0.01$) with OR = 7.2 and animal brought-in from outside ($p < 0.06$) with OR = 3.06 were positively related to brucellosis. It was revealed that farmers' socio-economics, such as ethnicity and poverty, and animal movement patterns, such as grazing practices are significantly associated with epidemiology of brucellosis in the dry zone of Sri Lanka. Therefore, the "farmer factor" should be carefully considered in veterinary epidemiological studies and animal disease control plans in the future.

■ 論文題目

スリランカの乾燥地帯における牛ブルセラ病の疫学と関連した生産者の社会経済学

■ 要 旨

調査地域において、牛ブルセラ病が、民族ではイスラムグループ、貧困、自由放牧、外部からの導入と関連していた。

■ 最終責任者 Hiroichi Kono (Corresponding Author)



Spatial dynamics of porcine epidemic diarrhea (PED) spread in the southern Kyushu, Japan



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ABSTRACT

Porcine epidemic diarrhea (PED) was detected for the first time in seven years in Japan in October 2013 in Okinawa Prefecture. By December 2013, PED had spread into Miyazaki and Kagoshima Prefectures in the southern part of Kyushu, one of the regions with the highest farm density in the country. The objective of the study here was to assess the spatial dynamics of PED spread during the 8 months of the epidemic in the southern part of Kyushu between December 2013, the month observed first case in the studied region, and July 2014. Information on location and capacity of all farms in the prefectures ($n = 1269$) was obtained from a government database containing demographic information for livestock producers. Additionally, data on PED detection (positive or negative) was obtained from the regional Livestock Hygiene Service Center. The Cuzick-Edwards (CE) test, the Knox test, the directional test, and the permutation model of the scan statistic were used to assess the spatio-temporal distribution of the epidemic. PED cumulative farm level incidence was 19.5% (248/1269) through the study period. The highest density of positive farms was observed in the most farm-populated areas of the prefecture. The CE test revealed an extensive degree of spatial clustering, with clustering of positive sites being significant ($P < 0.01$) up to the 35th level of neighborhood (approximately 5 km in the studied data). The observed-to-expected ratio of cases was maximized at short spatio-temporal distances, with values of the observed-to-expected ratio of cases maximized when the thresholds were set at 2 km and 10 days, respectively. A significant ($P < 0.01$) direction of spread was detected towards the northeastern direction. The permutation model detected five significant ($P < 0.01$) clusters occurring at different stages of the epidemic wave. The strong spatio-temporal clustering of PED-infected farms during the first 6 months of the epidemic in the southern part of Kyushu is consistent with results obtained elsewhere and demonstrates the rapid spread of the virus in naïve populations.

■ 論文題目

南九州における豚流行性下痢症拡散の地理的ダイナミクス

■ 要 旨

鹿児島県、宮崎県での PED 流行では、養豚場密集地域で拡大が速く、顕著な時空間クラスターが確認された。感染は北東に向かって移動した。

■ 最終責任者 Yosuke Sasaki (Corresponding Author)



Animal Insurance and Farmer's Behavior in Vietnam

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ABSTRACT

Most poor people in developing countries make their living from agriculture; hence, their livelihood is subject to various risks such as livestock disease, flooding, drought, and fluctuations in the price of agricultural products. One method of dealing with risk is insurance. However insurance markets in developing countries are seriously impacted by adverse selection and moral hazard, derived from information asymmetry. Livestock insurance was introduced in Vietnam as a pilot project in 2011–2013. We examine the factors behind the decision to take out insurance, from the viewpoint of moral

hazard or adverse selection in Vietnam. The results suggest that, if there are few trustworthy people locally, the rate of time discounting is lower and farmers will purchase insurance coverage. Further, the higher the number of calvings, and therefore the older the cows owned by a farmer, the more likely he/she is to take out livestock insurance. The analysis of results also reveals a low level of existence of adverse selection and moral hazard with respect to livestock insurance in the surveyed areas. The incidence of livestock diseases covered by insurance is currently low. Many dairy farmers expressed their wish for insurance coverage to be expanded to include other diseases such as mastitis and hoof disease. Expanding the range of diseases covered by insurance would introduce an additional financial burden. As a consequence, the system of surveillance and penalties would need to be strengthened.

■ 論文題目 ベトナムにおける家畜保険と農家の行動

■ 要 旨

発展途上国での農業は災害や病気等のリスクに脆弱である。保険制度はこのようなリスクに対して有効であるがモラルハザード等が問題である。本研究では家畜保険に関連して対象疾病とその発生頻度、ベトナムの酪農家のディスカウントレート、モラルハザード等について調査した。

■ 最終責任者 Hiroichi Kono (Corresponding Author)

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- 1) Bacteriological evaluation of composted manure solids prepared from anaerobic digested slurry for hygienic recycled bedding materials for dairy cows.

Okamoto E, Miyanishi H, Nakamura A, Kobayashi T, Kobayashi N, Terawaki Y, Nagahata H.

Anim. Sci. J. doi:10.1111/asj.12962. 2017.

II. その他<Others>

- 1) Control of *Pseudomonas mastitis* on a large dairy farm by using slightly acidic electrolyzed water.

Kawai K, Shinozuka Y, Uchida I, Hirose K, Mitamura T, Watanabe A, Kuruhara K, Yuasa R, Sato R, Onda K, Nagahata, H.

Anim. Sci. J. 88: 1601-1605. 2017.

- 2) *Mycoplasma bovis* escapes bovine neutrophil extracellular traps.

Gondaira S, Higuchi H, Nagahata H. *Vet. Microbiol.* 199: 68-73. 2017.

ORIGINAL ARTICLE

Bacteriological evaluation of composted manure solids prepared from anaerobic digested slurry for hygienic recycled bedding materials for dairy cows

Eiryu OKAMOTO,¹ Hiroki MIYANISHI,^{2,*} Akinobu NAKAMURA,² Tomoyuki KOBAYASHI,³ Norihiko KOBAYASHI,³ Yoshinori TERAOKA⁴ and Hajime NAGAHATA⁵ 

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ABSTRACT

Changes in mastitis-causing pathogens, pH and water content in composted manure solids (CMS) prepared from digested slurry were evaluated during turning at 2-day intervals for 8 days (C1–C4). The numbers of streptococci, coagulase-negative staphylococci and coliforms were 2.6×10^1 , 1.7×10^2 and 1.0×10^1 colony-forming units (cfu)/g in CMS (C4) (summer), and these counts were markedly lower ($P < 0.05$) than those in CMS (C0 and C1). The bacterial counts ranged from 10^1 to 1.7×10^2 cfu/g in CMS (C4) (summer) and were within approved levels, $< 1 \times 10^6$ cfu/g, indicating a minimal mastitis risk. The temperatures in CMS (C1–C4) increased to 63°C–74°C in summer and 67°C–70°C in winter. The mean pH values in CMS (C0–C4) were 9.2 in summer and 8.7 in winter, and water contents ranged from 61.7% to 69.6% in summer and 73.2% to 66.2% in winter. The significant decrease of pathogenic bacteria in CMS appears to be closely related to temperature $> 63^\circ\text{C}$ for 8 days, pH 8.7–9.2, and water content 62% to 73%. This study demonstrates that prepared CMS has value as a recycled material with the potential to alleviate udder health issues in dairy cows.

Key words: composted manure solid, digested slurry, mastitis-causing pathogen, recycled bedding material, udder health.

■ 論文題目

乳牛糞尿スラリーの嫌気分解および好氣的分解を経た固形物の微生物学的評価

■ 要 旨

乳牛糞尿スラリーの嫌気分解固形物の好氣的処理後の乳房炎原因菌、pH および水分含量を好氣的分解処理前及び処理後 8 日間 (2 日間隔) での切り換えしを行い評価した。Streptococci, CNS および Coliform は 2.6×10^1 , 1.7×10^2 , 1×10^1 cfu/g であり、分解前に比較して有意に低値であった。微生物数は 4 日間で $10^1 \sim 1.7 \times 10^2$ cfu/g であり許容範囲とされる 1×10^6 cfu/g 未満であり乳房炎感染に対するリスクは軽微なものとみられた。分解過程での温度は夏季で 63–74 度、pH 9.2、水分 62–73% であった。コンポスト化再生敷料の衛生学的性状が示された。

■ 最終責任者 Hajime Nagahata (Corresponding Author)



ORIGINAL ARTICLE

Control of *Pseudomonas* mastitis on a large dairy farm by using slightly acidic electrolyzed water

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ABSTRACT

The disinfection effect of slightly acidic electrolyzed water (SAEW) use in a farm where *Pseudomonas* mastitis has spread was evaluated. Despite the application of antibiotic therapy and complete cessation of milking infected quarters, numerous new and recurrent *Pseudomonas aeruginosa* clinical mastitis infections (5.8–7.1% of clinical mastitis cases) occurred on the farm from 2003 to 2005. Procedural changes and equipment modifications did not improve environmental contamination or the incidence of *Pseudomonas* mastitis. To more thoroughly decontaminate the milking parlor, an SAEW system was installed in 2006. All milking equipment and the parlor environment were sterilized with SAEW (pH 5–6.5, available chlorine 12 parts per million) before and during milking time. After adopting the SAEW system, the incidence of clinical and subclinical *Pseudomonas* mastitis cases decreased significantly ($P < 0.0001$) and disappeared. These findings suggest that SAEW effectively reduced the incidence of mastitis in a herd contaminated by *Pseudomonas* species. This is the first report to demonstrate the effectiveness of disinfection by SAEW against mastitis pathogens in the environment.

Key words: Disinfectant, economic losses, *Pseudomonas* mastitis, slightly acidic electrolyzed water.

■ 論文題目

微酸性電解水をもちいた大規模酪農場で発生していた緑膿菌性乳房炎の制御

■ 要 旨

臨床型乳房炎の5.8～7.1%の割合で緑膿菌性乳房炎が長期にわたり発生していた農場において、搾乳システム、搾乳パーラ環境に微酸性電解水を使用してその制御効果を検討した。微酸性電解水の供給により緑膿菌性乳房炎は有意に減少しその発生が終息した。微酸性電解水による乳房炎原因菌に対する消毒作用の有効性が示された。

■ 最終責任者 Kazuhiro Kawai (First Author)



Mycoplasma bovis escapes bovine neutrophil extracellular traps



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ABSTRACT

Mycoplasma bovis is a significant pathogen in bovine infections including mastitis, pneumonia, arthritis and otitis media, and is the cause of large economic losses in beef and dairy farms. During infection with *M. bovis*, recruited neutrophils are not sufficient to eradicate *M. bovis* from the infection site. The release of neutrophil extracellular traps (NETs) is one of the innate immune responses of neutrophils but the effect of *M. bovis* on NET formation by bovine neutrophils has not yet been clarified. The objective of our research was to examine the effect of *M. bovis* on NET formation and the killing activity of bovine neutrophils. We showed that NETs were not detected following stimulation of neutrophils by *M. bovis* alone or with Phorbol 12-myristate 13-acetate (PMA). Reactive oxygen species production is essential for NET formation but the levels in neutrophils stimulated with *M. bovis* at multiplicity of infections of 10, 100, and 1000 were similar to those of unstimulated cells. NET formation induced by PMA stimulated neutrophils disappeared following the addition of *M. bovis* but this phenomenon was not observed when ethylenediaminetetraacetic acid (EDTA) was added. *M. bovis* colony forming units were significantly decreased by the addition of EDTA in the presence of NETs. Our results suggested that *M. bovis* infection alone did not induce NETs and that *M. bovis* nucleases, as hypothesis-based, contributed to resistance against the killing activity of NETs.

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■ 論文題目

M. bovis はウシ好中球の extracellular traps を逃れる

■ 要 旨

M. bovis はウシの乳房炎、関節炎および中耳炎の原因微生物であり、農場に大きな経済損失をもたらす。好中球は *M. bovis* を十分に排除することができないが、その詳細なメカニズムについては十分に解明されていない。本研究において、好中球の主要な殺菌系である Neutrophil extracellular traps (NETs) は *M. bovis* 感染時に誘導されないことが示された。また、PMA 刺激で形成された NETs も *M. bovis* によって消去されることも明らかになった。このことから、*M. bovis* はウシ好中球の殺菌系に強く抵抗する性質を有することが明らかになった。

■ 最終責任者 Hidetoshi Higuchi (Corresponding Author)

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1) *Mycoplasma bovis* escapes bovine neutrophil extracellular traps.

Gondaira S, Higuchi H, Nishi K, Iwano H, Nagahata H.

Vet. Microbiol. 199: 68-73. 2017.

II. その他<Others>

1) Natural larval *Echinococcus multilocularis* infection in a Norway rat, *Rattus norvegicus*, captured indoors in Hokkaido, Japan.

Fukumoto S, Yamada S, Fushikida M, Toyada S, Nishikawa T, Higuchi H, Ueno H, Ueda H, Sugiyama H, Morishima Y.

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Mycoplasma bovis escapes bovine neutrophil extracellular traps



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■ 論文題目

*M. bovis*はウシ好中球の extracellular traps を逃れる

■ 要 旨

*M. bovis*はウシの乳房炎、関節炎および中耳炎の原因微生物であり、農場に大きな経済損失をもたらす。好中球は *M. bovis* を十分に排除することができないが、その詳細なメカニズムについては十分に解明されていない。本研究において、好中球の主要な殺菌系である Neutrophil extracellular traps (NETs)は *M. bovis* 感染時に誘導されないことが示された。また、PMA 刺激で形成された NETs も *M. bovis* によって消去されることも明らかになった。このことから、*M. bovis*はウシ好中球の殺菌系に強く抵抗する性質を有することが明らかになった。

■ 最終責任者 Hidetoshi Higuchi (Corresponding Author)



NOTE

Parasitology

Natural larval *Echinococcus multilocularis* infection in a Norway rat, *Rattus norvegicus*, captured indoors in Hokkaido, Japan

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ABSTRACT: Natural infection with larval *Echinococcus multilocularis* was recognized in one of eight Norway rats, *Rattus norvegicus*, caught indoors in 2009 in Ebetsu, Hokkaido, northern Japan. Cystic lesions were found in the right median and lateral lobes of the liver, with numerous alveolar cysts in the periphery of the lesions. Protoscolices were formed within large cysts. The laminated layers of the cysts were positive for PAS staining. Nested PCR using the primers specific for *Taenia* mitochondrial 12S rDNA yielded a 250-bp product, and the sequence of the PCR product matched that of *E. multilocularis* isolates from Hokkaido and Germany. This is the third natural alveolar hydatidosis in *R. norvegicus* in Japan.

KEY WORDS: alveolar hydatidosis, *Echinococcus multilocularis*, indoor infection, Norway rat, protoscolex

■ 論文題目

北海道内で検出された建造物内で捕獲された多包条虫幼虫(多包虫)ドブネズミ *Rattus norvegicus* 自然感染例

■ 要 旨

2009年に北海道江別市の建造物内で捕獲された8頭のドブネズミ、*Rattus norvegicus*の1頭に *Echinococcus multilocularis* の幼虫による自然感染が認められた。嚢胞性病変は肝臓の右中央および側葉に見られ、病変の周辺には多数の繁殖形成が見られた。原頭節は大きな嚢胞中で形成された。嚢胞のクチクラ層はPAS染色陽性であった。*Taenia*科条虫 mitochondrial 12S rDNAに特異的なプライマーを用いたPCR反応により、250bpの産物を得、PCR産物の配列は、北海道およびドイツの *E. multilocularis* 分離株の配列と一致した。これは、日本の *R. norvegicus* における3番目にあたり、さらに世界で初めての室内感染が示唆された自然感染多包虫症例であった。

■ 最終責任者 Shin-ichiro Fukumoto (First Author)

ORIGINAL RESEARCH

Increase of cells expressing PD-1 and PD-L1 and enhancement of IFN- γ production via PD-1/PD-L1 blockade in bovine mycoplasmosisShinya Goto¹, Satoru Konnai¹, Tomohiro Okagawa¹, Asami Nishimori¹, Naoya Maekawa¹, Satoshi Gondaira², Hidetoshi Higuchi², Masateru Koiwa², Motoshi Tajima², Junko Kohara³, Satoshi Ogasawara⁴, Yukinari Kato⁵, Yasuhiko Suzuki^{6,7}, Shiro Murata¹, & Kazuhiko Ohashi¹¹Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan²School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan³Hokkaido Research Organization, Agriculture Research Department, Animal Research Center, Shintoku, Japan⁴Department of Regional Innovation, Tohoku University Graduate School of Medicine, Sendai, Japan⁵Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, Sendai, Japan⁶Division of Bioresources, Research Center for Zoonosis, Hokkaido University, Sapporo, Japan⁷Global Station for Zoonosis Control, Global Institution for Collaborative Research and Education (GI-CORE), Hokkaido University, Sapporo, Japan**Keywords**Bovine mycoplasmosis, IFN- γ , immunosuppression, PD-1, PD-L1**Correspondence**Dr. Satoru Konnai, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.
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This research was supported by JSPS KAKENHI, grants from the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries, and Food Industry, Japan (grant 260588 to S.K.) and the NARO, Bio-oriented Technology Research Advancement Institution (the special scheme project on regional developing strategy: grant 16817557 to S.K.). This work was supported in part by the Platform for Drug Discovery, Informatics, and Structural Life Science from Japan Agency for Medical Research and development, AMED.

Received: 27 February 2017; Revised: 6 May 2017; Accepted: 10 May 2017

Abstract**Introduction:** Bovine mycoplasma, chiefly *Mycoplasma bovis*, is a pathogen that causes pneumonia, mastitis, arthritis, and otitis media in cattle. This pathogen exerts immunosuppressive effects, such as the inhibition of interferon production. However, the mechanisms involved in bovine mycoplasmosis have not been fully elucidated. In this study, we investigated the role of the programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway in immunosuppression in bovine mycoplasmosis.**Methods:** In the initial experiments, we used enzyme-linked immunosorbent assay to measure interferon- γ (IFN- γ) from peripheral blood mononuclear cells (PBMCs) isolated from cattle with mycoplasmosis.**Results:** Expectedly, IFN- γ production significantly decreased in cattle with mycoplasmosis compared with that in clinically healthy cattle. Concomitantly, flow cytometric analysis revealed that the proportions of PD-1⁺CD4⁺ and PD-L1⁺CD14⁺ cells significantly increased in peripheral blood of the infected cattle. Interestingly, the number of PD-1⁺CD4⁺ and PD-1⁺CD8⁺ T cells were negatively correlated with IFN- γ production from PBMCs in bovine mycoplasmosis. Additionally, blockade of the PD-1/PD-L1 pathway in vitro by anti-bovine PD-1- and anti-bovine PD-L1 antibodies significantly upregulated the production of IFN- γ from anti-mycoplasma-specific cells.**Conclusions:** These results suggest that the PD-1/PD-L1 pathway could be involved in immune exhaustion of bovine mycoplasma-specific T cells. In conclusion, our study opens up a new perspective in the therapeutic strategy for bovine mycoplasmosis by targeting the immunoinhibitory receptor pathways.

■ 論文題目

ウシマイコプラズマ感染症における PD-1 and PD-L1 発現細胞の増加と PD-1/PD-L1 の阻害を介した IFN γ 産生の促進

■ 要 旨

ウシマイコプラズマ感染症で認められる免疫抑制についてPD-1/PD-L1との関連性を検討した。IFN- γ の産生がマイコプラズマ感染症で正常牛に比較して有意に低下した。また、PD-1+ CD4+ and PD-L1+ CD14+ cells は感染個体の末梢血で有意に上昇した。興味深いことにマイコプラズマ感染症ではPD-1+ CD4+ and PD-1+ CD8+ T cells の数値はIFN- γ 産生量と負の相関関係を示した。さらに、PD-1/PD-L1 pathwayをそれらの抗体で抑制するとIFN- γ 産生量の増加が確認された。以上のことより、ウシマイコプラズマ感染症ではマイコプラズマ特異的Tcellの疲弊化においてPD-1/PD-L1の関係が示唆された。

■ 最終責任者 Satoru Konnai (Corresponding Author)

Original Article

***Mycoplasma bovis* isolates from dairy calves in Japan have less susceptibility than a reference strain to all approved macrolides associated with a point mutation (G748A) combined with multiple species-specific nucleotide alterations in 23S rRNA**Toyotaka Sato, Hidetoshi Higuchi, Shin-ichi Yokota, Yutaka Tamura First published: 13 June 2017 [Full publication history](#)DOI: 10.1111/1348-0421.12490 [View/save citation](#)Cited by (CrossRef): 0 articles  [Check for updates](#)  [Citation tools](#) ▼[Funding information](#)View Issue TOC
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ABSTRACT

Erythromycin, tylosin and tilmicosin are approved for use in cattle in Japan, the latter two being used to treat *Mycoplasma bovis* infection. In this study, 58 *M. bovis* isolates obtained from Japanese dairy calves all exhibited reduced susceptibility to these macrolides, this widespread reduced susceptibility being attributable to a few dominant lineages. All 58 isolates contained the G748A variant in both the *rrl3* and *rrl4* alleles of 23S rRNA, whereas a reference strain (PG45) did not. G748 localizes in the central loop of domain II (from C744 to A753) of 23S rRNA, which participates in binding to mycinose, a sugar residue present in both tylosin and tilmicosin. A number of *in vitro*-selected mutants derived from *M. bovis* PG45 showed reduced susceptibility to tylosin and tilmicosin and contained a nucleotide insertion within the central loop of domain II of *rrl3* (U747–G748Ins_CU/GU or A743–U744Ins_UA), suggesting that mutations around G748 confer this reduced susceptibility phenotype. However, other *Mycoplasma* species containing G748A were susceptible to tylosin and tilmicosin. Sequence comparison with *Escherichia coli* revealed that *M. bovis* PG45 and isolates harbored five nucleotide alterations (U744C, G745A, U746C, A752C and A753G) in the central loop of domain II of 23S rRNA, whereas other *Mycoplasma* species lacked at least two of these five nucleotide alterations. It was therefore concluded that G748 mutations in combination with species-specific nucleotide alterations in the central loop of domain II of 23S rRNA are likely sufficient to reduce susceptibility of *M. bovis* to tylosin and tilmicosin.

■ 論文題目

日本の子牛より分離された *Mycoplasma bovis* は点変異 (G748) により基準株よりマクロライドに対する感受性が低い

■ 要 旨

国内で分離されたマクロライド低感受性の *M. bovis* について薬剤感受性に関わる遺伝子配列を調べた。全58株において23S rRNAの*rrl3* および *rrl4* alleles両者においてG748A variantを含むことが示された。しかし基準株 (PG45) においてこれらの変異は確認されなかった。In vitroにおける *M. bovis* PG45の変異株でtylosin と tilmicosinの変異とともに、*rrl3* (U747–G748Ins_CU/GU or A743–U744Ins_UA) のcentral loop of domain IIに塩基の挿入が確認された。このことから、G748周辺の変異はtylosin and tilmicosinに対する感受性の低下に関連することが示された。

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- 1) Relevance of serum concentrations of non-esterified fatty acids and very low-density lipoproteins in nulli/primiparous and multiparous cows in the close-up period.

Oikawa S, Saitoh-Okumura H, Tanji M, Nakada K.

J. Vet. Med. Sci. 79:1656-1659. 2017.

II. その他<Others>



NOTE

Internal Medicine

Relevance of serum concentrations of non-esterified fatty acids and very low-density lipoproteins in nulli/primiparous and multiparous cows in the close-up period

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ABSTRACT. Serum concentrations of non-esterified fatty acids (NEFA) and very low-density lipoprotein (VLDL) in close-up dairy cattle were compared in relation to parity. Data were obtained from 37 nulli/primiparous (NP) and 24 multiparous (MU, parity: 2–7) cows between 14 days and 1 day prepartum. A positive correlation ($r=0.684$, $P<0.01$) was found between serum NEFA and VLDL concentrations in NP cows. Among the VLDL constituents, the NEFA concentration was particularly correlated with the triglyceride (TG) concentration ($r=0.658$, $P<0.01$). However, no significant correlation was found between the concentrations of NEFA and VLDL or VLDL-TG in MU cows ($r=-0.028$ and 0.307). These results suggest the presence of higher hepatic secretion of NEFA-derived VLDL in NP cows.

KEY WORDS: cattle, non-esterified fatty acids (NEFA), parity, very low-density lipoprotein (VLDL)

■ 論文題目

乾乳期の未経産と初産牛および経産牛における血中非エステル型脂肪酸と超低比重リポ蛋白質濃度の関連性

■ 要 旨

乾乳期の血中非エステル型脂肪酸 (NEFA) と超低比重リポ蛋白質 (VLDL) 濃度の産次による関連性を検討した。37 頭の未経産および初産 (NP) 牛と 24 頭の経産 (MU) 牛 (2~7 産) において分娩 1~14 日前に血液を採取した。NP 牛では NEFA と VLDL 濃度に有意な相関 ($r = 0.684$) が認められた。特に、VLDL の成分では中性脂肪 (TG) との間に 0.658 の有意な相関が見られた。しかしながら MU 牛において、NEFA と VLDL および VLDL-TG との間に有意な相関が見られなかった ($r = -0.028$, 0.307)。この結果は、NP 牛では NEFA 由来の VLDL が肝臓からより多く分泌されることを示唆している。

■ 最終責任者 Shin Oikawa (First and Corresponding Author)

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II. その他<Others>

1) Influence of maternal remifentanil concentration on fetal-to-maternal ratio in pregnant ewes.

Sato M, Masui K, Sarentonglaga B, Yamaguchi M, Fukumori R, Nagao Y, Sago H, Sumikura H.

J. Anesth. 31: 517-522. 2017



Influence of maternal remifentanyl concentration on fetal-to-maternal ratio in pregnant ewes

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Abstract

Purpose Maternal remifentanyl infusion is used for minimally invasive fetal surgery or ex-utero intrapartum treatment. The fetal-to-maternal (F/M) ratio of remifentanyl concentration at various dosing regimens is useful to manage remifentanyl effects. The aim of this study was to investigate the F/M ratio of remifentanyl at various concentrations. **Methods** Five pregnant ewes received continuous remifentanyl infusion under propofol anesthesia. The remifentanyl infusion rate was increased by 0.4 µg/kg/min every 15 min. The response to tail clamping in fetuses was assessed immediately before the change of infusion rate. Arterial remifentanyl concentrations in the mother and fetus were determined at each tail clamp. After observing a loss of response to tail clamping, remifentanyl infusion was terminated and the concentrations were assessed.

Results The median remifentanyl maximum infusion rate and maternal concentration were 3.0 µg/kg/min (range

2.4–3.6) and 21.6 (range 18.0–29.9) ng/mL, respectively. During continuous infusion, the F/M ratio was 0.15 (0.07–0.17), and the slope of the linear regression for the F/M ratio versus infusion rate in each individual was $-0.001 \pm 0.012/\mu\text{g kg min}$ ($P = 0.876$ vs hypothetical value of 0). The F/M ratio at the first sampling point in the elimination phase [0.33 (0.07–0.65)] was higher ($P = 0.033$) than at the last sampling point during continuous infusion [0.15 (0.06–0.17)].

Conclusion The F/M ratio was constant at a steady state regardless of the remifentanyl concentration up to 29.9 ng/mL, and increased in the elimination phase in pregnant ewes.

Keywords Remifentanyl · Pharmacokinetics · Fetal-to-maternal ratio

■ 論文題目 妊娠羊の血中レミフェンタニル濃度が胎子／母体濃度比率に及ぼす影響

■ 要 旨

麻酔薬のレミフェンタニルは児娩出を伴わない手術や体内治療的帝王切開術で使用される。レミフェンタニルの投与量調節を行い、胎子／母体 (F/M) 濃度の設定による麻酔管理技術への応用を目的として、本研究では妊娠羊を用いて母体レミフェンタニル濃度と F/M の関係性を解析した。その結果、母体レミフェンタニル濃度が 0.29 ng/mL までは、F/M 比が一定であること、投与終了後に比が上昇する、つまり、母体濃度の減少に対して胎子濃度の減少が遅いことが示された。

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- 1) Relevance of serum concentrations of non-esterified fatty acids and very low-density lipoproteins in nulli/primiparous and multiparous cows in the close-up period.

Oikawa S, Okumura-Saitoh ., Tanji M, Nakada K.
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NOTE

Internal Medicine

Relevance of serum concentrations of non-esterified fatty acids and very low-density lipoproteins in nulli/primiparous and multiparous cows in the close-up period

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ABSTRACT. Serum concentrations of non-esterified fatty acids (NEFA) and very low-density lipoprotein (VLDL) in close-up dairy cattle were compared in relation to parity. Data were obtained from 37 nulli/primiparous (NP) and 24 multiparous (MU, parity: 2–7) cows between 14 days and 1 day prepartum. A positive correlation ($r=0.684$, $P<0.01$) was found between serum NEFA and VLDL concentrations in NP cows. Among the VLDL constituents, the NEFA concentration was particularly correlated with the triglyceride (TG) concentration ($r=0.658$, $P<0.01$). However, no significant correlation was found between the concentrations of NEFA and VLDL or VLDL-TG in MU cows ($r=-0.028$ and 0.307). These results suggest the presence of higher hepatic secretion of NEFA-derived VLDL in NP cows.

KEY WORDS: cattle, non-esterified fatty acids (NEFA), parity, very low-density lipoprotein (VLDL)

■ 論文題目

クローズアップ期の未経産および経産牛の血清中非エステル型脂肪酸および超低密度リポ蛋白質濃度の評価

■ 要 旨

乳牛の未経産および経産牛の分娩前の血清中非エステル型脂肪酸 (NEFA) および超低密度リポ蛋白質 (VLDL) 濃度の関係を明らかにする目的で、分娩前 14 日から 1 日までの NEFA および VLDL を測定した。未経産牛では NEFA と VLDL は正の相関性が認められ、VLDL を構成する中性脂肪 (VLDL-TG) と、特に相関性が認められた。一方、経産牛は NEFA と VLDL、NEFA と VLDL-TG との間に関連性は認められなかった。このことは、未経産は肝臓での高い NEFA 誘導性 VLDL の分泌が存在することを示唆している。

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- 1) A transvaginal endoscopy-based technique for performing ovarian examinations in sows.

Okuyama M W, Sugiura T, Moriyoshi M, Yamashita K, Tamura J, Katagiri S.

J. Reprod. Dev. 63: 617-622. 2017.

—Technology Report—

A transvaginal endoscopy-based technique for performing ovarian examinations in sows

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Abstract. For examining pig ovaries, which have complex structures, laparoscopy is a useful technique, but requires general anesthesia; therefore, it cannot be performed repeatedly within a short period of time. We report a transvaginal endoscopy-based technique for conducting ovarian examinations without general anesthesia. Sows were sedated in pig stalls. Using a colonoscope, the vaginal wall was punctured with a trocar. To avoid the trocar being caught in the broad ligament of the uterus or the connective tissue around the vagina, the trocar was inserted close to the external uterine os and between the 2:00 and 3:00 or the 9:00 and 10:00 positions (in a clockwise direction). Then, a urethroscope was inserted into the abdomen, and an examination was carried out after the ovaries had been moved towards the urethroscope camera via rectal palpation. This less invasive procedure may allow repeated examinations and will increase our understanding of ovarian dynamics in pigs.

Key words: Ovarian observation, Sows, Transvaginal endoscopy

(J. Reprod. Dev. 63: 617–622, 2017)

■ 論文題目

経産豚の卵巣を観察するための経膣法による内視鏡検査技術について

■ 要 旨

複雑な構造を持っている豚の卵巣を検査するために、腹腔鏡検査は有益な技術であるが、全身麻酔を必要とするまで、短期間に繰り返し実施することは難しかった。今回我々は、全身麻酔なしで卵巣の検査を実施するための基礎技術として経膣法による内視鏡検査を実施したので報告する。供試豚（経産豚）は、専用のストールに入れ鎮静処置を施した。外陰部より結腸鏡を膣内に挿入し、膣壁に套管針で穴を開けた。その穴から尿道鏡を腹腔内部に挿入して、直腸検査により手で保持した卵巣を尿道鏡カメラで映し出し、検査観察を実施した。本法は豚に対する侵襲性が少なく、繰り返し検査することが可能であることから、短期間に変化する豚の卵巣の形態を理解する上で有用であることがわかった。

■ 最終責任者 Minami W Okuyama (First Author)

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- 1) Increase of cells expressing PD-1 and PD-L1 and enhancement of IFN- γ production via PD-1/PD-L1 blockade in bovine mycoplasmosis.
Goto S, Konnai S, Okagawa T, Nishimori A, Maekawa N, Gondaira S, Higuchi H, Koiwa M, Tajima M, Kohara J, Ogasawara S, Kato Y, Suzuki Y, Murata S, Ohashi K.
Immun. Inflamm. Dis. 10: 1-9. 2017.
- 2) Intrauterine infection with bovine leukemia virus in pregnant dam with high viral load.
Sajiki Y, Konnai S, Nishimori A, Okagawa T, Maekawa N, Goto S, Nagano M, Kohara J, Kitano N, Takahashi T, Tajima M, Mekata H, Horii Y, Murata S, Ohashi K.
J. Vet. Med. Sci. 79: 2036-2039. 2017.

Increase of cells expressing PD-1 and PD-L1 and enhancement of IFN- γ production via PD-1/PD-L1 blockade in bovine mycoplasmosis

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Keywords

Bovine mycoplasmosis, IFN- γ , immunosuppression, PD-1, PD-L1

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Immunity, Inflammation and Disease (2017)

Abstract

Introduction: Bovine mycoplasma, chiefly *Mycoplasma bovis*, is a pathogen that causes pneumonia, mastitis, arthritis, and otitis media in cattle. This pathogen exerts immunosuppressive effects, such as the inhibition of interferon production. However, the mechanisms involved in bovine mycoplasmosis have not been fully elucidated. In this study, we investigated the role of the programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway in immunosuppression in bovine mycoplasmosis.

Methods: In the initial experiments, we used enzyme-linked immunosorbent assay to measure interferon- γ (IFN- γ) from peripheral blood mononuclear cells (PBMCs) isolated from cattle with mycoplasmosis.

Results: Expectedly, IFN- γ production significantly decreased in cattle with mycoplasmosis compared with that in clinically healthy cattle. Concomitantly, flow cytometric analysis revealed that the proportions of PD-1⁺CD4⁺ and PD-L1⁺CD14⁺ cells significantly increased in peripheral blood of the infected cattle. Interestingly, the number of PD-1⁺CD4⁺ and PD-1⁺CD8⁺ T cells were negatively correlated with IFN- γ production from PBMCs in bovine mycoplasmosis. Additionally, blockade of the PD-1/PD-L1 pathway in vitro by anti-bovine PD-1- and anti-bovine PD-L1 antibodies significantly upregulated the production of IFN- γ from anti-mycoplasma-specific cells.

Conclusions: These results suggest that the PD-1/PD-L1 pathway could be involved in immune exhaustion of bovine mycoplasma-specific T cells. In conclusion, our study opens up a new perspective in the therapeutic strategy for bovine mycoplasmosis by targeting the immunoinhibitory receptor pathways.

■ 論文題目

牛マイコプラズマ症における PD-1/PD-L1 結合阻害による両分子発現細胞の増加と IFN- γ 産生増強

■ 要 旨

牛のマイコプラズマ感染症において、PD-1/PD-L1 作用によって抗原特異的 T-cell の免疫枯渇が起こっている可能性が示唆された。その作用を阻害したところ、両分子発現細胞が増加し、IFN- γ 産生量も増加した。免疫反応を抑制する受容体結合過程を標的にすることによって、牛のマイコプラズマ感染症の新たな治療法の確立が可能になるかもしれない。

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NOTE

Virology

Intrauterine infection with bovine leukemia virus in pregnant dam with high viral load

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ABSTRACT. Enzootic bovine leukemia is caused by the bovine leukemia virus (BLV). BLV is transmitted vertically or horizontally through the transfer of infected cells via direct contact, through milk, insect bites and contaminated iatrogenic procedures. However, we lacked direct evidence of intrauterine infection. The purpose of this study was to confirm intrauterine BLV infection in two pregnant dams with high viral load by cesarean delivery. BLV was detected in cord and placental blood, and the BLV in the newborns showed 100% nucleotide identity with the BLV-*env* sequence from the dams. Notably, a newborn was seropositive for BLV but had no colostral antibodies. In this study, we presented a direct evidence of intrauterine BLV transmission in pregnant dam with a high proviral load. These results could aid the development of BLV control measures targeting viral load.

KEY WORDS: bovine leukemia virus, high proviral load, intrauterine infection, vertical transmission

■ 論文題目

牛白血病ウイルス重度感染妊娠牛における子宮内感染

■ 要 旨

水平感染が主な伝播様式とされる牛白血病ウイルス (BLV) の垂直感染の可能性を検討した。BLV 重度感染妊娠牛から帝王切開により摘出した新生子牛と臍帯血、胎盤などから母牛と同一のウイルスが検出された。垂直感染の可能性が示され、今後の BLV 対策の改善に役立つと考えられた。

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- 1) Fluoroscopic observation of the development of displacement of abomasum in dairy cows.

Itoh M, Aoki T, Sakurai Y, Sasaki N, Inokuma H, Kamawamoto S, Yamada K.

J. Vet. Med. Sci. 79: 1952–1956. 2017

II. その他<Others>



NOTE

Surgery

Fluoroscopic observation of the development of displaced abomasum in dairy cows

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ABSTRACT. To clarify the mechanisms underlying the development of displaced abomasum (DA), we repositioned DA in 12 cows by rolling and observed the course of redispacement using X-ray fluoroscopy. Displacement of the abomasum was observed to follow three patterns: displacement to the left side of the abdominal cavity through the gap formed at the reticulum, atrium of the rumen and ventral sac of the rumen (n=7); displacement to the left side after cranial movement along the diaphragm (n=3); displacement to the right side after cranial movement along the diaphragm (n=2). These differences were considered to be associated with the rumen volume. To the best of our knowledge, this is the first study reporting the visual observation of the course of DA in dairy cows.

KEY WORDS: dairy cow, displaced abomasum, fluoroscopy

■ 論文題目

乳牛の第四胃変位のエックス線透視による観察

■ 要 旨

乳牛の第四胃の変位様式を明らかにするために、12頭の第四胃変位牛をローリングによって整腹し、その後の第四胃の変位様式をエックス線透視によって観察した。第四胃の変位は、第四胃が第二胃、第一胃前房と第一胃腹囊から形成される空隙から左腹腔へ変位する様式 (n=7)、頭方へ移動し横隔膜に沿って左方へ変位する様式 (n=3) および頭方へ移動し横隔膜に沿って右方へ変位する様式 (n=2) の3様式が観察された。この様式の違いは第一胃の容積との関連によると考えられた。乳牛の第四胃変位の変位様式を観察した初めての報告である。

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- 1) Plasma Endotoxin Activity in Eastern Grey Kangaroos (*Macropus giganteus*) with Lumpy Jaw Disease.
Sotohira Y, Suzuki K, Otsuka M, Tsuchiya M, Shimamori T, Nishi Y, Tsukano K, Asakawa M.
J. Vet. Med. Sci. 79: 1138-1141. 2017
- 2) Plasma lead, silicon and titanium concentrations are much higher in Green sea turtle from suburban coast than in those from rural coast in Okinawa, Japan.
Tsukano K, Suzuki K, Noda J, Yanagisawa M, Kameda K, Sera K, Nishi Y, Shimamori T, Morimoto Y, Yokota H, Asakawa M.
J. Vet. Med. Sci. 79: 2043-2047. 2017.

II. その他<Others>

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Shimamori T, Noda J, Tsukano K, Sera K, Yokota H, Koiwa M, Suzuki T, Suzuki K.
Jpn. J. Vet. Res. 65: 29-37. 2017.
- 2) Effect of Acetate Ringer's Solution with or without 5% Dextrose Intravenously Administered to Diarrheic Calves.
Tsukano K, Kato S, Sarashina S, Abe I, Ajito T, Ohtsuka H, Suzuki K.
J. Vet. Med. Sci. 79: 795-800. 2017.
- 3) Stress assessment using hair cortisol of kangaroos affected by lumpy jaw disease.
Sotohira Y, Suzuki K, Sano T, Arai C, Asakawa M, Hayashi H.

J. Vet. Med. Sci. 79: 852-854. 2017.

- 4) Increase in branched chain amino acids due to acidemia in neonatal calves with diarrhea.

Tsukano K, Inoue H, Suzuki K.

Vet. Rec. Open 4: e000234. 2017.

- 5) Rehydration and catabolic preventive effects depend on the composition of oral electrolyte solutions for diarrheic calves.

Tsukano K, Ajito T, Abe I, Sarashina S, Suzuki K.

J. Vet. Med. Sci. 79: 1776-1779. 2017.

- 6) Association between the levels of stress markers and the onset of kangaroo disease (lumpy jaw disease) in captive kangaroos.

Sotohira Y, Okui H, Suzuki K, Asakawa M, Sano T.

J. Zoo Biol. 1: 17-20. 2017.



NOTE

Wildlife Science

Plasma endotoxin activity in Eastern grey kangaroos (*Macropus giganteus*) with lumpy jaw disease

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ABSTRACT. Progressive pyogranulomatous osteomyelitis involving the mandible or maxilla of captive macropods, referred to as "Lumpy jaw disease (LJD)", is one of the most significant causes of illness and death in captive macropods. The aim of the present study was to evaluate the relationship between the severity of LJD and plasma endotoxin activity in kangaroos. Plasma samples obtained from moderate (n=24) and severe LJD (n=12), and healthy kangaroos (n=46), were diluted 1:20 in endotoxin-free water and heated to 80°C for 10 min. Plasma endotoxin activity was measured using the Limulus amoebocyte lysate (LAL)-kinetic turbidimetric (KT) assay. Plasma endotoxin activity was higher in kangaroos with severe LJD (0.199 ± 0.157 EU/ml) than in those with moderate LJD (0.051 ± 0.012 EU/ml, $P < 0.001$) and healthy controls (0.057 ± 0.028 EU/ml, $P < 0.001$). Our results suggest that the severity of LJD in captive macropods may be related to the plasma endotoxin activity.

KEY WORDS: captive, endotoxin, lumpy jaw, macropod, severity

■ 論文題目

Lumpy Jaw Disease 罹患オオカンガルーの血漿中エンドトキシン活性値

■ 要 旨

下顎骨または上顎骨を含む進行性骨髄炎（Lumpy jaw disease、LJD）は有袋類において重要な疾患である。本研究の目的は、カンガルーにおける LJD の重篤度と血漿エンドトキシン活性値との関係性を評価することであった。中等度（n=24）、重度の LJD 罹患（n=12）および健常カンガルー（n=46）から得た血漿をエンドトキシンの汚染のない注射用水で 20 倍希釈し、80°C で 10 分間加熱した。血漿エンドトキシン活性値は、リムルス（LAL）比濁時間法（KT）を用いて測定した。血漿エンドトキシン活性は、中等度 LJD（ 0.051 ± 0.012 EU/ml、 $P < 0.001$ ）および健常（ 0.057 ± 0.028 EU/ml、 $P < 0.001$ ）よりも重度 LJD カンガルーで有意に高値を示した（ 0.199 ± 0.157 EU/ml）。動物園で飼育されている有袋類の LJD の重篤度と血漿中エンドトキシン活性値は関連性があることが示唆された。

■ 最終責任者 Kazuyuki Suzuki (Corresponding Author)



NOTE

Wildlife Science

Plasma lead, silicon and titanium concentrations are considerably higher in green sea turtle from the suburban coast than in those from the rural coast in Okinawa, Japan

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ABSTRACT. The purpose of this study was to compare the concentration of trace elements in the plasma of sea turtles that inhabited the suburban (Okinawa Main Island, n=8) and the rural coast (Yaeyama Island, n=57) in Okinawa, Japan. Particle induced X-ray emission allowed detection of 20 trace and major elements. The wild sea turtles in the suburban coast in Okinawa were found to have high concentrations of Pb, Si and Ti in the plasma when compared to the rural area but there were no significant changes in the Al, As and Hg concentrations. These results may help to suggest the status of some elements in a marine environment. Further, monitoring the plasma trace and major element status in sea turtles can be used as a bio-monitoring approach by which specific types of elements found here could indicate effects that are related to human activities.

KEY WORDS: marine pollution, Okinawa Main Island, particle induced X-ray emission, sea turtle, Yaeyama Island

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■ 論文題目

沖縄郊外の沿岸に生息するアオウミガメの血漿中 Pb、ケイ素およびチタン濃度は地方の沿岸に生息するウミガメよりも高値である

■ 要 旨

本研究の目的は、沖縄の郊外（沖縄本島、n = 8）と地方（八重山島、n = 57）に生息したウミガメの血漿中の微量元素の濃度を比較することである。粒子誘起 X 線分析法により 20 種類の微量および主要元素の検出が可能であった。沖縄郊外に生息している野生のウミガメは地方のそれに比べて血漿中 Al、As、Hg 濃度には大きな差は認められなかったものの、Pb、Si、Ti 濃度が有意に高値であった。これらの結果は、海洋環境の状態を評価するのに役立つものと思われる。さらに、ウミガメの血漿中微量元素および主要元素の状態をモニタリングすることは、本知見によって差が認められた Pb、Si、Ti が人間の活動に関連するものであり、人間活動による影響に対するバイオモニタリングアプローチとして応用できる。

■ 最終責任者 Kazuyuki Suzuki (Corresponding Author)

Particle-Induced X-ray Emission Analysis of Serum Trace and Major Elements in Cattle with Acute Coliform Mastitis

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Abstract

The aim of the present study was to examine the applicability of the direct determination of trace and major element concentrations in serum samples collected from Holstein dairy cattle with acute coliform mastitis ($n = 53$) compared with a healthy control group ($n = 39$). Twenty-eight elements (Na, Mg, Al, Si, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ce, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, and Pb) were detected by particle-induced X-ray emission (PIXE). Significant differences were observed in serum K, Fe, Zn, and Br concentrations, but not in those of the remaining twenty-four elements. Furthermore, serum Fe concentrations ($0.751 \pm 0.583 \mu\text{g/ml}$, $n = 18$) were significantly lower in dairy cattle with a poor prognosis than in those with a good prognosis ($0.945 \pm 0.393 \mu\text{g/ml}$, $n = 35$, $P < 0.05$) and healthy controls ($1.458 \pm 0.391 \mu\text{g/ml}$, $n = 39$, $P < 0.01$). We proposed a diagnostic cut-off point for serum Fe concentrations of $< 0.82 \mu\text{g/ml}$ based on receiver operating characteristic (ROC) curves in order to identify cattle with a poor prognosis. The results of the present study indicated that assessing the elemental composition of serum, particularly iron, is a promising prognostic tool for determining the outcomes of cattle with severe acute coliform mastitis.

Key Words: Acute coliform mastitis, Dairy cattle, Iron, Particle-induced X-ray emission, Prognosis

■ 論文題目

急性大腸菌性乳房炎牛の血清微量および主要元素における粒子励起 X 線分析

■ 要 旨

急性大腸菌乳房炎 ($n = 53$) 罹患ホルスタイン種乳牛の血清中微量および主要元素動態を粒子励起 X 線分析 (PIXE) により測定して健常牛 ($n = 39$) と比較した。PIXE 法により 28 元素が検出された。その結果、血清 K、Fe、Zn および Br 濃度に有意差が認められたが、残りの 24 元素のそれには有意差は認められなかった。さらに、予後不良の乳牛の血清 Fe 濃度 ($0.751 \pm 0.583 \mu\text{g/ml}$, $n=18$) は、予後良好 ($0.945 \pm 0.393 \mu\text{g/ml}$, $n=35$, $P < 0.05$) および健常乳牛 ($1.458 \pm 0.391 \mu\text{g/ml}$, $n=39$, $P < 0.01$) よりも有意に低値であった。予後不良牛を同定するための ROC 曲線によれば、血清 Fe 濃度が $0.82 \mu\text{g/ml}$ 未満が診断カットオフ値となり得た。本研究により、血清中微量元素、特に Fe の動態を評価することは、重度の急性大腸菌性乳房炎牛の有用な予後診断ツールであることを示した。

■ 最終責任者 Jun Noda (Corresponding Author)



FULL PAPER

Internal Medicine

Effect of acetate Ringer's solution with or without 5% dextrose administered intravenously to diarrheic calves

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ABSTRACT. The objectives of this study were to evaluate the effects of intravenous acetate Ringer's solution, with or without dextrose, on diarrheic calves with either experimentally induced or spontaneous diarrhea. In the experimental model, diarrhea was induced in nine healthy calves by administering cold milk (below 4°C) twice a day for 2 days. The calves were randomly assigned to the isotonic saline (ISS), acetated Ringer's (AR) or acetated Ringer's with 5% dextrose (ARD) groups, with three calves assigned to each group. The calves received 80 ml/kg of their designated solution, at a flow rate of 20 ml/kg/hr. Infusion of ISS, AR and ARD were all found to be safe and effective in increasing plasma volume. Intravenous (IV) infusion of ISS resulted in the acidification secondary to dilution, while AR and ARD infusion inhibited acidification. In addition, prevention of catabolism was observed only with IV infusion of ARD. Sixteen calves with spontaneous diarrhea were enrolled in the clinical study. The calves were randomly assigned to the AR or ARD groups, with eight calves being assigned to each group. The calves received 100 ml/kg of their designated solution, at a flow rate of 25 ml/kg/hr. Intravenous infusion of AR and ARD was found to be effective in increasing plasma volume and inhibiting acidification. Only infusion of ARD prevented catabolism, but it also led to hyperglycemia. Our results suggest that a solution containing dextrose may be beneficial for wasting diarrheic calves.

KEY WORDS: acetate Ringer's solution, beta-hydroxybutyrate, calf, catabolism, diarrhea

■ 論文題目

5%ブドウ糖配合または非配合酢酸リンゲル液の下痢症子牛に対する有効性

■ 要 旨

実験的下痢または自然下痢症例を用いてブドウ糖配合（ARD）または非配合酢酸リンゲル液（AR）の有効性を評価した。9頭の健常子牛に低温ミルク（4℃以下）を1日2回、2日間投与して下痢を発症させ、各群3頭に80ml/kgの等張生理食塩水（ISS）、ARまたはARDを無作為に20ml/kg/hrの投与速度で静脈内投与した。ISS、ARおよびARDの静脈内投与は血漿量を増加させるのに安全で効果的であることが示唆された。異化予防効果は、ARD群でのみで観察された。16頭の下痢を呈した子牛を各群8頭に無作為に100ml/kgのARまたはARDを25ml/kg/hrで静脈内投与したところ、循環血漿量の増加および酸性化の予防効果が認められたが、ARD群のみが異化作用を予防した。これらの結果は、5%ブドウ糖配合酢酸リンゲル液が下痢症子牛を消耗に対して有用であることを示唆している。

■ 最終責任者 Kenji Tsukano (Corresponding Author)



NOTE

Wildlife Science

Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease

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ABSTRACT. The aim of this study was to objectively assess stress of kangaroos affected by lumpy jaw disease (LJD) using plasma and hair cortisol concentrations. The plasma and hair samples were collected from kangaroos with LJD and healthy controls. Collected hair samples were extracted with methanol after washing with isopropanol, following which they were processed with the cortisol enzyme immunoassay kit. The plasma cortisol concentration of LJD animals tended to be higher than that of the control. Ventral hair cortisol, but not dorsal hair, of LJD animals was significantly higher than that of the control. In conclusion, stress in kangaroos infected with LJD could be assessed by measuring ventral hair cortisol.

KEY WORDS: hair cortisol, kangaroo, lumpy jaw disease, objectively assessment, stress

■ 論文題目

lumpy jaw disease (LJD)罹患カンガルーの被毛中のコルチゾールを用いたストレス評価

■ 要 旨

本研究の目的は、血漿および被毛中コルチゾールを用いて lumpy jaw disease (LJD) 罹患カンガルーのストレスを客観的に評価することである。LJD 罹患カンガルーおよび健常個体より血液および被毛を採取し、血漿および被毛から抽出した溶液中のコルチゾール濃度を ELISA キットにより測定した。LJD 罹患カンガルーの血漿中コルチゾール濃度はコントロールよりも高い傾向があった。LJD 罹患カンガルーの腹側被毛中コルチゾール含有量はコントロールよりも有意に高い値を示した。以上のことより、LJD に罹患したカンガルーのストレスは腹側被毛中コルチゾール含有量を測定することにより評価することができることが示唆される。

■ 最終責任者 Hideaki Hayashi (Corresponding Author)

Increase in branched-chain amino acids due to acidemia in neonatal calves with diarrhoea

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ABSTRACT

The aim of this study was to investigate the relationships between acid-base status and plasma branched-chain amino acids (BCAA) concentration in calves with diarrhoea for intravenous nutrition, especially with amino acid solution in calves with diarrhoea. Thirty-four Holstein calves aged 11.0±5.9 days old were enrolled in this study. In 10 of 34 calves exhibiting clinical signs of diarrhoea, severe dehydration and acidemia were observed (severe group: pH: 7.04±0.11, base excess (BE): -17.4±4.5) based on blood gas analysis. In 7 of 34 calves exhibiting clinical signs of diarrhoea, mild dehydration and acidemia were observed (mild group: pH: 7.29±0.06, BE: 0.0±5.2). The other 17 calves did not exhibit dehydration or acidemia (pH: 7.41±0.02, BE: 11.2±3.5) based on clinical signs and blood gas analysis. The plasma concentration of BCAA was significantly higher in the severe group than in the other groups. In addition, the blood pH and plasma concentrations of BCAA ($r=-0.41$, $P<0.05$) were significantly and negatively correlated. As calves with metabolic acidosis have increased plasma BCAA concentrations due to hypermetabolic states of proteolysis, amino acid solutions containing low concentrations of BCAA may be useful to gradually correct the negative nitrogen balance.

mild decrease in extracellular pH is sufficient to activate proteolysis in human beings.⁶ If plasma amino acid profiles of diarrhoeic calves are similar to those in human beings with acidemia, a greater energy supply may be needed for diarrhoeic calves with acidemia. However, intravenous nutrition for diarrhoeic calves with proteolysis-induced acidemia is poorly understood. The aim of this study was to investigate the relationships between acid-base status and plasma BCAA concentration in calves with diarrhoea. These data may be useful for intravenous nutrition, especially with amino acid solution in calves with diarrhoea.

MATERIALS AND METHODS

All procedures were performed in accordance with the Good for the Care and Use of Laboratory Animals of the School of Veterinary Medicine at Rakuno Gakuen University (approval #VH16C1) and the National Research Council.⁷ Thirty-four Holstein

■ 論文題目

下痢症新生子牛のアシデミアによる分枝鎖アミノ酸の増加

■ 要 旨

本研究の目的は、下痢子牛、特に下痢子牛へのアミノ酸輸液を用いた静脈栄養の評価を行うため、酸塩基平衡状態と血漿分枝鎖アミノ酸 (BCAA) 濃度との関係を調査することであった。11.0±5.9日齢のホルスタイン種子牛34頭を供試した。血液ガス分析に基づいて下痢の臨床的徴候を示す34頭中10頭の子牛において重度の脱水および酸血症が観察された (重度群: pH: 7.04±0.11、塩基過剰 (BE): -17.4±4.5)。同様に7頭で軽度の脱水および酸血症が観察された (軽度群: pH: 7.29±0.06、BE: 0.0±5.2)。他の17頭の子牛は脱水または酸血症 (pH: 7.41±0.02、B-11.2±3.5) を示さなかった。血漿BCAA濃度は重症群で有意に高かった。さらに、血液pHおよび血漿BCAA濃度 ($r = -0.41$, $P < 0.05$) は有意な負の相関があった。代謝性アシドーシス子牛では、蛋白代謝が高いために血漿BCAA濃度が上昇するため、低濃度のBCAAを含むアミノ酸輸液剤は、負の窒素バランスを緩徐に補正するため有用である。

■ 最終責任者 Kenji Tsukano (Corresponding Author)



NOTE

Internal Medicine

Rehydration and catabolic preventive effects depend on the composition of oral electrolyte solutions for diarrheic calves

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ABSTRACT. In this study, two commercially available oral electrolyte solutions (OES) with high sodium (CF) or with high glucose and glycine (SL), and two prototype OES were evaluated in terms of rehydration and preventing catabolism. Prototype OES based on CF were prepared by doubling the glucose amount (CFG) or by doubling both glucose and glycine (CFGG). Thirty-two diarrheic calves were randomly assigned four groups with eight calves in each group. Blood volume increased with CF and CFGG compared with that of other OES. The catabolic preventive effect was excellent in CFGG and SL. Our results suggest that both the amount of sodium, glucose, and glycine, and ratio of these factors aid dehydration and provide energy.

KEY WORDS: calf, catabolism, diarrhea, oral electrolyte solutions, sodium

■ 論文題目

下痢性子牛に対する経口輸液剤の組成に基づいた再水和および異化予防効果

■ 要 旨

本研究では、高ナトリウム (CF) または高グルコースおよびグリシン (SL) を有する 2 つの市販経口電解質溶液 (OES) および 2 種類のプロトタイプ OES を、再水和および異化予防の観点から評価した。CF をベースに調整したプロトタイプ OES は、グルコース量 2 倍 (CFG)、またはグルコースとグリシン両者を 2 倍 (CFGG) に組成・調製した。32 頭の下痢性子牛を無作為各群 8 頭 4 群に割り当てた。CF は他の OES と比較して循環血液量の改善に優れ、異化予防効果は CFGG と SL において優れていた。これらの結果は、ナトリウム、グルコース、およびグリシンの組成量、およびこれらの比率が脱水を改善、栄養付加の特徴を決定している。

■ 最終責任者 Kenji Tsukano (Corresponding Author)



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Journal of Zoo Biology

<http://www.escijournals.net/JZB>



USE OF DIAZEPAM-KETAMINE IN PREVENTION OF CAPTURE MYOPATHY IN THE OSTRICH (*STRUTHIO CAMELUS*): A CASE REPORT

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ABSTRACT

Capture or exertional myopathy is an attendant complication of manual restraint in ratites, besides physical injuries that handlers may suffer. This work was carried out to restrain and immobilize two ostriches (*Struthio camelus*) in a bid to facilitate their clinical examination and transportation from one location to another, without subjecting the birds to capture myopathy that arises from the stress and exertion associated with physical restraint and capture. Two ostriches, male and female, weighing 120kg and 105kg respectively, were requested to be immobilized for relocation from Onireke to Jericho area in Oyo State, Nigeria. The birds were fasted overnight, administered with diazepam (3mg/kg) orally and ketamine (10mg/kg) intramuscularly. Mild sedation was achieved with diazepam after one hour. The birds were successfully transported. Complete recovery was achieved at about 8 hours post-administration of ketamine. Anaesthetic agents did not alter the haematologic parameters. We conclude that the diazepam-ketamine combination is generally safe for ratites and that oral administration of diazepam accompanied by intramuscular administration of ketamine provides restraint in ratites while also avoiding the risk of capture myopathy. It is suggested that the current dose of diazepam might need to be increased if the oral route is to be employed so as to shorten the onset of sedation and increase the depth of sedation.

Keywords: Capture myopathy, chemical restraint, ostrich.

■ 論文題目

飼育下カンガルーにおけるカンガルー病 (LJD) 発症と各種ストレスマーカーの関連性

■ 要 旨

到津の森公園ならびにひびき動物ワールドで飼育されているカンガルー38頭(健常群; 13頭、LJD群; 25頭)を対象に血清中コルチゾール濃度、酸化ストレス値(d-ROMs)および抗酸化力(BAP)の比較を行った。さらに唾液中コルチゾール濃度の測定も併せて行った。酸化ストレス値ならびに血清中コルチゾール濃度はLJD群で優位に高値を示したが、その他の値は両群間に有意な差は認められなかった。LJDに罹患することでストレス関連マーカーの値に変化は認められるものの、比較的大きな個体差が存在することを明らかにすることができた。

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RESEARCH PAPER

Plethysmography variability index for prediction of fluid responsiveness during graded haemorrhage and transfusion in sevoflurane-anaesthetized mechanically ventilated dogs

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■ 論文題目

セボフルラン麻酔下で調節呼吸した犬において段階的に脱血および返血した際の非侵襲的輸液反応性指標としての脈波変動指標の評価

■ 要 旨

犬における非侵襲的な動的輸液反応性指標としての脈波変動指標 (PVI) を侵襲的な動的輸液反応性指標の脈波変動率 (PPV) および一回拍出量変動率 (SVV) と比較し、PVI の有用性を実験的に評価した。健康なビーグル犬 6 頭をセボフルラン麻酔して調節呼吸で呼吸管理し、静脈血液を 5mL/kg ずつ段階的に 30mL/kg まで脱血し、その後 5mL/kg ずつ段階的に返血した。この間の PVI、PPV、および SVV の変化を ROC 解析および Bland-Altman 解析で比較した。PVI は輸液反応性指標として PPV とほぼ同等の中等度の精度を示し、輸液反応性を判断するカットオフ値は PVI > 12% であった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



Stroke volume variation (SVV) and pulse pressure variation (PPV) as indicators of fluid responsiveness in sevoflurane anesthetized mechanically ventilated euvolemic dogs

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■ 論文題目

セボフルラン麻酔下で調節呼吸した正常血液量の犬における輸液反応性指標としての一回拍出量変動率 (SVV) および脈波変動率 (PPV) の評価

■ 要 旨

犬における侵襲的な動的輸液反応性指標としての脈波変動率 (PPV) および一回拍出量変動率 (SVV) の有用性を実験的に評価した。健康なビーグル犬 6 頭をセボフルラン麻酔して調節呼吸で呼吸管理し、過剰輸液によって中心静脈圧 (CVP) を段階的に増加させ、PPV および SVV の変化を ROC 解析で比較した。SVV および PPV の変化は過剰輸液を反映しており、輸液反応性を判断するカットオフ値は SVV > 11% および PPV > 7% であった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)

ANESTHETIC MANAGEMENT OF AN INDO-PACIFIC BOTTLENOSE DOLPHIN (*TURSIOPS ADUNCUS*) REQUIRING SURGICAL DEBRIDEMENT OF A TAIL ABSCESS

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Ph.D., Haruka Koga, Yasuharu Izumisawa, D.V.M., Ph.D., and Kazuto Yamashita, D.V.M., Ph.D.

Abstract: This report describes the anesthetic management of a 14-yr-old, 160-kg, female Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) that underwent surgical debridement for a refractory subcutaneous abscess twice within a 6-mo interval. The animal was otherwise in good physical condition at each anesthetic procedure. Following premedication with intramuscular midazolam and butorphanol, anesthesia was induced with propofol and maintained with sevoflurane by intubation. During surgery ventilation was controlled. Blood pressure was indirectly estimated using either oscillometric or pulse oximetry. Presumed hypotension was managed by adjusting the sevoflurane concentration and infusion of dopamine. During recovery, the dolphin regained adequate spontaneous respiration following intravenous administration of flumazenil and doxapram. The dolphin was extubated at 85 min and 53 min after the first and second surgeries, respectively. Successful weaning from the ventilator and initiation of spontaneous respiration was the most important complication encountered. Establishment of a reliable blood pressure measurement technique is critical to success for anesthesia in this species.

Key words: Blood pressure, bottlenose dolphin, general anesthesia, sevoflurane, *Tursiops aduncus*, ventilator weaning.

■ 論文題目

尾部膿瘍の外科的鮮創を目的としたミナミバンドウイルカの全身麻酔

■ 要 旨

尾部に皮下膿瘍を発症したミナミバンドウイルカ(雌, 14歳, 体長239cm, 体重160kg)を6ヶ月間隔で二度全身麻酔し、外科的鮮創を実施した。麻酔前投薬としてミダゾラムおよびブトルファノールを筋肉内投与し、プロポフォールを静脈内投与して麻酔導入した。麻酔導入後、経口的に気管挿管し、酸素-セボフルラン吸入麻酔で麻酔維持した。麻酔回復期には、ドキサプラム投与後に自発呼吸が回復し、最初の外科手術ではセボフルラン吸入停止後85分目、二回目の外科手術では53分目に気管チューブを抜管した。イルカの全身麻酔では、陸生乳類と比較して麻酔回復期の調節呼吸からの離脱と自発呼吸への移行が困難であった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



FULL PAPER
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Interaction between maropitant and carprofen on sparing of the minimum alveolar concentration for blunting adrenergic response (MAC-BAR) of sevoflurane in dogs

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■ 論文題目

犬のセボフルラン minimum alveolar concentration for blunting adrenergic response (MAC-BAR) の減少効果におけるマロピタントとカルプロフェンの相互作用

■ 要 旨

ニューロキニン 1 (NK₁) 受容体拮抗によって制吐作用と鎮痛作用を発揮するマロピタントとシクロオキシゲナーゼ-2 (COX-2) 阻害によって消炎解熱鎮痛作用を発揮する非ステロイド系抗炎症薬のカルプロフェンの犬における鎮痛効果の相互作用をセボフルラン MAC-BAR を指標にビーグル犬 6 頭を用いて実験的に検討した。セボフルラン MAC-BAR は、生理食塩液を投与した対照群で 3.37 ± 0.56 %、マロピタント 1mg/kg 皮下投与群で 2.88 ± 0.73 %、カルプロフェン 4mg/kg 皮下投与群で 2.96 ± 0.38 %、およびマロピタント 1mg/kg-カルプロフェン 4mg/kg 併用皮下投与群で 2.81 ± 0.51 %であった。セボフルラン MAC-BAR はマロピタントまたはカルプロフェンの単独投与で有意に減少したが、マロピタントとカルプロフェンの併用では相加作用は認められなかった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)

—Technology Report—

A transvaginal endoscopy-based technique for performing ovarian examinations in sows

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Abstract. For examining pig ovaries, which have complex structures, laparoscopy is a useful technique, but requires general anesthesia; therefore, it cannot be performed repeatedly within a short period of time. We report a transvaginal endoscopy-based technique for conducting ovarian examinations without general anesthesia. Sows were sedated in pig stalls. Using a colonoscope, the vaginal wall was punctured with a trocar. To avoid the trocar being caught in the broad ligament of the uterus or the connective tissue around the vagina, the trocar was inserted close to the external uterine os and between the 2:00 and 3:00 or the 9:00 and 10:00 positions (in a clockwise direction). Then, a urethroscope was inserted into the abdomen, and an examination was carried out after the ovaries had been moved towards the urethroscope camera via rectal palpation. This less invasive procedure may allow repeated examinations and will increase our understanding of ovarian dynamics in pigs.

Key words: Ovarian observation, Sows, Transvaginal endoscopy

(J. Reprod. Dev. 63: 617–622, 2017)

■ 論文題目

雌豚の卵巣検査のための経膣内視鏡検査法

■ 要 旨

腹腔鏡による豚の卵巣検査は有用な方法であるが、全身麻酔が必要であり、反復検査は困難である。本研究では、全身麻酔の必要ない経膣内視鏡による豚の卵巣検査法を検討した。雌豚を鎮静後、膣壁をトロカーで穿孔して、時計周りに 2:00 と 3:00 または 9:00 と 10:00 の間に配置した。これらのトロカーを通して内視鏡を腹腔内に挿入し、直腸から卵巣を触知し、卵巣を内視鏡に近づけて観察した。

■ 最終責任者 Seiji Katagiri (Corresponding Author)

RESEARCH PAPER

Association between preoperative characteristics and risk of anaesthesia-related death in dogs in small-animal referral hospitals in Japan

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■ 論文題目

日本の動物二次診療施設における犬の周術期麻酔関連偶発死亡例の発生状況

■ 要 旨

日本の動物二次診療施設における犬の周術期麻酔関連偶発死亡例の発生状況を調査した。2010年4月1日から2011年3月31日に18施設で実施された犬の全身麻酔例4,323例を調査対象とした。全体の麻酔関連偶発死亡発生率は0.65%であった。そのうち75%は罹患疾患に関連して死亡し、多くが術後に死亡していた。

■ 最終責任者 Takaharu Itami (Corresponding Author)

Comparison between indocyanine green angiography and fluorescein angiography in normal cats

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Abstract

Objective To study a new approach to indocyanine green (ICG) angiography for contrasting the ocular fundus in cats.

Animals studied Six healthy laboratory cats.

Procedures Fluorescein (FLUO) and ICG angiography were performed using an infra-red-sensitive charged coupled device-equipped fundus camera on sedated cats.

Results At 12.3 ± 3.4 s after ICG administration, the choroidal arteries could be seen extending radially from the optic disk. The choroidal veins became apparent at 16.2 ± 4.1 s alongside the choroidal arteries. Gradual fading of the choroidal vessels began 5.8 ± 1.5 min postdye administration and diffuse fluorescence of the fundus appeared. Diffuse fluorescence of the optic disk faded at about 18.8 ± 2.9 min. Mean arterial blood pressure at 1 and 3 min after ICG administration showed no significant change when compared to pre-administration ($P > 0.05$). However, 5 min ($P = 0.054$) and 10 min ($P < 0.05$) postadministration, a significant drop in blood pressure occurred. The time lapse between FLUO administration and its appearance in the ocular fundus was 15.7 ± 3.8 s. Retinal veins became apparent at 22.0 ± 3.6 s alongside retinal arteries. At 31.2 ± 4.1 s, full venous fluorescence was visualized throughout the entire fundus.

Conclusions While FLUO angiography shows only the retinal vessels, ICG angiography enabled visualization of the choroidal vasculature. ICG angiography provides clear resolution while remaining reliable and simple; thus, a combination of ICG and FLUO angiography shows promise as a diagnostic aid for clinical evaluation of various chorioretinal diseases in cats.

Key Words: cat, choroid, fluorescein, fundus, indocyanine green, ocular angiography

■ 論文題目

正常猫のインドシニアングリーン血管造影とフルオレセイン血管造影の比較

■ 要 旨

猫の眼底血管造影検査におけるインドシニアングリーン (ICG) の有用性を検討するために従来のフルオレセイン (FLUO) と比較した。FLUO では網膜血管のみを造影するのに対し、ICG では脈絡膜血管を造影できた。FLUO 造影と ICG 造影を組み合わせることで、猫の脈絡膜疾患の診断精度を高めることができる。

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II. その他<Others>

- 1) Stroke volume variation (SVV) and pulse pressure variation (PPV) as indicators of fluid responsiveness in sevoflurane anesthetized mechanically ventilated euvoletic dogs.

Endo Y, Tamura J, Ishizuka T, Itami T, Hanazono K, Miyoshi K, Sano T, Yamashita K, Muir WW.

J. Vet. Med. Sci. 79:1437-1445. 2017.

- 2) Interaction between maropitant and carprofen on sparing of the minimum alveolar concentration for blunting adrenergic response (MAC-BAR) of sevoflurane in dogs.

Fukui S, Ooyama N, Tamura J, Umar MA, Ishizuka T, Itami T, Miyoshi K, Sano T, Yamashita K.

J. Vet. Med. Sci. 79:502-508. 2017.



Stroke volume variation (SVV) and pulse pressure variation (PPV) as indicators of fluid responsiveness in sevoflurane anesthetized mechanically ventilated euvolemic dogs

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■ 論文題目

セボフルラン麻酔下で調節呼吸した正常血液量の犬における輸液反応性指標としての一回拍出量変動率 (SVV) および脈波変動率 (PPV) の評価

■ 要 旨

犬における侵襲的な動的輸液反応性指標としての脈波変動率 (PPV) および一回拍出量変動率 (SVV) の有用性を実験的に評価した。健康なビーグル犬 6 頭をセボフルラン麻酔して調節呼吸で呼吸管理し、過剰輸液によって中心静脈圧 (CVP) を段階的に増加させ、PPV および SVV の変化を ROC 解析で比較した。SVV および PPV の変化は過剰輸液を反映しており、輸液反応性を判断するカットオフ値は SVV > 11% および PPV > 7% であった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



FULL PAPER

Surgery

Interaction between maropitant and carprofen on sparing of the minimum alveolar concentration for blunting adrenergic response (MAC-BAR) of sevoflurane in dogs

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■ 論文題目

犬のセボフルラン minimum alveolar concentration for blunting adrenergic response (MAC-BAR) の減少効果におけるマロピタントとカルプロフェンの相互作用

■ 要 旨

ニューロキニン 1 (NK₁) 受容体拮抗によって制吐作用と鎮痛作用を発揮するマロピタントとシクロオキシゲナーゼ-2 (COX-2) 阻害によって消炎解熱鎮痛作用を発揮する非ステロイド系抗炎症薬のカルプロフェンの犬における鎮痛効果の相互作用をセボフルラン MAC-BAR を指標にビーグル犬 6 頭を用いて実験的に検討した。セボフルラン MAC-BAR は、生理食塩液を投与した対照群で 3.37 ± 0.56 %、マロピタント 1mg/kg 皮下投与群で 2.88 ± 0.73 %、カルプロフェン 4mg/kg 皮下投与群で 2.96 ± 0.38 %、およびマロピタント 1mg/kg-カルプロフェン 4mg/kg 併用皮下投与群で 2.81 ± 0.51 %であった。セボフルラン MAC-BAR はマロピタントまたはカルプロフェンの単独投与で有意に減少したが、マロピタントとカルプロフェンの併用では相加作用は認められなかった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)

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1) Comparison between indocyanine green angiography and fluorescein angiography in normal cats.

Hayashi M, Maehara S, Ito Y, Yamashita K, Kubo A, Nakade T.
Vet. Ophthalmol. 20: 310-315. 2017.

II. その他<Others>

Comparison between indocyanine green angiography and fluorescein angiography in normal cats

Miri Hayashi, Seiya Maehara, Yosuke Ito, Kazuto Yamashita, Akira Kubo and Tetsuya Nakade

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Abstract

Objective To study a new approach to indocyanine green (ICG) angiography for contrasting the ocular fundus in cats.

Animals studied Six healthy laboratory cats.

Procedures Fluorescein (FLUO) and ICG angiography were performed using an infra-red-sensitive charged coupled device-equipped fundus camera on sedated cats.

Results At 12.3 ± 3.4 s after ICG administration, the choroidal arteries could be seen extending radially from the optic disk. The choroidal veins became apparent at 16.2 ± 4.1 s alongside the choroidal arteries. Gradual fading of the choroidal vessels began 5.8 ± 1.5 min postdye administration and diffuse fluorescence of the fundus appeared. Diffuse fluorescence of the optic disk faded at about 18.8 ± 2.9 min. Mean arterial blood pressure at 1 and 3 min after ICG administration showed no significant change when compared to pre-administration ($P > 0.05$). However, 5 min ($P = 0.054$) and 10 min ($P < 0.05$) postadministration, a significant drop in blood pressure occurred. The time lapse between FLUO administration and its appearance in the ocular fundus was 15.7 ± 3.8 s. Retinal veins became apparent at 22.0 ± 3.6 s alongside retinal arteries. At 31.2 ± 4.1 s, full venous fluorescence was visualized throughout the entire fundus.

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Key Words: cat, choroid, fluorescein, fundus, indocyanine green, ocular angiography

■ 論文題目

正常猫におけるインドシアニングリーン蛍光眼底造影とフルオレセイン蛍光眼底造影の造影像の比較

■ 要 旨

正常猫において、インドシアニンググリーン蛍光眼底造影とフルオレセイン蛍光眼底造影の造影像を比較した。フルオレセイン蛍光眼底造影では網膜血管のみが描出されたのに対し、インドシアニンググリーン蛍光眼底造影では脈絡膜血管の描出が可能であった。これらの蛍光眼底造影を組み合わせて検査することは、猫の網脈絡膜疾患の病態把握に有用であると考えられた。

■ 最終責任者 Seiya Maehara (Corresponding author)

伴侶動物内科学 II ユニット

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- 1) The Aldosterone Receptor Antagonist Eplerenone Inhibits
Isoproterenol-induced Collagen-I and 11 β -HSD1 Expression in Rat
Cardiac Fibroblasts and the Left Ventricle.
Hori Y, Touei D, Saitoh R, Yamagishi M, Kanai K, Hoshi F, Itoh N.
Biol. Pharm. Bull. 40: 1716. 2017.

II . その他<Others>

Regular Article

The Aldosterone Receptor Antagonist Eplerenone Inhibits Isoproterenol-Induced Collagen-I and 11 β -HSD1 Expression in Rat Cardiac Fibroblasts and the Left Ventricle

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β -Adrenergic receptor (β -AR)-induction of collagen-I synthesis is partially mediated by the cardiac mineralocorticoid receptor (MR) system. However, it remains unclear whether the selective MR antagonist, eplerenone, inhibits collagen-I synthesis induced by β -AR stimulation. We investigated the effects of eplerenone on the responses to a non-selective β -AR agonist, isoproterenol, which induced collagen-I synthesis in primary cardiac fibroblasts (CFs) and the left ventricle. mRNAs encoding the MR and 11 β -hydroxysteroid dehydrogenase type I (11 β -HSD1) were evident in the left ventricle and primary CFs. mRNAs encoding the CYP family 11 subfamily B member 2 (CYP11-B2) were not detected, even after isoproterenol treatment. *In vivo*, isoproterenol induced collagenous fiber accumulation in the left ventricle. The phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), 11 β -HSD1 levels, and mRNA/protein levels of collagen-I increased upon exposure to isoproterenol, but these increases were inhibited by eplerenone co-treatment. In primary CFs, isoproterenol increased the phosphorylation of ERK1/2 and the expression levels of both 11 β -HSD1 and collagen-I; these isoproterenol-attributable effects were inhibited by co-treatment with eplerenone and PD98059, a specific inhibitor of mitogen-activated protein kinase/ERK kinase activity. The results suggest that 11 β -HSD1 but not CYP11-B2 is expressed in primary CFs. Eplerenone inhibited isoproterenol-induced ERK1/2 phosphorylation and expression of 11 β -HSD1 and collagen-I in primary CFs, as well as the progression of cardiac fibrosis in the left ventricle. Therefore, eplerenone inhibited the isoproterenol-induced increases in 11 β -HSD1 and collagen-I expression in primary CFs, and progression of cardiac fibrosis in the left ventricle.

■ 論文題目

ラット心臓線維芽細胞および左心室におけるアルドステロン受容体阻害薬(エプレレノン)を用いたイソプロテレノール誘発コラーゲン I 産生の抑制

■ 要 旨

交感神経 β 受容体はミネラルコルチコイド受容体を介してコラーゲン I の産生を調節していることが示唆されているが、アルドステロン受容体阻害薬(エプレレノン)が交感神経 β 受容体刺激によるコラーゲン I 産生および心臓線維化を抑制する機序は不明である。心臓線維芽細胞ではアルドステロン誘導酵素(CYP11-B2)の発現を確認できなかったが、グルココルチコイド誘導酵素(11 β -HSD1)の mRNA/蛋白発現を確認した。交感神経 β 受容体作動薬(イソプロテレノール)の生体投与では左心室の線維化が認められ、コラーゲン I と 11 β -HSD1 の発現も増加していたが、エプレレノンはこれらの作用を抑制した。ラット心臓線維芽細胞において、イソプロテレノールはコラーゲン I および 11 β -HSD1 の産生を誘導したが、エプレレノンならびに ERK 阻害薬はこれらの作用を抑制した。これらのことからラット心臓線維芽細胞には 11 β -HSD1 が発現しており、エプレレノンは交感神経 β 受容体刺激による 11 β -HSD1 を抑制することでコラーゲン I の産生および心臓線維化を抑制することが示唆された。

■ 最終責任者 Yasutomo Hori (Corresponding Author)

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- 1) Effects of serum amyloid A on matrix metalloproteinase-9 production in feline lymphoma-derived cell lines.

Tamamoto T, Ohno K, Goto-Koshino Y, Tsujimoto H.

Vet. Immunol. Immunopathol. 187: 10-13. 2017.

II. その他<Others>

- 1) Assesment of severity and changes in C-reactive protein concentration and various biomarkers in dogs with pancreatitis.

Sato T, Ohno K, Tamamoto T, Oishi M, Kanemoto H, Fukushima K, Goto-Koshino Y, Takahashi M, Tsujimoto H.

J. Vet. Med. Sci. 79: 35-40. 2017.



Short communication

Effects of serum amyloid A on matrix metalloproteinase-9 production in feline lymphoma-derived cell lines



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ABSTRACT

Serum amyloid A (SAA) concentration and plasma matrix metalloproteinase-9 (MMP-9) levels are increased in cats with lymphoma. In the present study, the association between SAA and MMP-9 production was evaluated using recombinant feline SAA (rfSAA) and three feline lymphoma-derived cell lines: 3201, M54, and MCC. MMP-9 mRNA expression was significantly increased by rfSAA stimulation only in MCC cells. Secreted MMP-9 protein in culture media was confirmed by gelatin zymography, with clear bands of MMP-9 detected in MCC cells following rfSAA stimulation. A significant increase in semi-quantified MMP-9 levels was observed with 5 and 25 µg/ml of rfSAA stimulation. The infiltrative activities of feline lymphoma cells, assessed by the matrigel transwell assay, showed that rfSAA stimulated cell infiltration in MCC cells, in addition to MMP-9 expression. Although the response to rfSAA stimulation varied between cell lines, the results showed that rfSAA can stimulate MMP-9 production and infiltration of feline lymphoma-derived cells. The findings of this study have identified a novel role for SAA in the progression of some forms of feline lymphoma.

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■ 論文題目

猫リンパ腫由来細胞株の matrix metalloproteinase-9 産生における血清アミロイド A の影響

■ 要 旨

血中血清アミロイド A (SAA) 濃度と matrix metalloproteinase-9 (MMP-9) 濃度は、リンパ腫に罹患した猫においてしばしば増加している。SAA と MMP-9 はともにリンパ腫の増悪因子であることが知られているが、本研究では両者の関連性について猫リンパ腫由来細胞株を用いて検討した。猫リンパ腫由来細胞株の培養液中に組み替え猫 SAA を添加すると、一部の株では MMP-9 の遺伝子産生およびタンパク産生が濃度依存的に増加することが明らかとなった。また、リンパ腫由来細胞株の浸潤性も増加させることがわかった。本研究の結果より、SAA は一部のリンパ腫において、MMP-9 の産生を促進することで病態の進行や増悪に関与していると考えられる。

■ 最終責任者 Takashi Tamamoto (Corresponding Author)



FULL PAPER

Internal Medicine

Assesment of severity and changes in C-reactive protein concentration and various biomarkers in dogs with pancreatitis

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ABSTRACT. Canine pancreatitis is a relatively common disorder, and its mortality rate remains high. However, prognostic factors for pancreatitis based on evidence are limited. Moreover, the relationship between changes in C-reactive protein (CRP) concentration—an important prognostic factor for human patients with acute pancreatitis—and the prognosis of dogs with pancreatitis has not been widely studied. Therefore, we examined prognostic factors for canine pancreatitis during the first medical examination and evaluated the usefulness of serial CRP measurements during hospitalization. Sixty-five dogs met the inclusion criteria, including 22 that were hospitalized and treated. In Study 1, a multivariate analysis revealed that three factors—decreased platelet count and a marked (greater than 1,000 µg/l) elevation of specific canine pancreatic lipase (Spec cPL) concentration at the first medical examination, as well as elevated blood urea nitrogen (BUN) and/or creatinine (CRE) level—were significantly different between the survivors and nonsurvivors. Moreover, CRP concentrations on the third and fourth days were significantly different between the two groups in Study 2. An evaluation of the decreased platelet count, remarkable elevation of Spec cPL concentration at the first medical examination, elevation of BUN and/or CRE as well as serial CRP concentration measurements may be useful for predicting the prognosis of canine pancreatitis.

KEY WORDS: canine, CRP, pancreatitis, Spec cPL

■ 論文題目

膵炎に罹患した犬における重症度の評価と C 反応性蛋白および各種生化学指標の変化

■ 要 旨

膵炎は犬において一般的な疾患であり、その致死率は依然として高い。しかし、エビデンスに基づいた予後因子に関する情報はほとんどない。今回、犬の膵炎診断時における予後指標として CRP の経時的測定の有用性を検討した。入院 3 日目と 4 日目の CRP の値が生存群と死亡群で有意に異なっていた。これらに注意することで、膵炎の予後予測が可能になると考えられる。

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IL-7 and procalcitonin are useful biomarkers in the comprehensive evaluation of the severity of acute cholangitis

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Abstract

Background The incidence of biliary tract infection (BTI), especially healthcare-associated cholangitis, is increasing. However, there are few reports concerning biomarkers of acute cholangitis. We therefore performed an exhaustive investigation of several biomarkers.

Methods We retrospectively measured 11 cytokines, six chemokines and procalcitonin (PCT), and endotoxin activity assay (EAA) values (IRB: 110512019) of 61 samples with acute cholangitis.

Result The 28-day mortality rate was 9.8%. The levels of most cytokines and chemokines were significantly correlated with each other. A low IL-7 level was found to predict blood culture positivity. Low IL-7 level was also found to predict disseminated intravascular coagulation. Low IL-7 levels and a high PCT level were found to be predictors of severe cholangitis. The 28-day mortality in the group of patients with an IL-7 level of ≤ 6.0 and a PCT level of >0.5 was 18.2%. It was significantly higher than in the other group.

Conclusion The combined use of IL-7 and PCT may be useful for evaluating severe acute cholangitis; these results

may suggest that severe acute cholangitis is affected by immunosuppressive changes.

Keywords Acute cholangitis · Cytokines · Endotoxin activity assay · Interleukin-7 · Procalcitonin

■ 論文題目 IL-7およびプロカルシトニンが急性胆管炎の重症度の総合評価における有用なバイオマーカーである

■ 要 旨

胆道感染症(BTI)、特に医療関連胆管炎の発生率は増加している。しかし、急性胆管炎のバイオマーカーに関する報告は少ないため、いくつかのバイオマーカーを網羅的に探索した。61症例の急性胆管炎患者において、11種のサイトカイン、6種のケモカインおよびプロカルシトニン(PCT)およびエンドトキシリアクティブアッセイ(EAA)値を測定した。28日死亡率は9.8%であった。ほとんどのサイトカインおよびケモカインレベルは互いに有意に相関していた。低IL-7レベルは血液培養陽性を予測することが見出された。低IL-7レベルはまたDICも予測することが判明した。IL-7レベルが低く、PCTレベルが高いことが重度の胆管炎の予後因子であることが判明した。IL-7レベル ≤ 6.0 およびPCTレベル >0.5 であった患者群における28日死亡率は18.2%であり、それは他の群より有意に高かった。IL-7とPCTとの併用は重度の急性胆管炎の評価に有用であり、これらの結果は、重度の急性胆管炎が免疫抑制性変化によって影響されることを示唆した。

■ 最終責任者 Itaru Endo (Corresponding Author)

Haptoglobin Reduces Inflammatory Cytokine INF- γ and Facilitates Clot Formation in Acute Severe Burn Rat Model

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Haptoglobin exerts renal protective function by scavenging free hemoglobin from the urine and blood stream in patients with hemolytic disorders. Recent studies elucidate the relationships between haptoglobin and inflammation. In addition, coagulopathy is often induced by systemic inflammation characterized by the presence of vascular endothelial damage. We hypothesize that haptoglobin might have an anti-inflammatory effect and affect hypercoagulability using rat burn model. Thirty anesthetized rats of six-weeks of age received over 30% full-thickness scald burn on the dorsal skin surface. All rats were injected with either haptoglobin (Hpt) or normal saline (NS) intraperitoneally. The rats were divided into three groups: 1) control group (NS 20 mL/kg); 2) low concentration of Hpt group, L-Hpt, (Hpt 4 mL (80 U)/kg+NS 16 mL/kg); and 3) high concentration of Hpt group, H-Hpt, (Hpt 20 mL (400 U)/kg). While under anesthesia, all rats were euthanized by exsanguination at 6 hours (N=5) and 24 hours (N=5). Inflammatory and anti-inflammatory cytokines were measured and whole-blood viscoelastic tests were performed by thromboelastometry (ROTEM). Haptoglobin significantly reduced free hemoglobin 24 hours after the injury. Improvement of hematuria was confirmed in the H-Hpt group. There were no differences in thrombin-antithrombin complex and plasmin- $\alpha 2$ plasmin inhibitor complex. The haptoglobin tended to decrease interferon-gamma (INF- γ) in H-Hpt group. ROTEM findings of the L-Hpt group showed significantly higher clot firmness and shorter time to maximum clot formation velocity than the control group. Haptoglobin reduced INF- γ , and accelerated speed of clot formation in acute phase of severe burn. (J Nippon Med Sch 2017; 84: 64-72)

Key words: haptoglobin, severe burn, INF- γ , clot formation, thromboelastometry

■ 論文題目 ハプトグロビンは急性重症熱傷モデルラットにおいて炎症性サイトカイン INF- γ を減少させ、凝固形成を促進させる

■ 要 旨

ハプトグロビンは溶血性疾患患者の尿や血流から遊離ヘモグロビンを除去することによって腎保護機能を発揮する。最近の研究でハプトグロビンと炎症との関係が解明された。凝固障害は血管内皮損傷を特徴とする全身性炎症によって誘導される。我々はハプトグロビンがラット火傷モデルを用いて抗炎症効果を有し、凝固亢進に影響を及ぼすと仮定した。麻酔下で6週齢ラット(30匹)の背部皮膚表面に30%以上の全層熱傷を施した。全てのラットにハプトグロビン(Hpt)または生理食塩水(NS)を腹腔内注射した。ラットを1)対照群(NS 20mL/kg)、2)低濃度Hpt群、L-Hpt(Hpt 4mL(80U)/kg+NS 16mL/kg)、3)高濃度Hpt群、H-Hpt(Hpt 20mL(400U)/kg)の3群に分けた。6時間後(N=5)および24時間後(N=5)に麻酔下で放血殺した。炎症性および抗炎症性サイトカインを測定し、全血粘弾性試験をトロンボエストロメトリー(ROTEM)を用いて行った。ハプトグロビンは損傷24時間後に遊離ヘモグロビンを有意に減少させ、H-Hpt群では血尿の改善が確認された。トロンビン-アンチトロンビン複合体およびプラスミン- $\alpha 2$ プラスミン阻害複合体には差異はなかった。ハプトグロビンはH-Hpt群でINF- γ を減少させる傾向にあった。L-Hpt群のROTEM所見では対照群よりも有意な凝固硬度の上昇と最大凝固形成速度までの時間の短縮を示した。ハプトグロビンはINF- γ を減少させ、重度火傷の急性期における凝固形成の速度を加速する。

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The alpha-lipoic acid derivative DHLHZn: a new therapeutic agent for acute lung injury in vivo

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Abstract

Objective and design An animal experiment was performed to demonstrate the anti-inflammatory effects of an alpha-lipoic acid (ALA) derivative, dihydrolipoyl histidinate zinc complex (DHLHZn) for acute lung injury (ALI) and to investigate the mechanism of action.

Material Rats were randomly divided into three experimental groups: control group ($n = 17$), DHLHZn(–) group ($n = 11$, ALI model rats), and DHLHZn(+) group ($n = 12$, ALI model rats treated by DHLHZn).

Treatment Lipopolysaccharides (LPS, 10 mg/kg) were administered intratracheally in the DHLHZn(–) group and the DHLHZn(+) group. For the DHLHZn(+) group, DHLHZn (100 mg/kg) was administered intraperitoneally 2 h prior to LPS administration.

Methods Four hours after LPS administration, bronchoalveolar lavage fluid (BALF) and lung tissue were collected. The findings were analyzed using the Mann-Whitney *U* test.

Results Total number of cells, number of neutrophils and lymphocytes, levels of various inflammatory cytokines, and NF- κ B p65 concentration of BALF were significantly lower in the DHLHZn(+) group than in the DHLHZn(–) group ($p < 0.05$). ALI pathology scores were significantly lower in the DHLHZn(+) group than in the DHLHZn(–) group ($p < 0.001$).

Conclusions Anti-inflammatory effects of DHLHZn for ALI were demonstrated by BALF and histopathological findings. The mechanism of action of DHLHZn was considered to be via inhibition of the NF- κ B signaling pathway. DHLHZn is thus suggested to be a new prophylactic agent for ALI.

■ 論文題目 α -リポ酸誘導体 DHLHZn: in vivo での急性肺傷害のための新しい治療薬

■ 要 旨

α -リポ酸(ALA)誘導体であるジヒドロリポ酸ヒスチジン酸亜鉛複合体(DHLHZn)の急性肺傷害(ALI)に対する抗炎症効果を実証し、その作用機序を調べるために動物実験を行った。ラットを対照群($n=17$)、DHLHZn(–)群($n=11$, ALI モデルラット)および DHLHZn(+)群($n=12$, DHLHZn で処理された ALI モデルラット)の 3 群に分けた。リポ多糖類(LPS, 10mg/kg)を DHLHZn(–)群および DHLHZn(+)群に気管内投与した。DHLHZn(+)群では LPS 投与 2 時間前に DHLHZn(100mg/kg)を腹腔内投与した。LPS 投与 4 時間後に気管支肺胞洗浄液(BALF)および肺組織を採取した。結果は Mann-Whitney *U* 検定を用いて分析した。BALF の全細胞数、好中球およびリンパ球数、種々の炎症性サイトカインレベルおよび NF- κ B p65 濃度は DHLHZn(+)群で DHLHZn(–)群より有意に低かった($p < 0.05$)。ALI 病理スコアは DHLHZn(+)群で DHLHZn(–)群より有意に低かった($p < 0.001$)。ALI に対する DHLHZn の抗炎症効果は、BALF および組織病理学的所見によって実証された。DHLHZn の作用機序は、NF- κ B シグナル伝達経路の阻害によるものと考えられた。したがって、DHLHZn は ALI の新しい予防剤であることが示唆された。

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Letter to the Editor

Evaluation of urinary IL-6 in neonates with septic shock treated with polymyxin B-immobilized fiber column

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■ 論文題目 ポリミキシン B 固定化繊維カラムで処置した敗血症性ショックを有する新生児における尿中 IL-6 の評価

■ 要 旨

現在でも新生児敗血症は世界中の罹患率および死亡率に大きく影響を与えている。敗血症は IL-6 などの炎症性メディエーターの産生と放出による SIRS に随伴する。最近、新生児においても敗血症性ショックに対する PMX-DHP の臨床的有効性が報告されている。そこで我々は敗血症性ショックを発症した超低体重児 (ELBWI) における PMX-DHP を評価し、尿 IL-6 (uIL-6) と血清 IL-6 (sIL-6) の相関性を分析した。2 人の ELBWI に SIRS に伴う敗血症性ショックの治療のために PMX-DHP を 24 時間以内に施行した。PMX-DHP の前後に uIL-6 と sIL-6 を測定し、uIL-6 はクレアチニン (Cr) 濃度で標準化した。uIL-6/mgCr と sIL-6 との間に強い正の相関が見られた (0.98, $P < 0.01$)。両患者ともに PMX-DHP で著明な臨床的改善がみられ、神経学的および呼吸的値はほぼ正常範囲内にあった。新生児における敗血症性ショックに対する PMX-DHP の有効性は uIL-6 を用いて判断することができた。uIL-6 の経時的評価と 2 人の患者が合併症なしに生存したことを考慮すると、PMX-DHP は敗血症性ショックに有効であると考え。uIL-6 が採血を必要とせずに測定することができることから、PMX-DHP で処置した敗血症性ショックを有する ELBWI に有用であると考え。

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RESEARCH PAPER

Plethysmography variability index for prediction of fluid responsiveness during graded haemorrhage and transfusion in sevoflurane-anaesthetized mechanically ventilated dogs

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Abstract

Objective To examine the accuracy of plethysmography variability index (PVI) as a noninvasive indicator of fluid responsiveness in hypovolaemic dogs.

Study design Prospective experimental study.

Animals Six adult healthy sevoflurane-anaesthetized Beagle dogs.

Methods Dogs were anaesthetized with 1.3-fold their individual minimum alveolar concentration of sevoflurane. The lungs were mechanically ventilated after neuromuscular blockade with vecuronium bromide. Cardiopulmonary variables including mean arterial blood pressure (MAP), central venous pressure (CVP), transpulmonary thermodilution cardiac output (TPTDCO), stroke volume (SV), perfusion index (PI), pulse pressure variation (PPV), stroke volume variation (SVV) and PVI were determined during six stages of graded venous blood withdrawal (5 mL kg⁻¹ increments) and six stages of graded blood infusion (5 mL kg⁻¹ increments). The cardiopulmonary variables were analysed using paired *t* test or Wilcoxon signed rank test. Correlations between PPV and SVV or PVI were analysed by linear regression. The accuracy of PPV, SVV and PVI for

predicting fluid responsiveness was examined by using receiver operating characteristic curve analysis. A value of *p* < 0.05 was considered statistically significant.

Results Blood withdrawal resulted in significant increases in PPV and PVI and decreases in MAP, CVP, TPTDCO, SV and PI. Blood infusion resulted in significant increases in MAP, CVP, TPTDCO, SV and PI and decreases in PPV and PVI. PPV and PVI showed a relevant correlation (*p* < 0.001, *r*² = 0.62) and threshold values of PPV ≥ 16% (sensitivity 71%, specificity 82%) and PVI ≥ 12% (sensitivity 78%, specificity 72%) for identifying fluid responsiveness. SVV did not change.

Conclusions and clinical relevance Noninvasive measurement of PVI predicted fluid responsiveness with moderate accuracy equal to PPV in sevoflurane-anaesthetized mechanically ventilated dogs. Provisional threshold values for identification of fluid responsiveness were PPV ≥ 16% and PVI ≥ 12%. Clinical trials are needed to confirm these threshold values in dogs.

Keywords dogs, fluid responsiveness, plethysmography variability index, pulse pressure variation, stroke volume variation.

論文題目 セボフルラン麻酔下で機械的人工呼吸されたイヌにおける段階的失血および輸血中の輸液反応性予測のための脈波変動指数

要 旨

低血圧症のイヌにおける輸液応答性の非侵襲的指標としての脈波変動指数(PVI)の精度を調べることを目的とした。最小肺泡濃度の1.3倍でセボフルラン麻酔した6匹の健康成ビーグル犬を使用した。臭化ベクロニウムで神経筋遮断し、機械的換気された。平均動脈圧(MAP)、中心静脈圧(CVP)、肺動脈心拍出量(TPTDCO)、心拍出量(SV)、灌流指数(PI)、脈圧変動(PPV)、心拍出量変動(SVV)およびPVIの心肺変量は6段階の血液吸引と6段階の血液注入(5mL/kgの増減)で測定した。失血はPPVおよびPVIの有意な増加をもたらした。MAP、CVP、TPTDCO、SVおよびPIの有意な減少をもたらした。輸血はMAP、CVP、TPTDCO、SVおよびPIの有意な増加をもたらした。PPVおよびPVIの有意な減少をもたらした。PPVおよびPVIは、輸液反応性を同定するそれぞれの閾値PPV≥16%(感度71%、特異度82%)およびPVI≥12%(感度78%、特異度72%)で関連相関を示した(*p*<0.001、*r*²=0.62)。SVVは変化しなかった。PVIの非侵襲的測定はセボフルラン麻酔下の機械的換気された犬のPPVを中程度の精度で輸液反応性を予測した。輸液応答性を識別するための暫定的な閾値はPPVが16%以上であり、PVIが12%以上であった。犬のこれらの閾値を確認するために臨床試験が必要である。

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Research paper

Does motilin peptide regulate gastrointestinal motility of zebrafish? An *in vitro* study using isolated intestinal stripsTakio Kitazawa^{a,*}, Maria Yoshida^a, Hiroki Teraoka^a, Hiroyuki Kaiya^b^a School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan^b Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka 565-8565, Japan

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Distribution

ABSTRACT

Motilin (MOT), a 22-amino-acid peptide hormone produced in the duodenal mucosa, stimulates gastrointestinal motility in mammals and birds, and it is a mediator of interdigestive motor complexes. Recently, expression of MOT-like peptide (MOTLP) and its receptor mRNAs was identified in zebrafish. The aim of the present study was to determine whether the zebrafish MOTLP (zfMOTLP, HIAFSPKEMRELREKE) affects zebrafish gastrointestinal motility, with comparison to the effect of human MOT, in which five amino acids are identical to zfMOTLP at positions 5, 9, 15, 16, and 17. zfMOTLP caused small contractions of the rabbit duodenum and chicken ileum but, the sensitivity was about 3000-times lower than that of human MOT. zfMOTLP-induced contraction in the rabbit duodenum was decreased by pretreatment of the MOT receptor antagonist GM109, indicating that zfMOTLP could bind to the MOT receptor. zfMOTLP (3–100 nM) increased the intracellular Ca^{2+} concentration in zfMOT receptor-expressing HEK293 cells, but human MOT did not cause responses even at 100 nM. In *in vitro* study using isolated zebrafish gastrointestinal strips, zfMOTLP caused only small contractions even at high doses (1–10 μ M). zfMOT receptor mRNA is detected in the gastrointestinal tract and brain to almost the same extent, and the expression level (40–70 copies/100 ng total RNA) is much lower than that in the chicken gastrointestinal tract. These results suggest that the MOTLP/MOT receptor system is present in zebrafish, but its physiological role for regulation of gastrointestinal motility might be not significant due to the weak contractile activity and low expression level of the receptor.

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■ 論文題目

モチリンはゼブラフィッシュ消化管運動を調節しているか？摘出標本を用いての検討

■ 要 旨

ゼブラフィッシュで遺伝子レベルで存在が認められているモチリン様物質（17 アミノ酸ペプチド）の作用を種々の脊椎動物の消化管で検討した。モチリン様物質はモチリンが活性を示すウサギ十二指腸、ニワトリ回腸を収縮させたが、モチリンが収縮を誘起しないマウス腸管では収縮は認められなかった。ゼブラフィッシュ腸管はモチリン様物質の高濃度（1–10 μ M）で収縮を誘起した。この時、同様な濃度のモチリンでも収縮が誘起されたが、いずれの物質による収縮も極めて弱かった。ゼブラフィッシュ腸管にはモチリン受容体 mRNA が存在していたが発現レベルは低かった。モチリン受容体発現細胞においてモチリン様物質は 3–100 nM で興奮を誘起した。結論として、ゼブラフィッシュにはモチリンシステムが存在するものの受容体発現量が低値であることからその消化管運動調節系としての意義は低いと考えられた。

■ 最終責任者 Takio Kitazawa (First and Corresponding Author)

Neuropeptide Y (NPY) inhibits spontaneous contraction of the mouse atrium by possible activation of the NPY1 receptor

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Email: tko-kita@rakuno.ac.jp**Summary**

1. Neuropeptide Y (NPY) causes various central and peripheral actions through activation of G-protein-coupled NPY receptors. Although a species-dependent difference in cardiac actions of NPY has been reported, the responses to NPY have not been examined in mice, widely used experimental animals. This study aimed to clarify the responses to NPY and the receptor subtype involved in the responses in mouse atrium.
2. Neuropeptide Y caused negative inotropic and negative chronotropic actions in spontaneous beating right atria. Negative inotropic actions were more marked than negative chronotropic actions. Therefore, negative inotropic actions were studied in detail for evaluation of the NPY-induced cardiac actions in mouse atrium.
3. Neuropeptide Y-induced negative inotropic actions were not affected by atropine but were abolished in the atria from pertussis toxin-treated mice.
4. In isolated atrial preparations from reserpine-treated mice, NPY-induced negative inotropic actions were significantly attenuated.
5. [Leu31, Pro34]-NPY, but not peptide YY, was effective in decreasing spontaneous contraction in atrial preparations.
6. Although Y_1 , Y_2 , Y_4 and Y_5 receptor mRNAs were expressed almost equally in the brain, NPY₁ receptor mRNA was dominantly expressed in the atrium.
7. In conclusion, NPY caused negative inotropic and chronotropic actions through activation of the Y_1 receptor in the mouse atrium. A high expression level of Y_1 mRNA in the atrium suggests a functional role of NPY in the regulation of mouse cardiac contraction.

KEYWORDSmouse atrium, negative inotropic action, neuropeptide Y, Y_1 receptor

■ 論文題目 NPY1 受容体介した neuropeptide Y (NPY) のマウス心房筋収縮抑制作用

■ 要 旨

NPY の心筋収縮に与える影響には動物種差が認められる。これまでマウス心房における NPY の作用は検討されていなかったため今回検討を行った。NPY はマウス心房筋の自発収縮（収縮力、頻度）を抑制した。この抑制は心収縮力の抑制（変力作用）で著明であった。陰性変力作用は、アトロピンでは抑制されず、百日咳毒素処理で消失したことから、Gi タンパク質が関与する反応であることがわかった。NPY1 受容体の作動薬により NPY と同様な抑制作用が認められた。また、心房筋では NPY1 mRNA の発現レベルが最も高かった。以上のことから、マウス心房で NPY は NPY1 受容体を興奮させ、陰性の変力作用および変時作用を起こすことが明らかになった。

■ 最終責任者 Takio Kitazawa (Corresponding Author)

The chicken is an interesting animal for study of the functional role of ghrelin in the gastrointestinal tract

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Abstract. Ghrelin has been identified in vertebrates from fish to mammals, and it has multiple biological activities including gastrointestinal (GI) motor-stimulating action. In some non-mammalian vertebrates, we examined the effects of ghrelin on contractility of the isolated GI tract as well as the mRNA expression of growth hormone secretagogue-receptor 1a (GHS-R1a) to determine whether the motor-stimulating action of ghrelin is common in vertebrates. The expression level of GHS-R1a mRNA differed depending on the species and on the GI region (stomach, small intestine, and colon). GI region-dependent expression of GHS-R1a mRNA was remarkable in chickens, and the expression levels changed depending on age. In a functional study, ghrelin did not cause contraction of unstimulated GI strips in fish (goldfish and rainbow trout) or amphibians (bullfrog and Japanese fire belly newts) even using their homologous ghrelin. In avian species, ghrelin caused contraction of the unstimulated GI tract of the chicken but not of the Japanese quail, and the responses to ghrelin in the chicken GI tract decreased with aging. Our *in vitro* studies show that the motor-stimulating action of ghrelin is not conserved across vertebrates and that the chicken is a unique animal species for evaluation of the GI-stimulating action of ghrelin of different age.

Key words: Ghrelin, Gastrointestinal contraction, GHS-R1a, Non-mammals, Chicken

■ 論文題目

ニワトリはグレリンの消化管機能（運動）に対する影響を調べる上でユニークな動物種である。

■ 要 旨

グレリンは多くの脊椎動物の胃粘膜から分離精製されるペプチドホルモンで3位のアミノ酸（一般的にはセリン）が脂肪酸修飾を受けているのが特徴である。この物質の消化管収縮に与える影響には動物種差が著明である。すなわち、魚類や両生類では胃腸管収縮はグレリンによって全く影響を受けないが、鳥類と哺乳類では電気刺激誘発性収縮がグレリンにより増強される。加えてニワトリでは非刺激胃腸管標本がグレリンにより濃度依存性の収縮を誘起し、この反応には平滑筋性のグレリン受容体の関与が知られている。このような非刺激標本でのグレリン誘発性収縮および平滑筋性グレリン受容体はこれまでニワトリでしか存在が認められていない。このことは、ニワトリがグレリンの消化管収縮機能を検討するうえで極めて興味ある動物であることを示している。

■ 最終責任者 Takio Kitazawa (First and Corresponding Author)

Letter

Dexamethasone-induced hepatomegaly and steatosis in larval zebrafish

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[Recommended by Chiharu Tohyama]

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ABSTRACT — Fish hepatobiliary syndrome, characterized by hepatomegaly and fatty liver, has been frequently reported in many cultured fish species and has caused a dramatic economic loss in China. Glucocorticoids are thought to be important non-nutritional factors for hepatomegaly and fatty liver development. In the present study, a dexamethasone-induced zebrafish model of fatty liver and hepatomegaly was established, and the role of glucocorticoid receptor (GR) in the development of hepatomegaly and fatty liver was investigated using developing zebrafish. Exposure of larval zebrafish at 5 days post fertilization (dpf) to dexamethasone for 24 hr caused significant increases of liver size and number of fish with hepatic steatosis at 6 dpf. The increase of liver size caused by dexamethasone was significantly reversed by treatment with RU486, a GR antagonist, and by gene knock-down with a morpholino against the GR. The dexamethasone-induced hepatic steatosis was also inhibited by treatment with RU486. Overall, the results highlight larval zebrafish as a useful model for stress-induced liver failure.

Key words: Glucocorticoid, Hepatomegaly, Liver, Steatosis, Zebrafish

■ 論文題目

ゼブラフィッシュ稚魚におけるデキサメサゾン誘発性肝肥大と線維症

■ 要 旨

肝肥大と脂肪肝を特徴とする肝・胆道疾患は養殖魚における疾患として頻繁に発生し、経済的に大きな打撃をあたえる。ストレスホルモンである副腎皮質ホルモンは非栄養性の肝肥大や脂肪肝の原因として重要である。魚類モデルとしても有用なゼブラフィッシュ稚魚においてデキサメサゾンは肝肥大と脂肪蓄積を起こし、その作用はグルココルチコイド受容体のノックダウンで抑制された。ゼブラフィッシュ稚魚は魚類の肝肥大、脂肪肝モデルとして有用であることが示唆された。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)



RESEARCH ARTICLE

Functional expression and comparative characterization of four feline P450 cytochromes using fluorescent substrates

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Abstract

1. Cytochrome P450s (CYP) are a major group of metabolizing enzymes for xenobiotics in humans and other mammals. The properties of CYP isoforms in the domestic cat, an obligate carnivore, are largely unknown at present. In this study, we studied relative expression in tissues and enzymatic properties of nine significant feline CYP isoforms.
2. CYP2E2 transcript was most abundant in the feline liver, followed by CYP2A13 and 2E1. Transcripts of CYP3A131, 1A2 and 1A1 were also present in the liver, while CYP2D6 and 3A132 were only slightly expressed. CYP3A131 was a major transcript in the small intestine.
3. Four major CYP isoforms in the feline liver and small intestine (CYP1A2, CYP2A13, CYP2E2 and CYP3A131) were heterologously expressed in *Escherichia coli* to generate functional monooxygenase systems. We carried out screenings of 17 test compounds known to be inhibitors of CYP isoforms in other mammals as well as two anticancer drugs to assess the activity modulation of feline CYP isoforms using fluorogenic substrates. These CYP isoforms showed similar selectivity to counterparts in other mammals against inhibitors as a whole but with many exceptions.
4. The present study suggests the usefulness of the feline CYP recombinant system to obtain chemical affinity information and possible drug interactions in CYP metabolism of domestic cats.

Keywords

Carnivore, cytochrome P450, domestic cats, drug interaction, felid, xenobiotics

History

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Accepted 1 November 2016

■ 論文題目

4種のネコシトクローム P450 サブタイプの発現系の構築と機能の検討

■ 要 旨

ネコは主要なコンパニオンアニマルであるにもかかわらず、その代謝についての知識は不足している。本研究ではネコ肝臓におけるシトクローム P450 サブタイプの発現量を明らかにした上で、主要な CYP1A2、2A13、2E2、3A131 の大腸菌発現系を構築し、蛍光基質特異性と種々の薬物による阻害について検討した。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)



RESEARCH ARTICLE

Characterization of feline cytochrome P450 2B6

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Abstract

1. Little is known about drug metabolism in carnivores. Although the domestic cat (*Felis catus*) is an obligate carnivore and is the most common companion animal, usage and dosage of many drugs are determined according to information obtained from humans and dogs. We determined the complete cDNA sequence of CYP2B6 from the feline lung.
2. Feline CYP2B6 consists of 494 deduced amino acids, showing highest identity with the dog CYP2B ortholog, followed by those of horse, pig, primate and human.
3. Feline CYP2B6 transcripts were expressed predominantly in the lung and slightly in the small intestine but not in the liver without significant sex-dependent differences. Western blot analysis with an anti-human CYP2B6 antibody confirmed the presence of CYP2B protein in the lung but not in the liver.
4. Feline CYP2B6 proteins heterologously expressed in *Escherichia coli* metabolized several substrates specific to human CYP2B6, including 7-ethoxy-4-(trifluoromethyl) coumarin (EFC). The metabolic activity was strongly inhibited by medetomidine and atipamezole, potent inhibitors of canine CYP2B11 (now officially CYP2B6) as well as by ticlopidine and sertraline, inhibitors selective to human CYP2B6.
5. The results suggest that feline CYP2B6 is a functional CYP2B ortholog that plays a role in the local defense mechanism in the cat respiratory system and intestine.

Keywords

Carnivore, cytochrome P450 2B, domestic cats, felid, xenobiotics

History

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Published online 4 March 2016

■ 論文題目

ネコのシトクローム P450 2B6 に関する検討

■ 要 旨

ネコは主要なコンパニオンアニマルであるにもかかわらず、その代謝についての知識は不足している。本研究ではネコの CYP2B6 を新たにクローニングし、その組織発現量や大腸菌発現系を用いて代謝活性を明らかにした。ネコ肝臓は CYP2B サブタイプを全くあるいはほとんど発現していない顕著な特性を示すことがわかった。

■ 最終責任者 Hiroki Teraoka (Corresponding Author)

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- 1) Incidence of anaphylactic reactions after propofol administration in dogs.
Onuma M, Terada M, Ono S, Murakami A, Ishida T, Sano T
J. Vet. Med. Sci. 79: 1446-1452. 2017.
- 2) Retrospective investigation of cardiopulmonary resuscitation outcome in
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J. Vet. Med. Sci. 79: 852-854. 2017.



FULL PAPER

Surgery

Incidence of anaphylactic reactions after

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ABSTRACT. Propofol is an anesthetic agent suspended in an emulsion system that includes egg yolk lecithin and soybean oil, because of which, there is concern about the use of propofol in patients allergic to these substances. We examined the association between propofol administration and incidence of adverse events in dogs with allergy to egg yolk lecithin and soybean oil. On the basis of the findings of an allergen-specific immunoglobulin E (IgE) test, 14 dogs with high levels (high-IgE group) and 7 dogs with low levels (normal-IgE group) of IgE were selected. Following intravenous administration of propofol, the incidence of anaphylactic reactions and plasma histamine concentrations under general anesthesia maintained with isoflurane throughout surgery were compared between the two groups. The frequency of anaphylactic reactions and plasma histamine concentrations were compared by the chi-square test and Student t-test, respectively. The statistical significance for both tests was set at $P < 0.05$. In the high- and normal-IgE groups, the average frequencies of anaphylactic reactions after propofol administration were 21.4 and 14.3%, and the mean plasma histamine concentrations were 167.9 ± 94.5 nM and 65.7 ± 40.3 nM, respectively. Animals of neither groups experienced shock-like symptoms. These results revealed that propofol might be relatively safe, although careful perioperative anesthesia monitoring and standby protocols are required when using propofol in dogs with a history of allergic diseases or high chicken- or soybean-specific IgE levels.

KEY WORDS: anesthesia, dog, histamine, immunoglobulin-E, propofol-induced anaphylaxis

■ 論文題目

犬におけるプロポフォール投与によるアナフィラキシー様症状の発生状況

■ 要 旨

卵黄レシチンや大豆成分を含み精製されているプロポフォールを麻酔導入薬として用いた際の犬におけるアナフィラキシー様症状の発生状況を調査した。対象犬は 14 頭で IgE が高く、残りの 4 頭は IgE 値が低値であった。アナフィラキシー発生状況はそれぞれのグループにおいて 21.4%、14.3%であり、血中のヒスタミン濃度はそれぞれ 167.9 ± 94.5 nM、 65.7 ± 40.3 nM であった。以上のことより、プロポフォールは麻酔導入薬として非常に安全性の高い薬物であるが、過去にアレルギー症状を呈したことがある症例や、鳥肉もしくは卵に対する特異的 IgE 値が高値である症例においては注意深いモニタリングが必要である。

■ 最終責任者 Tadashi Sano (Corresponding Author)



NOTE

Surgery

Retrospective investigation of cardiopulmonary resuscitation outcome in 146 exotic animals

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ABSTRACT. The outcomes of cardiopulmonary resuscitation (CPR) were retrospectively evaluated in 146 exotic animals including 20 pet birds, 47 rabbits, 34 hamsters, 18 ferrets, 7 turtles and 20 other small mammals in cardiopulmonary arrest (CPA) at presentation or during hospitalization at an animal clinic. The rates of return of spontaneous circulation, survival after CPR and discharge were 9.3, 2.3 and 1.2%, respectively. The mean success rate of CPR in animals included in this study was lower than those previously reported in dogs and cats. This might have been because of the challenges in effective chest compression, airway management and monitoring as well as establishment of intravenous catheterization route in exotic animals.

KEY WORDS: cardiopulmonary arrest, cardiopulmonary resuscitation, exotic animals, survival rate

■ 論文題目

エキゾチックアニマル 146 症例の心肺蘇生の成績調査

■ 要 旨

心肺停止 (CPA) に陥った 146 症例のエキゾチックアニマルの心肺蘇生 (CPR) の成績を回顧的に調査した。146 症例の内訳はペットバードが 20 例、ウサギが 47 例、ハムスターが 34 例、フェレットが 18 例、カメが 7 例、その他の小型哺乳類が 20 例であった。自己心拍再開は 9.3%、CPA 後の生存率は 2.3%そして生存退院は 1.2%であった。これらの成績はヒトならびに犬・猫と比較して非常に低く、その原因として効果的な胸部圧迫 (心臓マッサージ) の困難さをはじめ、気道の管理、モニタリングそして薬物投与経路としての静脈路の確保の困難さが原因であると考えられた。

■ 最終責任者 Tadashi Sano (Corresponding Author)



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USE OF DIAZEPAM-KETAMINE IN PREVENTION OF CAPTURE MYOPATHY IN THE OSTRICH (*STRUTHIO CAMELUS*): A CASE REPORT

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ABSTRACT

Capture or exertional myopathy is an attendant complication of manual restraint in ratites, besides physical injuries that handlers may suffer. This work was carried out to restrain and immobilize two ostriches (*Struthio camelus*) in a bid to facilitate their clinical examination and transportation from one location to another, without subjecting the birds to capture myopathy that arises from the stress and exertion associated with physical restraint and capture. Two ostriches, male and female, weighing 120kg and 105kg respectively, were requested to be immobilized for relocation from Onireke to Jericho area in Oyo State, Nigeria. The birds were fasted overnight, administered with diazepam (3mg/kg) orally and ketamine (10mg/kg) intramuscularly. Mild sedation was achieved with diazepam after one hour. The birds were successfully transported. Complete recovery was achieved at about 8hours post-administration of ketamine. Anaesthetic agents did not alter the haematologic parameters. We conclude that the diazepam-ketamine combination is generally safe for ratites and that oral administration of diazepam accompanied by intramuscular administration of ketamine provides restraint in ratites while also avoiding the risk of capture myopathy. It is suggested that the current dose of diazepam might need to be increased if the oral route is to be employed so as to shorten the onset of sedation and increase the depth of sedation.

Keywords: Capture myopathy, chemical restraint, ostrich.

■ 論文題目

飼育下カンガルーにおけるカンガルー病 (LJD) 発症と各種ストレスマーカーの関連性

■ 要 旨

到津の森公園ならびにひびき動物ワールドで飼育されているカンガルー38頭 (健常群; 13頭、LJD群; 25頭) を対象に血清中コルチゾール濃度、酸化ストレス値 (d-ROMs) および抗酸化力 (BAP) の比較を行った。さらに唾液中コルチゾール濃度の測定も併せて行った。酸化ストレス値ならびに血清中コルチゾール濃度は LJD 群で優位に高値を示したが、その他の値は両群間に有意な差は認められなかった。LJD に罹患することでストレス関連マーカーの値に変化は認められるものの、比較的大きな個体差が存在することを明らかにすることができた。

■ 最終責任者 Tadashi Sano (Corresponding Author)

RESEARCH PAPER

Plethysmography variability index for prediction of fluid responsiveness during graded haemorrhage and transfusion in sevoflurane-anaesthetized mechanically ventilated dogs

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■ 論文題目

セボフルラン麻酔下で調節呼吸した犬において段階的に脱血および返血した際の非侵襲的輸液反応性指標としての脈波変動指標の評価

■ 要 旨

犬における非侵襲的な動的輸液反応性指標としての脈波変動指標（PVI）を侵襲的な動的輸液反応性指標の脈波変動率（PPV）および一回拍出量変動率（SVV）と比較し、PVIの有用性を実験的に評価した。健康なビーグル犬6頭をセボフルラン麻酔して調節呼吸で呼吸管理し、静脈血液を5mL/kgずつ段階的に30mL/kgまで脱血し、その後5mL/kgずつ段階的に返血した。この間のPVI、PPV、およびSVVの変化をROC解析およびBland-Altman解析で比較した。PVIは輸液反応性指標としてPPVとほぼ同等の中等度の精度を示し、輸液反応性を判断するカットオフ値はPVI>12%であった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



Stroke volume variation (SVV) and pulse pressure variation (PPV) as indicators of fluid responsiveness in sevoflurane anesthetized mechanically ventilated euvolemic dogs

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■ 論文題目

セボフルラン麻酔下で調節呼吸した正常血液量の犬における輸液反応性指標としての一回拍出量変動率 (SVV) および脈波変動率 (PPV) の評価

■ 要 旨

犬における侵襲的な動的輸液反応性指標としての脈波変動率 (PPV) および一回拍出量変動率 (SVV) の有用性を実験的に評価した。健康なビーグル犬 6 頭をセボフルラン麻酔して調節呼吸で呼吸管理し、過剰輸液によって中心静脈圧 (CVP) を段階的に増加させ、PPV および SVV の変化を ROC 解析で比較した。SVV および PPV の変化は過剰輸液を反映しており、輸液反応性を判断するカットオフ値は SVV > 11% および PPV > 7% であった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)



FULL PAPER
Surgery

The Journal of
Veterinary
Medical
Science

Interaction between maropitant and carprofen on sparing of the minimum alveolar concentration for blunting adrenergic response (MAC-BAR) of sevoflurane in dogs

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■ 論文題目

犬のセボフルラン minimum alveolar concentration for blunting adrenergic response (MAC-BAR) の減少効果におけるマロピタントとカルプロフェンの相互作用

■ 要 旨

ニューロキニン 1 (NK₁) 受容体拮抗によって制吐作用と鎮痛作用を発揮するマロピタントとシクロオキシゲナーゼ-2 (COX-2) 阻害によって消炎解熱鎮痛作用を発揮する非ステロイド系抗炎症薬のカルプロフェンの犬における鎮痛効果の相互作用をセボフルラン MAC-BAR を指標にビーグル犬 6 頭を用いて実験的に検討した。セボフルラン MAC-BAR は、生理食塩液を投与した対照群で 3.37 ± 0.56 %、マロピタント 1mg/kg 皮下投与群で 2.88 ± 0.73 %、カルプロフェン 4mg/kg 皮下投与群で 2.96 ± 0.38 %、およびマロピタント 1mg/kg-カルプロフェン 4mg/kg 併用皮下投与群で 2.81 ± 0.51 %であった。セボフルラン MAC-BAR はマロピタントまたはカルプロフェンの単独投与で有意に減少したが、マロピタントとカルプロフェンの併用では相加作用は認められなかった。

■ 最終責任者 Kazuto Yamashita (Corresponding Author)

RESEARCH PAPER

Association between preoperative characteristics and risk of anaesthesia-related death in dogs in small-animal referral hospitals in Japan

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■ 論文題目

日本の動物二次診療施設における犬の周術期麻酔関連偶発死亡例の発生状況

■ 要 旨

日本の動物二次診療施設における犬の周術期麻酔関連偶発死亡例の発生状況を調査した。2010年4月1日から2011年3月31日に18施設で実施された犬の全身麻酔例4,323例を調査対象とした。全体の麻酔関連偶発死亡発生率は0.65%であった。そのうち75%は罹患疾患に関連して死亡し、多くが術後に死亡していた。

■ 最終責任者 Takaharu Itami (Corresponding Author)



NOTE

Wildlife Science

Stress assessment using hair cortisol of kangaroos affected by the lumpy jaw disease

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ABSTRACT. The aim of this study was to objectively assess stress of kangaroos affected by lumpy jaw disease (LJD) using plasma and hair cortisol concentrations. The plasma and hair samples were collected from kangaroos with LJD and healthy controls. Collected hair samples were extracted with methanol after washing with isopropanol, following which they were processed with the cortisol enzyme immunoassay kit. The plasma cortisol concentration of LJD animals tended to be higher than that of the control. Ventral hair cortisol, but not dorsal hair, of LJD animals was significantly higher than that of the control. In conclusion, stress in kangaroos infected with LJD could be assessed by measuring ventral hair cortisol.

KEY WORDS: hair cortisol, kangaroo, lumpy jaw disease, objectively assessment, stress

■ 論文題目

カンガルー病 (Lumpy Jaw Disease; LJD) 罹患カンガルーの被毛中コルチゾール濃度を用いたストレス状態の評価

■ 要 旨

カンガルー病罹患カンガルーのストレス状態を評価するために血液中および被毛中コルチゾール濃度を健常カンガルーと比較した。血液中コルチゾール濃度および腹部被毛中のコルチゾール濃度は LJD 罹患カンガルーにおいて健常個体と比較して高値を示した。腹部の被毛はカンガルーのストレス状態を評価するのに有用であることが示された。

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- 1) Ubiquitin-specific protease 2 modulates the lipopolysaccharide-elicited expression of proinflammatory cytokines in macrophage-like HL-60 cells.

Kitamura H, Ishino T, Shimamoto Y, Okabe J, Miyamoto T, Takahashi E, Miyoshi I.

Mediat. Inflamm. 2017:6909415. 2017.

- 2) Macrophage ubiquitin-specific protease 2 modifies insulin sensitivity in obese mice.

Saito N, Kimura S, Miyamoto T, Fukushima S, Amagasa M, Shimamoto Y, Nishioka C, Okamoto S, Toda C, Washio K, Asano A, Miyoshi I, Takahashi E, Kitamura H.

Biochem. Biophys. Rep. 26: 322-329. 2017.

Research Article

Ubiquitin-Specific Protease 2 Modulates the Lipopolysaccharide-Elicited Expression of Proinflammatory Cytokines in Macrophage-like HL-60 Cells

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We investigated the regulatory roles of USP2 in mRNA accumulation of proinflammatory cytokines in macrophage-like cells after stimulation with a toll-like receptor (TLR) 4 ligand, lipopolysaccharide (LPS). Human macrophage-like HL-60 cells, mouse macrophage-like J774.1 cells, and mouse peritoneal macrophages demonstrated negative feedback to USP2 mRNA levels after LPS stimulation, suggesting that USP2 plays a significant role in LPS-stimulated macrophages. USP2 knockdown (KD) by short hairpin RNA in HL-60 cells promoted the accumulation of transcripts for 25 of 104 cytokines after LPS stimulation. In contrast, limited induction of cytokines was observed in cells forcibly expressing the longer splice variant of USP2 (USP2A), or in peritoneal macrophages isolated from *Usp2a* transgenic mice. An ubiquitin isopeptidase-deficient USP2A mutant failed to suppress LPS-induced cytokine expression, suggesting that protein ubiquitination contributes to USP2-mediated cytokine repression. Although USP2 deficiency did not accelerate TNF receptor-associated factor (TRAF) 6-nuclear factor- κ B (NF- κ B) signaling, it increased the DNA binding ratio of the octamer binding transcription factor (Oct)-1 to Oct-2 in TNF, CXCL8, CCL4, and *IL6* promoters. USP2 decreased nuclear Oct-2 protein levels in addition to decreasing the polyubiquitination of Oct-1. In summary, USP2 modulates proinflammatory cytokine induction, possibly through modification of Oct proteins, in macrophages following TLR4 activation.

■ 論文題目

ユビキチン特異的プロテアーゼ2は、マクロファージ様細胞HL-60におけるリポポリサッカライドによる炎症性サイトカインの発現を変化させる。

■ 要 旨

我々は、Toll様受容体 (TLR4) リガンドであるリポポリサッカライド(LPS)刺激後のマクロファージ様細胞での炎症性サイトカインのmRNA蓄積におけるUSP2の調節的役割を調査した。HL-60細胞、J774.1細胞、マウス腹腔内マクロファージでは、LPS刺激後にUSP2 mRNAの減少を生じた。USP2ノックダウンしたHL-60細胞では、LPS刺激後に104個のサイトカインうち25個の転写物の蓄積が生じた。反対に、USP2の長いスプライシングバリエーション(USP2A)を強制発現させた細胞や*Usp2a*トランスジェニックマウスの腹腔内マクロファージでは、サイトカインの誘導が制限された。ユビキチンイソペプチダーゼ欠損USP2A変異体は、LPSによるサイトカイン発現を抑制せず、TRAF 6-NF κ Bシグナリングを速めることもなかったが、TNF, CXCL8, CCL4およびIL-6プロモーターにおけるOct2に対するOct1のDNA結合比率を増加させた。USP2はOct 1のポリユビキチン化の減少に加えて核内のOct2タンパク質のレベルを減少させた。要約すると、USP2はTLR4の活性化後のマクロファージにおいて炎症性サイトカインの誘導を変化させる。これにはOctタンパク質の修飾を介している可能性がある。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)



Macrophage ubiquitin-specific protease 2 modifies insulin sensitivity in obese mice



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ABSTRACT

We previously reported that ubiquitin-specific protease (USP) 2 in macrophages down-regulates genes associated with metabolic diseases, suggesting a putative anti-diabetic role for USP2 in macrophages. In this study, we evaluate this role at both cellular and individual levels. Isolated macrophages forcibly expressing *Usp2a*, a longer splicing variant of USP2, failed to modulate the insulin sensitivity of 3T3-L1 adipocytes. Similarly, macrophage-selective overexpression of *Usp2a* in mice (*Usp2a* transgenic mice) had a negligible effect on insulin sensitivity relative to wild type littermates following a three-month high-fat diet. However, *Usp2a* transgenic mice exhibited fewer M1 macrophages in their mesenteric adipose tissue. Following a six-month high-fat diet, *Usp2a* transgenic mice exhibited a retarded progression of insulin resistance in their skeletal muscle and liver, and an improvement in insulin sensitivity at an individual level. Although conditioned media from *Usp2a*-overexpressing macrophages did not directly affect the insulin sensitivity of C2C12 myotubes compared to media from control macrophages, they did increase the insulin sensitivity of C2C12 cells after subsequent conditioning with 3T3-L1 cells. These results indicate that macrophage USP2A hampers obesity-elicited insulin resistance via an adipocyte-dependent mechanism.

■ 論文題目

マクロファージのユビキチン特異的プロテアーゼ2は、肥満マウスのインスリン感受性を修正する。

■ 要 旨

本研究で我々は、この役割を細胞および個体レベルで評価した。マクロファージでのUsp2a (USP2の長いスプライシングバリエント) を強制発現は、3T3-L1脂肪細胞のインスリン感受性を変化させず、同様にUsp2aトランジェニックマウスのマクロファージ選択的Usp2aの過剰発現は、高脂肪食給餌3ヶ月後のインスリン感受性に効果を示さなかった。しかし、Usp2aトランジェニックマウスでは、腸間膜脂肪のM1マクロファージが減少しており、高脂肪食給餌6ヶ月後の骨格筋と肝臓におけるインスリン抵抗性の進行が遅延し、個体レベルでのインスリン感受性が改善された。Usp2aを過剰発現したマクロファージの馴化培地は、C2C12筋管のインスリン感受性に直接的には影響を及ぼさなかったが、3T3-L1細胞との調整後の培地ではC2C12細胞のインスリン感受性を増加させた。これらの結果は、マクロファージUSP2Aは、肥満により誘発したインスリン抵抗性を脂肪細胞依存的な機序を経て阻止することを示している。

■ 最終責任者 Hiroshi Kitamura (Corresponding Author)

