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A1

Development of antiviral agents for recombinant feline-derived interferon protein

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The interferon-alpha (IFNα) selected for this study belongs to type I, which interferes with lymphocytes and macrophages. Feline panleukopenia virus (FPV) belongs to the *parvoviridae* family, an unenveloped single-stranded DNA virus (ssDNA) of the genus Parvovirus. This study would like to determine if interferon can successfully inhibit virus reproduction and achieve the effect of a short-term treatment against viral infections. The recombinant vector pET24a(+) and feline IFNα sequence are used to synthesize plasmids that were then transfected to competent cells DH5α. To confirm that the plasmids were successfully delivered into the competent cells, confirmation tests were conducted including colony selection by platin in antibiotic medium, plasmid extraction from selected clones, and protein expression and purification assays, including SDS-PAGE and Western blotting. For the in vitro tests, feline panleukopenia virus (FPV) was cultured with feline kidney cell line (Crandell Rees Feline Kidney, CRFK) and subjected to varying interferon treatments. The effects of different doses of interferon treatment were also tested.

Keywords: interferon, feline pan-leukopenia virus, antiviral

A2

Combine Radiation and Immunotherapy as A Treatment Modality of Canine Oral Melanoma

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Canine oral melanoma remains the most common malignant tumor in canine oral cavity, which has strong ability of local invasion and distant metastasis. The median survival time of untreated canine oral melanoma is about 2 months, and nowadays the major treatments are wide margin surgery and radiation therapy, while chemotherapy's effect is not that satisfied and the only FDA approved immunotherapy Oncept seems to have variable responses. In human melanoma, several immunotherapies were approved, and recent studies also focus on combination of immunotherapy and radiation therapy, which revealed a 36-50% clinical benefit by combining anti-CTLA4 antibody and radiation therapy. In canine patients, there is no study focus on combination of immunotherapy and radiation, thus my research hypothesizes that the combination will improve patient's overall survival and show clinical benefit. The immunotherapy we used is dendritic cell/tumor cell fusion vaccine, which had been proved to be effective in canine oral melanoma patients by significantly prolonging survival. My radiation protocol is 7.5-8.5 Gy, weekly, with regional lymph node included. One dose vaccine utilizes $1*10^7$ tumor cells and $1*10^7$ dendritic cells (cultured from healthy dog's blood) hybrid, four doses needed with 2 weeks interval. Vaccination will start after the 2nd radiation treatment. Response will be evaluated according to response evaluation criteria for solid tumor in dogs (v1.0) by VCOG, side effect of radiation therapy will be recorded according to toxicity criteria of the veterinary radiation therapy oncology group, while the side effect of immunotherapy will be monitored by physical and blood examination. Time to progression and overall survival will be recorded and analyzed, while some cytokines expression level and regulatory T cells level will also be detected and compared, to see if there is any prognostic indication. There are 5 cases recruited in this study, 4 died and 1 are still alive, 3 died of tumor related reason, 1 had sudden death without any local tumor progression or distant metastasis in last recheck. The alive 1 patient is progression free now. All patients had CR or PR after radiation therapy, 1 had lung metastasis noted at follow up CT 4 weeks after the last radiation treatment. 2 patients had local recurrence 79 days and 219 days after first radiation treatment. Overall survivals of the 4 dead patients are 83 days, 125 days, 152 days and 293 days. The alive patient has a follow-up of 660 days. The side effects of radiation were mild, no obvious side effect of immunotherapy was noted. The future work includes cytokine and immune cells analysis.

Keywords: canine, melanoma, radiation, immunotherapy

A3

The Different Perception of Dog Behavior Problems between Owner and Veterinarian——An Epidemiology Study

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The dogs were relinquished in Taiwan caused problems in society and government. The main reasons of relinquishment were possible because of barking, inappropriate elimination, accommodation problem, destroy furniture, dog owner passed away, diseases of dogs, economic impact and loss of interests. Therefore, canine behavioral problems had strongest effect on relinquished issue of dogs. To better understanding canine behavioral problems, epidemiology and clinical treatment were used in this study. A questionnaire surveys of veterinarians and dog owners were using snowball sampling method (filled out on the internet platform), with clinical follow-ups try to therapy the canine behavior problems may be to reduce the number of relinquishment and also avoid to do euthanasia. The contents of questionnaire included items of fear, aggression, excessive barking, compulsive, hyperactivity, destructive behavior, inappropriate elimination, noise phobia. Online questions survey including about 8 kinds of canine behaviors that could become be problem for owners, to investigate through (1) the prevalence of behavioral problems, (2) the most common happened behavioral problems, and (3) the relevant factors of each behavior. The study sample consisted of 1,671 data of dogs. Among all data of dogs, only 45.1% exhibited at least one behavioral problem. “noise phobia”, “excessive barking”, “obsessive/compulsive” were the most occurred in behavioral problems. A total of 50 veterinarians returned during 14-days questionnaire period, 56 and 16% of veterinarians recommended dog owners to go to see veterinary behaviorists and dog trainers for therapy in severe cases, respectively. While 14 and 14% of veterinarians referred all cases to veterinary behaviorists and never referred cases, respectively. Excessive barking, aggression, destructive behavior were the most frequent complaints regarding behavioral problems in dogs according to veterinarian survey result. Behavioral modification with drug therapy was considered the most effective treatment for canine behavioral problems. Some findings were similar to preview contributions, but this study supported some new data on the epidemiology of behavioral on problems in domestic dogs, which could be useful to improve animal welfare for reducing dog relinquishment and euthanasia by preventing undesirable behavioral problems.

Keywords: behavioral problems, relinquishment, euthanasia, veterinarians

A4

Host Protein WDR5 Localizes to CDV-induced Cytoplasmic Inclusion Bodies and Enhances Viral Replication

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Canine distemper virus (CDV), a contagious pathogen causing fatal systemic disease, still widely exists in wildlife reservoirs and unvaccinated domestic dogs. CDV belongs to *Morbillivirus*，*Paramyxoviridae*; this species also includes Measle virus (MeV). In virus-infected cells, both cytoplasmic and nucleus inclusion bodies (IBs) can be observed. Cytoplasmic IBs have been proved as viral replication machinery in many members of *Mononegavirales*, such as MeV, HPIV3, RSV. However, formation mechanisms of these IBs have yet been completely unraveled; especially, that of CDV IBs remains unknown. In MeV，nuceloprotein (N), phosphoprotein (P) and host protein WDR5, previously known as a gene expression regulator，localize in IBs. In our study, CDV N and P proteins were co-expressed in Vero cells to mimic virus-induced cytoplasmic IBs. Based on co-expression model, over-expressed WDR5 colocalized with N-P-induced IB-like puncta, which implies the interaction between WDR5 and N-P complex. To confirm this interaction, we performed co-immunoprecipitation and found WDR5 could be pulled down with N-P complex. To investigate the function of WDR5 in IBs, we knocked down endogenous WDR5 in host cells and found decreased viral protein production. Besides, we found WDR5 inhibitor (OICR-9429) partially blocked CDV infection in B95a cells. We presumed this blockage is due to the impairment of IBs. As expected, inhibitor-treated N-P co-expression model could hardly induce IB-like puncta, which indicates WDR5 is essential for IBs formation. Overall, we proved that similar to MeV, WDR5 localizes to CDV-induced cytoplasmic IBs to promote viral replication, and we found a promising anti-CDV drug, WDR5 inhibitor.

Keywords: canine distemper virus, inclusion bodies, WDR5, WDR5 inhibitor

A5

Beta-amyloid precursor protein as a biomarker for traumatic axonal injury in cats

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Traumatic brain injury (TBI) is one of the most common causes of death in veterinary forensic cases, and traumatic axonal injury (TAI) is almost a universal consequence of TBI. The pattern analysis for immunohistochemistry (IHC) against beta-amyloid precursor protein (βAPP) is a gold standard for early detecting axonal injuries in human forensic neuropathology. In animals, only several studies on TAI have been performed, but there is scanty research on TAI of dogs or cats. The study aims to detect early TAI in animals with TBI by βAPP IHC and to correlate the severity of head injury to histopathological and immunohistochemical results of TAI. Animal carcasses were collected from routinely forensic necropsy cases. Cats with TBI were separately grouped into four groups according to the severity of the head injury, while cats without TBI were selected for the control group. The entire brains of animals with TBI were collected and underwent appropriate fixation, and representative sections were selected following standardized serial sections. The histopathological examination for axonal degeneration and IHC against βAPP were performed. Positive signals of βAPP IHC, the axonal bulbs, in cats with TBI were quantified and correlated to the severity of the head injury. Thirty-nine cats with TBI and 20 cats without TBI are collected in this study. The results revealed that 64.1% of TBI cases demonstrated positive signals of axonal injuries. The axonal bulb count is remarkably increased in severer head injuries compared to milder head injuries. The shortest post-traumatic interval of the presence of axonal bulbs is between 0.5-2hr. The frequent locations where TAI presence in cats are similar to those in human medicine. Furthermore, the axonal bulb count may have a diagnostic value for determining the coup and contrecoup lesions. In conclusion, the research may shed light on the early detection of TAI in companion animals.

Keywords: traumatic brain injury, traumatic axonal injury, beta-amyloid precursor protein, veterinary forensic pathology, forensic neuropathology

A6

Evaluation of Indoxyl Sulfate Served as Prognostic Indicator of Acute Renal Failure Cats Treated with Peritoneal Dialysis: A Pilot Study

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Since there are no prognostic indicators that can be used effectively in cats with acute renal failure (ARF) on peritoneal dialysis, the purpose of this study is initially to assess whether the protein-bound urotoxin—Indoxyl sulfate(IS) has the potential used as a prognostic indicator of peritoneal dialysis. Pilot study was to explored if there is value for conducting large-scale trials. The 7 plasma samples of ARF cats undergoing peritoneal dialysis and healthy cats from animal hospital were collected for used in this study. The collected samples were divided into survival and non-survival groups, whereas the control group was non-azotemia cats. The body temperature, Creatinine(Cre), Symmetric Dimethylarginine(SDMA) and albumin of clinicalpathological values in these 7 samples were used as indicator for evaluation study. The IS concentration of plasma was quantified by high performance liquid chromatography method. The calibration curve of 500, 250, 100, 50, 25, 12.5, 6.25 μg/mL of IS concentration was $y=3.7145x+843.37$, $R^2=0.97$. The calibration curve was used to converting the IS concentration in plasma sample. A total of 7 samples were divided into 2 in the survival group, 2 in the non-survival group, and 3 in the control group. The plasma concentration of IS in ARF cats was significantly greater than that in non-azotemic cats, and IS was related to important biochemical indicators of nephropathy such as SDMA and Cre. The mean concentration of IS plasma in ARF cats before dialysis was as high as 200 ppm. After peritoneal dialysis, if the concentration of IS decreased significantly, it was more likely to survive within 10 days after dialysis than those with small decrease. It can be seen that IS maybe has potential as a biochemical indicator for the prognosis of dialysis for further exploration.

Keywords: feline, acute renal failure, prognostic indicator, peritoneal dialysis, indoxyl sulfate

A7

The possibility of transovarial transmission of *Bartonella henselae* in *Rhipicephalus sanguineus* ticks by using artificial membrane feeding system

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Bartonella henselae is a slow-growing, intraerythrocytic gram-negative bacterium that causes cat scratch disease (CSD) in humans. Cats are major reservoir of *B. henselae*, which is transmitted by cat fleas (*Ctenophephalides felis*). *Ixodes ricinus* tick has been experimentally demonstrated to be the competent vector for *B. henselae*. For *Rhipicephalus sanguineus* ticks, our previous study revealed the possibility of *B. henselae* transstadial transmission from *R. sanguineus* larvae to nymphs. The present study aims to investigate transovarial transmission of *B. henselae* in *R. sanguineus* ticks. Fifty *Bartonella*-negative *R. sanguineus* adults were fed on *B. henselae*-infected blood (10^6 CFU/ml) by using an artificial feeder for 14 days, and the other 40 adults were fed on non-infected blood as a control group. At the end of feeding, *B. henselae* DNA was detected in 100% (3/3) of midguts and 100% (3/3) of salivary glands of male ticks, and 60% (3/5) of midgut contents, 80% (4/5) of salivary glands, and 40% (2/5) of carcasses of the semi-engorged females, but not detected in male carcasses and female oviducts. *B. henselae* DNA was also detected in pooled tick feces collected during 14 days of feeding and the feces at day 1 to day 10 of post-feeding period. After oviposition, *B. henselae* DNA was detected in 33.3% (1/3) of salivary glands, while pooled eggs and larvae originated from infected females were all PCR-negative for *B. henselae*. Our findings provide the possibility of *B. henselae* acquisition by *R. sanguineus* adults during blood feeding. *B. henselae* could remain in the tick midguts, move to salivary glands and other tick tissues, and shed through feces. *B. henselae* DNA detected in salivary glands of oviposited female suggests the possibility of *B. henselae* persistence in salivary glands over the oviposition period of the ticks. Although *B. henselae* was not detected in eggs and unfed larvae by PCR, the further larval feeding needs to be performed to clarify whether *B. henselae* can be transmitted by *R. sanguineus* larvae.

Keywords: transovarial transmission, *Rhipicephalus sanguineus*, *Bartonella henselae*, artificial membrane feeding system

A8

The Distribution of Cerebral Microbleeds in Dogs with acute unilateral vestibular signs

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On magnetic resonance imaging (MRI), cerebral microbleeds (CMBs) are characterized as small (less than 5 mm in diameter), well-defined, round, intraparenchymal signal voids detected on T2* gradient-echo sequence or susceptibility-weighted imaging (SWI). Due to the small size, these lesions may be invisible on conventional T1- and T2-weighted images. Furthermore, compared with T2* gradient-echo sequence, SWI has been reported to be more sensitive and reliable in detecting CMBs. CMBs have been documented to occur in 9.3% of dogs undergoing brain MRI examination. The same study also reported that dogs with CMBs are usually older, and more frequently presented for vestibular signs. However, CMBs are also often recognized as incidental imaging findings. A study was carried out to investigate whether CMBs detected on SWI can potentially be the underlying cause for dogs presenting with acute non-progressive unilateral vestibular signs, mimicking the clinical presentation for idiopathic vestibular disease in dogs. Medical records of the National Taiwan University Veterinary Hospital were retrospectively searched. Dogs presenting with acute unilateral vestibular signs, with CMBs detecting on SWI, but no other significant intracranial disease identified on the clinical investigation to explain the vestibular signs were included in the study. The distribution of CMBs were analyzed, particularly focusing on the location potentially contributing to vestibular signs, i.e., vestibular nuclei in the brainstem, nodulus, flocculus, and fastigial nuclei in the cerebellum, caudal cerebellar peduncle, and the thalamus. From November 2019 to March 2021, 227 dogs underwent brain MRI including SWI examination. Out of 47 dogs with CMBs identified on SWI, 14 dogs fit with the inclusion criteria. All dogs were over 10 years old (mean 13.9 years, range 10-17). The majority of dogs were small to medium-sized breeds. Female dogs accounted for 50% of the population. The number of CMBs detected in each dog varied (mean 14.6, ranged 1-53), and ≥10 CMBs were identified in 43% of dogs. Regarding the CMBs distribution, no CMBs were detected in the vestibular nuclei or cerebellar area associated with vestibular signs. In two dogs, CMBs were identified in the thalamus. Interestingly, compared to the rest of population, the CMBs number were relatively high in these two dogs, and one dog with 3/28 CMBs detected in the thalamus while the other dog having 1/53 CMBs in the thalamus. Our results indicate, although presented in low percentage of dogs, CMBs potentially can contribute to the acute unilateral vestibular signs in dogs, mimicking the clinical presentation of idiopathic vestibular disease. However, large sample size is warranted to further support this finding.

Keywords: cerebral microbleeds, SWI, vestibular signs, idiopathic vestibular disease

A9

Atypical Magnetic Resonance Imaging Findings of Thoracolumbar Intervertebral Disc Herniation in Dogs

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Intervertebral disc herniation (IVDH) is a common spinal cord disease in dogs. The degenerated herniated disc materials typically present as hypointensity on T1-weighted and T2-weighted magnetic resonance (MR) images. However, over the past few years, several dogs with IVDH presenting atypical MR appearance were seen. The aim of this study is to describe the clinical characteristics and functional outcome in dogs with IVDH of atypical MR characteristics. Medical records of the National Taiwan University Veterinary Hospital were searched. Dog with surgically confirmed thoracolumbar IVDH affecting T3-L3 spinal cord segments and the herniated disc materials showing iso- or hyperintensity on T1-weighted and T2-weighted MR images were included in the study. The signalment, pre- and post-operative neurological function, site of IVDH, recovery outcome, and recovery days were recorded. A grading system was applied to describe the neurological function. The recovery outcome was defined as successful if a non-ambulatory patient regaining the ability to ambulate or if an ambulatory patient improving at least by one grade. The association between MR characteristics and outcome was analyzed. Out of 91 dogs with surgically confirmed thoracolumbar IVDH, 32 dogs with atypical MR appearance were discovered. Dachshund was the most common breed. The pre-operative neurological signs ranged from pelvic-limb ataxia to paraplegia with absent pain perception. The majority of dogs (84%) presented as in the non-ambulatory status, and 6% of dogs presented as paraplegia with absent pain perception. The most common site for IVDH was L1/L2 disc space (34%), followed by L2/L3 (31%). In one third of dogs, the herniated disc materials showed hyperintensity on T1-weighted image, while in the majority of dogs (94%), the disc materials were hyperintense on T2-weighted image. Successful outcome was recorded in 78% of dogs (25/32), and the median recovery days was 20 days (range 2-171 days). In the 22% of dogs with unsuccessful outcome, the follow-up time in the majority of dogs was short (median 20 days, range 12-635 days), indicating the long-term outcome potentially can be different from our results. The MR characteristics of herniated disc materials showing “T1 hyperintensity” or “strong contrast enhancement” was neither associated with pre-operative neurological grading nor the functional outcome. In two dogs, the pathological examination confirmed the chondroid metaplasia of the nucleus pulposus. In addition, no obvious calcification or hemorrhage was observed. Although the reason for the IVDH showing atypical MR appearance remains uncertain, this study demonstrated the importance to recognize IVDH as one differential diagnosis when extra-dural materials with T1 and T2 iso- to hyperintensity is observed on MR examination. The clinical characteristics and functional outcome of this type of IVDH seemed similar to typical IVDH. However, further study is warranted to confirm these findings.

Keywords: canine, intervertebral disc degeneration, T1-weighted, T2-weight, isointensity, hyperintensity

A10

18β-Glycyrrhetinic Acid Protected Against Cholestatic Liver Injury in Rats

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Cholestasis is one type of liver disease characterized by the imbalance between bile acid synthesis and secretion with a result of hepatic retention. Bile duct ligation (BDL) in rodents has been generated to cause extrahepatic cholestasis providing *in vivo* model for the investigation of pathogenic mechanisms and identification of therapeutic candidates. The clinical values of medicinal plants and natural extracts are increasingly focused. Those become an alternative strategy for the treatment of diseases. Studies also point out the importance of medicinal plants as sources of developing therapeutic drugs. Licorice displays multiple biological activities, including antioxidant, antiinflammation, anticancer, antidiabetes, and antilipidemia. It has also been implicated in the treatment of infants with biliary atresia. The hepatoprotective effects of Licorice Glycyrrhizin metabolite 18β-Glycyrrhetinic acid have been demonstrated in drug-induced cholestasis. In this study, the hepatoprotective effects and action mechanisms of 18β-Glycyrrhetinic acid centered on BDL-induced cholestasis were the focuses. Adult male Sprague-Dawley rats were allocated into four groups: Sham with Saline, BDL with Saline, Sham with 18β-Glycyrrhetinic acid, and BDL with 18β-Glycyrrhetinic acid. Rats were orall administrated with 18β-Glycyrrhetinic acid or Saline once daily for 4 weeks. BDL operation was conducted 1 week after the administration. Cholestatic rats displayed increased serum level of AST, ALT, total bile acids, total cholesterol, hepatocyte death, bile duct proliferation, hepatic extracellular matrix protein accumulation, hepatic fibrosis, hepatic α-smooth muscle actin (α-SMA)-positivity, hepatic immune cell infiltration, hepatic lipid peroxidation, and hepatic cytokine production. Oral 18β-Glycyrrhetinic acid alleviated cholestatic liver injury. Current findings indicate that 18β-Glycyrrhetinic acid could be of potential to be developed as candidate drug for the treatment of cholestatic liver injury.

Keywords: Bile acids; Cholestatic liver injury; Natural products

A11

Effect of *Antrodia Cinnamomea* Fruiting Body Ethanolic Extracts in Mice

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Antrodia cinnamomea, a Taiwan-specific mushroom, has been reported to have numerous biological activities including hepatoprotection, anti-inflammation, antihepatitis C virus activity, and anticancer activity. Different culture and extract method affect components in *Antrodia cinamoea* fruiting body extract (ACE). In our previous 7-day feeding study in rats revealed that hypertrophy and lipidosis in the adrenal glands and ovaries were found in the ACE treatment group. On the contrary, no significant toxic change was found in adrenal glands, ovaries and other organs in hamsters, even in 28-day feeding study. Previous study reveals that the effect of ACE differs in species. Still, the effect of ACE in mice is unknown. The aim of this study is to compare the effect induced by ACE among different strains of mice and elucidate the possible mechanisms. In our ACE feeding toxicity study, liver necrosis and chronic progressive nephropathy (CPN) were found in ICR and BALB/c mice by oral gavage 2 and 4 g/kg bw ACE for 7 days. There's no lesion in C57BL/6 mice after feeding ACE for 7 days, but same lesion can be found in 28-day feeding toxicology study. For excluding the possibility of sample contamination, mycotoxin analysis (include aflatoxin B1, ochratoxin A, citrinin) and microbiological examination (include *Salmonella*, *Clostridium piliforme*, mouse hepatitis virus) were detected All of the results were negative. According to the result, we suppose that among strains of mice, the expression of biotransformation enzymes in liver is varied. Further studies are needed to elucidate for the possible toxic mechanisms in mice.

Keywords: *Antrodia cinamoea,* ethanolic extracts, high dose, liver necrosis, nephropathy, mice

A12

Doxepin Leads to Obesity and Glucose Intolerance in Fat-Rich Diet–Fed Mice

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Metabolic syndromes is involved in the development of cardiac disease, diabetes, cerebrovascular disease, and kidney disease. Doxepin is a tricyclic antidepressant for treating major depressive disorder—may have positive effects on blood glucose levels and obesity. In our executed study, we treated male high-fat diet (HFD)-fed C57BL/6J mice with doxepin (5 mg/kg/day doxepin) for 6 weeks to understand the effect of doxepin on metabolic parameters, insulin profiles, glucose metabolisms and obesity changes. We noted these mice to exhibit high serum insulin levels, daily food efficiency, body weight, hepatic triglyceride levels, serum serotonin levels, liver, retroperitoneal and epididymal fat pad weight, and fatty acid regulation marker expression when compared with their counterparts (i.e., HFD-fed control mice). Furthermore, doxepin-treated revealed a marked increase in fatty liver scores and average fat cell size of retroperitoneal white adipose tissue and epididymal white adipose tissue, paralleling the decreasing activation of Akt (protein kinase B) and GLUT4 (glucose transporter 4) expression. Notably, the treated mice showed higher glucose tolerance and blood glucose levels but lower GLUT4 expression. In conclusion, doxepin may result in exacerbation of diabetes syndrome with glucose intolerance. These studies will provide a basis for the subsequent research of doxepin for obesity and diabetes, in order to solve the side effects caused obesity by psychosis in future.

Keywords: glucose tolerance, insulin, diabetes, doxepin, obesity

A13

Correlation of Blood Lead Concentration and Hair Lead concentration in Asiatic Black Bears (Ursus thibetanus)

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Lead (Pb) as a trace metal vital to the body, is a notable pollutant while overexposed which could result in various disorders in human and animals. Due to the effect biomagnification, larger animals are more prone to toxin accumulation in their body. Which means that Formosan black bears (*Ursus thibetanus Formosanus*) subspecies of Asiatic black bear, the largest wild carnivores in Taiwan, may face higher risk of lead toxicity. Animal hair has been used as bioindicator material for trace-metal contamination due to potential stress/anesthetic free collection process for wild animals and hair can be stored at ambient temperature for long periods of time without degradation, making them suitable for long-term and consecutive studies. The aim of the study was to discuss the potential of using hair as a bioindicator in the monitoring of lead toxicity in Asiatic Black Bears. 12 wild Formosan black bears and 11 captive Asiatic black bears (7Formosan black bears and 4 Asiatic black bears) were included in the present study. Blood samples were collected from all of the bears and hair samples were collected from 16 bears. Both blood and hair samples were submitted for ICP-MS test to determine Pb concentration. The outcome revealed that blood lead concentration in wild bears is significantly ($p=0.029$) higher than those in captive bears. Besides, a significant ($p=0.003$) positive correlation ($R^2=0.698$) was found between blood and hair lead concentration. These results indicated that wild Asiatic black bears in Taiwan might at greater risk of Pb poisoning than captive ones, and suggested that hair could be a potent biomarker for evaluating status of Pb toxicity in Asiatic black bears.

Keywords: Asiatic black bears (*Ursus thibetanus*), lead, hair

A14

Antimicrobial Resistance Profile of *Vibrio* Species from Stranding Sea Turtles in Taiwan

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Sea turtles are considered to be an indicator species which plays an important role in assessing the health of marine environment. *Vibrio* species is the most common Gram-negative bacteria in seawater, and some of them are pathogenic to sea turtles. The *Vibrio* sp. from wild sea turtles in Taiwan have found to be resistant to antibiotics, meaning that were exposed to risk of marine pollution, which may threaten the health of sea turtles. Fifty-one isolates of nasal cavity and cloaca were collected from 29 sea turtles including 22 *Chelonia mydas*, 2 *Lepidochelys olivacea*, and 5 *Eretmochelys imbricata* from 2018 to 2021 in this study. Polymerase chain reaction (PCR) was used to confirm the identities of a total of 51 *Vibrio* species using the genus-specific and species-specific primers and antimicrobial susceptibility testing was carried out by a standard disc diffusion method. Of these isolates, *V. harveyi* was the most dominant isolates (18/51, 35.29%) and follow by *V. alginolyticus* (15/51, 29.41%). The susceptibility profiles of 17 antimicrobial agents on the isolates revealed a high level of resistance for spiramycin, cefuroxime, amoxicillin, piperacillin, and amoxicillin and clavulanate. The *Vibrio* isolates of cloaca were more resistance to antimicrobial agents than nasal cavity. The occurrence of antimicrobial multiresistance (resistant to at least one drug in≥3 antimicrobial classes) patterns was observed in 92.15% of *Vibrio* isolates. This study aims to understand the antimicrobial resistance of *Vibrio* sp. from wild sea turtles in Taiwan, the results provide a reference for turtle medication and improves the medical quality of stranded sea turtles. Additionally, the antimicrobial-resistant *Vibrio* strains could be transmitted through the water environment to other aquatic animals and therefore constitutes a risk to marine pollution.

Keywords: sea turtles, *Vibrio* species, antimicrobial resistance, marine pollution

A15

Transcriptome Analysis of *Chelonia Mydas* with Fibropapillomatosis

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Fibropapillomatosis (FP) of green turtle (*Chelonia mydas*) is a tumor-forming disease. FP has been reported worldwide, threatening the survival of sea turtles. Tumors exists on body surface and visceral organs, impeded foraging and reduced visceral function of green turtles, resulting death from infirmity. So far, the etiology of FP has not been elucidated. Previous research generally considers the occurrence of FP is related to a type of alphaherpesvirus, Chelonia herpesvirus (ChHV5), associated with self-immunization of green turtle and also environmental factors such as water quality and ultraviolet rays. Our research analyses two blood samples of green turtles with and without FP provided by National Museum of Marine Biology and Aquarium (NMMBA) using de novo transcriptome assembly. In comparing the genetic expression, genes *Ankrd17* and *Hnrnpl* were highly expressed in blood sample from green turtle with FP. Pathways in cancer and the herpes simplex virus 1 infection exist in both sample of tumour and non-tumour green turtles but the two pathways were more enrich in blood sample from green turtle with FP. Real-time quantitative polymerase chain reaction (RT-*q*PCR) is used to detect the presence of highly expressed genes in blood sample from green turtle with FP. This study aims to deduce the possible pathogenesis of FP and hope to develop a way to help early identification of green turtles' population which may have higher risk of developing FP.

Keywords: *Chelonia mydas,* fibropapillomatosis, de no transcriptome assembly, Chelonia herpesvirus 5

A16

Measuring Acoustic Activity and Skin Cortisol as A Method to Evaluate Welfare in Captive Beluga Whales (*Delphinapterus leucas*)

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With the broadening of focus from animals on farms to zoos and aquaria, the field of welfare science and public concern for animal welfare grow nowadays. In captive animals, stress and its causes are topic of interest in welfare issues. Previous studies have showed that intrinsic and extrinsic stressors can cause psychological and physiological impacts to many mammals. Because stress could reflect the adverse effect in an animal's welfare, an objective method to assess animals' stress will be essential. Using both behavioral and physiological parameters as indicators to assess animal welfare quantitatively, rather than simply qualitatively, should be more valid indicator of animal welfare than behavioral measures alone and will prevent the occurrence of the bias cause by attribution. To validate this approach, acoustic activity and skin cortisol concentration have used to evaluate the animal welfare in captive beluga whales (*Delphinapterus leucas*) in Taiwan. The acoustic activity (5 min per hour) of three captive beluga whales has been recorded by transducer routinely, and are analyzed using audio editing software (Kaleidoscope, Wildlife Acoustics). The scrape skin samples have been collected non-invasively twice every week from all three animals. All animals had received pre-sampling training in order to collect scrape samples without or reducing potential stress. Cortisol was extracted using a modified skin steroid extraction technique delineated in previous study, and detected via commercially available enzyme immunoassays. The changes of acoustic activity and cortisol levels during specific events provided evidence that these two measurements could be applied for evaluating animal welfare in captive beluga whales. Using these two non-or less-invasive method simultaneously may contribute to identify and mitigate extrinsic stressors and improve management and husbandry for maintain the welfare in captive cetaceans.

Keywords: cetaceans, cortisol, stress, non-invasive

A17

Development and Evaluation of a Field-deployable Duplex Insulated Isothermal PCR for the Detection of *Toxoplasma gondii* in Stranded Cetaceans

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Toxoplasmosis is a zoonotic disease with veterinary and public health importance worldwide. *Toxoplasma gondii* (*T. gondii*) infection in cetaceans is an indicator of land-based biological pollution. Due to the fact that *T. gondii* diagnoses are often carried out in professional laboratories with non-movable equipment, many case reports were mainly from several countries. For facilitating the global surveillance of *T. gondii* infection in stranded cetaceans, we developed a field-deployable duplex insulated isothermal PCR (iiPCR) with automated magnetic bead-based DNA extraction for the detection of *T. gondii* in stranded cetaceans. It targets the B1 gene of *T. gondii* combined with β2-microglobulin (B2M) gene of cetaceans as an internal control. Compared with conventional real-time PCR assays, B1/B2M iiPCR assay showed comparable clinical sensitivity (770 copies in 25 mg tissue) for the detection of synthetic spike-in standards of *T. gondii* DNA in cerebrum, cerebellum, skeletal muscle samples. The B1/B2M iiPCR assay coupled with a field-deployable system provides a prompt (~1.5 h), feasible, highly sensitive and specific on-site diagnostic tool for *T. gondii* in stranded cetaceans, providing one approach to evaluating aquatic ecosystem health and early warnings about negative impacts on human and marine animals.

Keywords: *Toxoplasma gondii,* stranded cetacean, insulated isothermal PCR

A18

Cutaneous Conditions and Anthropogenic Scars in Chinese White Dolphin (*Sousa chinensis*) in Taiwan

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The Chinese White Dolphin (*Sousa Chinensis*) residing at the west coast of Taiwan is prone to human-induced harms. Direct human-induced harms, including entanglement and ship strikes, are mainly the result of fishing activities. The direct human-induced harms are susceptible to change the physiological status and behaviours of the dolphins immediately while indirect human-induced harms promote long-term effects towards individuals and population health. We analyzed the photos and categorized different conditions from boat observations of S. *chinensis* in Changhua in 2018 and 2019. The identities of the observed individuals are further analyzed, with 9% of calves, 72% of juveniles/ subadults and 19% of adults. The percentage of skin condition notations are higher in elder stages and that scar-bearing individuals occupied more than 80% of all age stages. Of all the scars, entanglement is the most common type of direct harm towards the dolphins. As for skin lesions, nodules, orange film and hypertrophic scar are the dominant skin problems. More than half of the individuals bear at least one skin lesion, indicating a negative impact on their immune function due to environmental stress.

Keywords: *Sousa chinensis*, skin condition, anthropogenic scars

A19

Evaluating Profective Efficacy of Four Heat-Shock Proteins as Recombinant Vaccine Against Photobacteriosis in Asian Seabass (*Lates calcarifer*)

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Photobacterium damselae subsp. *piscicida* (*Phdp*) is one of the major bacterial pathogens to aquaculture worldwide due to its wide host range, high mortality rate and ubiquitous distribution. Vaccination is the most useful and environmetally friendly to prevent the disease outbreak. In the current study, four HSPs of *Phdp*, including HSP90, HSP33, HSP70 and DnaJ were selected for developing recombinant proteins vaccine and the immunoprotective effects of these proteins against photobacteriosis were investigated in Asian seabass (*Lates calcarifer*) model. The fish immunized with different rHSPs resulted with 48.28%, 62,07%, 51,72%, and 31,03% relative percent survival, respectively. The high expression level of immune-related genes and antibody titer were observed in rHSP33 group. Taken together, our results suggested that HSP33 is a potential candidate for vaccine development against *Phdp* infection.

Keywords: *Photobacterium damselae* subsp. *piscicida*, Heat-shock proteins, recombinant proteins.

A20

Recombinant Protein Preparation and Functional Analysis of Grouper Interleukin-6 Receptor

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Interleukin-6 receptor (IL-6R) is the cytokine receptor of interleukin-6 (IL-6). In human medical research, IL-6 is considered to be an important cytokine related to innate and adaptive immune response. In the process of function, IL-6 firstly binds with IL-6R and the signaling molecule glycoprotein 130 (gp130) to form a hexamer, and then transmits signal into nucleus through the Janus kinase/signal transducer and activator (JAK-STAT) pathway to activate transcription of target genes. In addition, IL-6 is generally considered to be a pro-inflammation factor, but excessive inflammation may cause many diseases. Therefore, IL-6-related drugs are also being developed. For example, the immunosuppressive drug Tocilizumab can block IL-6 and IL-6R to combine and form the complex, it is widely used in the treatment of rheumatoid arthritis (RA). However, research of IL-6 is relatively insufficient in the fields of aquaculture and veterinary medicine compared with human medicine, including the research of completed gene cloning or function analysis, so it is necessary to study it. Our laboratory has previously successfully cloned the full-length gene sequences of *il-6*, *il-6r* and *gp130* extracellular domain of *Epinephelus coioides*. Therefore, this study prepared recombinant protein of IL-6R and try to analyze its functions, such as protein-protein interactions. Hope it can help evaluate signaling transduction in the future.

Keywords: interleukin-6, interleukin-6 receptor, glycoprotein 130, protein-protein interactions, *Epinephelus coioides*

A21

Exploring the Link between Cathepsin L and Immune System in the Orange-Spotted Grouper

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Cathepsin L (CTSL) is a lysosomal endopeptidase and ubiquitously present in the cytosol, nucleus, and extracellular matrix. CTSL participate in physiological and pathological processes by degrading proteins. The function of CTSL has been widely understood in mammal, but it is ambiguous in teleost. Since the function of CTSL is highly relative to its enzymatic activity, in order to understanding the role of CTSL in teleost immune system, we try to utilized two of recombinant CTSL base on the *Epinephelus coioides* (orange-spotted grouper) *ctsl* in this study. rCTSL was a complete protein precursor which include pro-peptide and catalytic domain, and rTCTSL was a truncated protein which loosed pro-peptide domain but retained catalytic domain. In the enzymatic activity assay, rCTSL has enzymatic activity but rTCTSL without it. The enzymatic activity of rCTSL was further analysis in various pH, and time, the results shown the maximal bioactivity of rCTSL was at pH 4, and at 45 °C, however, the bioactivity was detected at 25-55 °C. The result implied the structure of pro-peptide paly crucial role in CTSL catalytic activity. The *in vitro* bioactivity was measured by immune relative gene expression in quantitative real-time PCR (qPCR) under CTSL with rCTSL and without rTCTSL bioactivity. Pro-inflammatory molecular genes shown significantly increased at 6 h post-injection in head kidney and trunk kidney. The result indicated CTSL might participate involve in innate immunity especially their enzymatic activity might enhance the immune responses in immune-related organ of grouper.

Keywords: *Epinephelus coioides*, Cathepsin L, bioactivity

A22

Determination of Optimal Doses and Serum Minimum Effective Concentrations of Tricaine Methanesulfonate, 2-Phenoxyethanol, and Eugenol for Experimental Procedures in Nile Tilapia (*Oreochromis niloticus*)

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Anesthetics are frequently used as an efficient tool to prevent injuries, limit stress reactions and to promote welfare during handling procedures in aquaculture. However, limited studies exist on the efficacy of commonly used anesthetics tricaine methanesulfonate (MS-222), 2-phenoxyethanol (2-PE), and eugenol (EUG) in adult fish for handling purposes and the serum minimum effective concentrations (MECs) of each anesthetic was not known. The present study aimed to determine the optimal doses and MECs of MS-222, 2-PE, and EUG for handling procedures in adult Nile tilapia (400-600 g) cultured at 28°C. The fish were immersed in 3 different doses of each anesthetic and the minimal dose that could induce stage III anesthesia within 5 min, maintain anesthesia status for 3 min, and recover within 5 min (the criteria) was considered the optimal dose. The serum concentration of anesthetic immediately after the fish reached stage III anesthesia was defined as the MEC. The results revealed that the optimal doses of MS-222, 2-PE, and EUG in adult Nile tilapia cultured at 28°C were 300, 900, and 90 ppm, respectively. The MECs were similar across the 3 different doses, the average MECs of MS-222, 2-PE, and EUG were 70 ± 5, 263 ± 22, and 53 ± 4 μg/mL, respectively. The results demonstrated that all 3 anesthetic agents were capable to reach the anesthetic criteria for handling purposes in adult Nile tilapia, the most effective was EUG, working at a concentration 3-fold lower than MS-222 and 10-fold lower than 2-PE.

Keywords: anesthetics, MS-222, 2-phenoxyethanol, eugenol, Nile tilapia

A23

The Effects of Multiple Exposure to Tricaine Methanesulfonate, 2-Phenoxyethanol, or Eugenol on the Pharmacokinetics of Florfenicol in Nile Tilapia (*Oreochromis niloticus*) Following Single Oral Administration

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Anesthetics are used to mitigate physiological and biochemical disturbances during handling procedures in aquaculture, but itself can also induce effects which distort physiological and biochemical measurements integral to the experimental design. To date, few studies describing the influence of anesthesia on the pharmacokinetic (PK) properties of other agents are available. The objectives of the study were to evaluate the effects of multiple exposure to tricaine methanesulfonate (MS-222), 2-phenoxyethanol (2-PE), or eugenol (EUG) on the PK of florfenicol (FF) in Nile tilapia at 28°C. The fish were repeatedly exposed to 300 ppm MS-222, 900 ppm 2-PE, or 90 ppm EUG at prior to FF administration (15 mg/kg via single oral gavage) and each successive blood sampling. The serum concentrations of FF were analyzed by HPLC-UV method and the PK parameters of FF in each group were determined by 2-compartmental model and compared to the control group (without anesthetic). The results revealed that multiple exposures of the fish to MS-222 had no significant influence on the PK of FF. In contrast, the elimination half-life of 2-PE (9.73 h) and EUG groups (9.80 h) were significantly longer than the control (8.65 h). In addition, the absorption, distribution half-lives, and time to reach the maximum serum concentration (T_{max}) of the 2-PE group (0.31, 0.36, and 0.61 h, respectively) were significantly shorter than those of the control group (0.42, 0.49, and 0.92 h, respectively). Other major PK parameters including the serum concentration-time curve, volume of distribution, and clearance did not show statistical differences among the 4 groups. Based on these results, cautious should be made when use anesthetics for PK studies. Nevertheless, these PK alterations seem to post little importance from a clinical point of view. Therefore, it is advisable that researchers take into considerations the necessity and overall benefits before using anesthetics in the experimental protocol.

Keywords: anesthetics, MS-222, 2-phenoxyethanol, eugenol, florfenicol, Nile tilapia, pharmacokinetics

A24

Emergence of *Lactococcus Garvieae* in Taiwanese Open Water Cage Culture Systems

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This is the first research that successfully identifies a strain of *Lactococcus garvieae* in the populations of submerged cage-cultured cobia (*Rachycentron canadum*) in Taiwan. Affected fish displayed clinical signs like hemorrhagic eye and lethargy with acute mortality. Extensive degenerative and inflammatory changes in the liver, spleen, kidney and brain were also observed histopathologically. Four isolates recovered from liver, spleen, head kidney, posterior kidney, brain and muscle of cobia were all found to be gram-positive, non-motile, ovoid cocci, short-chain forming and α-haemolytic. The polymerase chain reaction assay for species-specific primers (pLG) and the internal transcribed spacer (ITS) region (G1&L1 primers; 430bp) confirmed all four isolates as *L. garvieae*. Transmission electron microscopy (TEM) observation of one representative *L. garvieae* isolate (AOD109191) and the multiplex PCR results did not reveal the presence of capsular gene cluster (CGC), thus categorizing it as KG+ phenotype. Capsule staining and TEM observations confirmed presence of hyaluronic acid like capsule, a possible virulence factor in KG+ phenotype *L. garvieae* isolates. Genetic homogeneity and geographic dissimilarity of all four isolates were assessed with fingerprinting methods. The pathogenic potential of representative isolate (AOD109191) was assessed through an intraperitoneal injection challenges in cobia (*Rachycentron canadum*) and tilapia (*Oreochromis niloticus*). The gross lesions and histopathological changes found in experimentally infected cobia and tilapia were similar to those seen in naturally infected fish. This confirms that *L. garvieae* induced “warm water lactococcosis” can cause outbreaks of diseases in cobia.

Keywords: cobia, *Rachycentron canadum*, *Lactococcosis, Lactococcous garvieae*, Capsule staining

A25

Epidemiological Validation of Fish Pathogenic *Lactococcus Garvieae* by Fingerprinting and Electron Microscopic Analysis

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Lactococcus garvieae is the etiological agent of Lactococcosis, an evolving disease affecting many fish species and causing significant economic losses worldwide, affecting both fish species in fresh water and salt water. Understanding pathogen relatedness is essential for determining the epidemiology of *L. garvieae* infections and aiding in the design of rational pathogen control methods. The primary goal of this study was to establish the relation between genotypic phenotypic and epidemiological associations. A comparative analysis of molecular typing is a method of choice for evaluating the molecular relationship of isolates for epidemiological investigation. In the present study, we used 45 *L. garvieae* isolates obtained from different farmed fish for phenotypic and genotypic analysis. The genotyping using *L. garvieae* isolates through DNA-based molecular methodologies include pulsed-field gel electrophoresis (PFGE). The macrofragment digestion of DNA using *ApaI* and *SmaI* restriction enzyme showed 15–22 resolvable bands ranging from 2 to 194 kb in PFGE. Taiwanese isolates were grouped into one clusters, where the three Japanese strain were of different cluster. The Electron microscopy observation of selected representative isolates of *L. garvieae* showed the presence of membrane vesicle like structure on the surface and varied stress response to variety of hostile environments was observed. This study provides epidemiological data of *L. garvieae* infections in farmed fish in Taiwan, and illustrating implications vaccination and infection mechanisms, which is necessary to develop comprehensive prevention and control strategies for the disease.

Keywords: *Lactococcosis, Lactococcous garvieae*, PFGE

A26

Immunostimulant binding with lipopolysaccaride test by ELISA method and protection effect against *Vibrio parahaemolyticus* challenged in *Litopenaeus vannamei* by adding β-Glucan, Montmorillonite and Sodium Alginate in feed.

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The immunostimulants of β-Glucan (βG), Montmorillonite (MMT) and Sodium Alginate (SA) were used to binding with lipopolysaccharide (LPS) test by enzyme-linked immunosorbent assay (ELISA) method. The results indicated that the optimal bound concentrations were shown 10 μg/mL of LPS could be bound with 1000 μg/mL, 500 μg/mL and 250 μg/mL of βG, 250 μg/mL of MMT with 2.5 μg/mL of LPS also 500 μg/mL of SA with 10 μg/mL of LPS, respectively. The study of dietary βG, MMT and SA on the growth and survival rate for challenged with *Vibrio parahaemolyticus* (*Vph)* in *Litopenaeus vannamei (Lv*) were assessed. Collect of healthy shrimps were cultivate for grouping with ten shrimps each tank in triplicated (negative control, positive control and treatment groups). The treated *Lv* showed significant different ($P < .05$) improvement lower of feed conversion ratio (FCR) D1(MMT 20 g/kg) 5.66 ± 0.98 compared to negative control *Lv* 7.57 ± 0.98. Higher of weight gain in D1 (MMT 20 g/kg) 3.24 ± 0.32 g compared to negative control *Lv* 2.44 ± 0.32 g significant different ($P < .05$). Survival rate (100%) of βG, MMT and SA fed groups were shown significant different ($P < .05$) compared to positive control *Lv* (50 %) at 5% significant level. *Vph* experiment resulted in 50% mortality for control positive groups *Lv* were totally protected with resulted in 100% survival against *Vph* and examined by polymerase chain reaction method. In histopathology study, most of sign by *Vph* infection was increased level of eosinophil cells. In addition, MMT group was significantly better based on all parameters. The higher group was D1 (MMT 20 g/kg) compared with the other groups. In brief, the results of this study were described that dietary immunostimulants of βG, MMT and SA successfully progresses their binding LPS, feed conversion ratio, growth rate, survival rate, intestinal bacterial study and immune parameters study (total hemocyte count and bactericidal activity) could help secures *Lv* against LPS and *Vph* challenged.

Keywords: β-Glucan, montmorillonite, sodium alginate, lipopolysaccaride, *Litopenaeus vannamei, Vibrio parahaemolyticus*

A27

Genotype, Phenotype and Virulence Gene Analyses of *Bacillus Cereus* Group from Chinese Softshell Turtle (*Pelodiscus sinensis*) in Taiwan

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Chinese softshell turtles (*Pelodiscus sinensis*) (CST) are susceptible to infections from bacteria belonging to the *Bacillus cereus* group (Bcg). The Bcg includes several closely related species, two of which, *B. cereus* and *B. thuringiensis*, are pathogens of aquatic animals or insects, however, these two bacteria are usually not discriminated against in clinical diagnostics. In the present study, we collected 57 Bcg isolates obtained from diseased CST from 2016 to 2019 in Kaoshiung and Pingtung, the areas with the most CST farms in Taiwan. All isolates were divided into 4 genotypes with two restriction enzymes *Sma*I and *Not*I in Pulsed-Field Gel Electrophoresis (PFGE) as well as Enterobacterial Repetitive Intergenic Consensus (ERIC) Polymerase Chain Reaction (PCR). Meanwhile, representative isolates from each genotype were subjected to phylogenetic tree analysis using 16S rDNA and *pycA* (pyruvate carboxylase) gene as phylogenetic markers and these isolates were seen to appear in different clades. In addition, PCR was performed targeting six selected virulent genes, four of which were detected in several isolates, including *hblC* of the hemolysin BL (HBL) complex (46/57), *nheA* of the nonhemolytic enterotoxin (NHE) complex (52/57), enterotoxin FM (*entFM*) (57/57) only one isolate was dectected cytotoxin K (*cytK*) (1/57), while two genes, cereulide synthetase gene (*ces*) and cereulide peptide synthase-like gene (*CER*) were not detected in any isolates. Disc diffusion method will be used to perform antibiotic susceptibility tests for antibiotics effectiveness against gram-positive bacteria including amoxicillin, ampicillin, doxytetracycline, oxytetracycline and erythromycin. All test strains show susceptible to doxytetracycline, oxytetracycline and erythromycin, however all strains show resistant to amoxicillin, ampicillin.

Keywords: *Bacillus cereus* group, PFGE, genotyping, epidemiology

A28

Demographic history and population genetic structure of *Anisakis pegreffii* in the cutlassfishes *Trichiurus japonicus* along the coast of mainland China and Taiwan

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Studying the genetic diversity of nematode parasite populations is crucial to understanding insight into the parasite's infection dynamics and inform on parasite phylogeography. Anisakiasis is a zoonotic disease caused by the consumption of infectious the third-stage larvae (L3) of *Anisakis* spp. carried by marine fish. In the present study, a total of 206 mitochondrial DNA sequences (cytochrome c oxidase 2, cox2) was used to study the genetic diversity, genetic structure, and historical demography of twelve *A. pegreffii* populations from *Trichiurus japonicas* in mainland China and Taiwan coastal waters. Two distinct evolutionary lineages of *A. pegreffii* and no significant genealogical branches corresponding to sampling localities, which suggested that the isolation in the marginal seas have shaped the patterns of phylogeographic distribution in the coast of mainland china during the glaciation lower sea levels. Further, pairwise *F*ST values and AMOVA showed that did not indicate any significant genetic differentiation among groups with no relation to the geographic area, which might be attributed to fewer barriers to gene flow as well as large population sizes. The results of mismatch distribution, neutrality test, and Bayesian skyline plot analyses showed that whole populations underwent population expansion during the Pleistocene. Analysis of demographic history revealed that *A. pegreffii* undergoes historical lineage diversification and admixture due to the secondary contact based on ABC analysis. The present research represents the first definitive population structure and demographic history across sampling locations of *A. pegreffi* in inshore waters of mainland China and Taiwan.

Keywords: *Anisakis pegreffii*, DIY-ABC, genetic diversity, mitochondria, *Trichiurus japonicas*

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A29

16S rRNA-Based Metagenomic analysis of the microbial community from Pond culture in Hunei, Kaohsiung, Taiwan

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Pond culture is one of the most critical and useful aquaculture production methods. It provides about 17% of the animal protein consumed by the global population in 2015, and has now become the world largest source of animal protein. Almost all aquaculture industries have outbreaks of diseases caused by pathogenic bacteria, and resulted in huge economic losses. Aquaculture is facing severe challenge to mitigate the adverse effects of intensive aquaculture on the aquaculture environment and increased prevalence of drug resistance, due to the overuse of antibiotics. Silver nanoparticles (AgNPs) as antibacterial agents offer a novel alternative to traditional antibiotics to against viruses and bacteria. In this study, high-throughput sequencing (HTS) technology of V3-V4 16S rRNA amplicons was used to investigate the microbial diversity and community structure of pond culture using silver nanoparticles in Hunei at three stages of aquaculture. The results showed that Cyanobacteria, Actinobacteria, Planctomycetes and Proteobacteria were the most dominant phyla at three stages. We also found the increase in the relative abundances of Cyanobacteria, Planctomycetes and Proteobacteria, and decrease in Actinobacteria at three stages. This research provides new data for microbial community in Taiwan aquaculture.

Keywords: High-throughput sequencing, Aquaculture, Microbiota

A30

Improving the efficacy of *Bovine Herpesvirus 1* subunit vaccine

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Bovine Herpesvirus 1 (BHV-1) causes many serious losses to cattle industry with a variety of clinical diseases. The main sources of infection are the droplets and secretions from the respiratory and reproductive organs. The BHV-1 can establish latency in trigeminal ganglia and similar sites related to the genital tract. Latent BHV-1 can become reactivate under stress state or after corticosteroid treatment. In the past, inactivated vaccines and modified live virus vaccines are used for prevention of BHV-1 infections in cattle. However, the immunization of modified live virus vaccine has the risk to produce latent infections. Inactivated vaccine is safe but poor immune response induction. Recently, subunit vaccines considered to be promising candidates for developing vaccinations against many pathogens, including BHV-1. In this present study, we aim to improve immunization by the combination of the inactivated and subunit vaccine. To prove this hypothesis, subunit vaccine based on glycoprotein gD was produced in the *E. coli* expression system. Inactivated BHV-1 was selected as vaccine additive candidate. The truncated version of glycoprotein gD was cloned into pET-32a(+) vector and then expressed in BL21(DE3) competent cells. The purified protein was collected and confirmed by SDS-PAGE and Western Blot assay. The combination and individual groups were vaccinated on mice model. Neutralizing assay was performed to evaluate the effectiveness of vaccination. Results showed that the highest titer of neutralizing antibodies is in the combination group, compared to individual groups. This revealed that the direction of immune response can be controlled by the addition of BHV-1.

Keywords: *Bovine Herpesvirus 1* (BHV-1), vaccine, immunization

A31

Type I hypersensitivity in Bovine Immune Cells induced by Bluetongue Virus Taiwan isolate BTV2/KM/2003

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Bluetongue (BT) is a fatal infectious hemorrhagic disease in ruminants, and is also listed as class A disease under the Office international des épizooties (OIE) Terrestrial Animal Health Code. Bluetongue virus (BTV) belongs to genus *Orbivirus* of the Reoviridae family, transmitted via *Culicoides* insect vector. Thus far 29 serotypes of BTV have been recognized worldwide, and two strains, BTV2/KM/2003 and BTV12/PT/2003, were isolated in Taiwan. We hypothesized that various BTV strains differ in their ability to induce clinical signs of variable severities. The aim of this study was to compare the interactions between BTVs and bovine immune cells, to demonstrate the strain differences in immunological perspective. The replication of BTV and cytokines expression profile in bovine peripheral blood mononuclear cells (PBMC), monocyte-derived macrophages (MDM) and macrophages reconstituted with autologous lymphocytes (MDM-Lymphocyte) were monitored. The replication curves of BTV2 were similar and without significant difference among PBMC, MDM and MDM-Lymphocyte. In PBMC and MDM-Lymphocyte, the expression of IL-4 mRNA was earlier than the cytokines of innate immunity and cell-mediated immunity (CMI). The IL-4 protein and IgE antibodies in the supernatant indicated that type I hypersensitivity was involved in BTV pathogenesis. However, the IL-1β, TNF-α and IL-12p40 mRNA increased in MDM (no lymphocytes) without inhibition. This result supports that lymphocytes and IL-4 mediated negative feedback in innate immunity and CMI. Noteworthily, the pathogenesis among BTV serotypes was also different, even in the same animal species. BTV2 and BTV12 were used to investigate the differential immunological effects on PBMC. BTV2 preferentially activated IL-4 (T helper 2 pathway) and likely feedback to inhibit the innate immunity. In contrast, BTV12 preferentially activated the innate immunity (TNF-α and IL-1β), with only minimal subsequent IL-4. The nonstructural protein 3 antibody (anti-BTV-NS3) fluorescent signals showed that monocytes in PBMC and MDM were the essential targets of BTV replication. Bioinformatics analysis revealed that the capability to induce IL-4 were attributed to the tip region of the VP2 protein, wherein a higher number of predicted peptide segments on BTVs were positively correlated with the allergy reported in cattle. Synthetic peptides of BTV2-VP2 induced significant IL-4 and IgE within 12–48 h post-infection (hpi) in PBMC, whereas those of BTV12 did not, consistent with the bioinformatics prediction. Bovine PBMCs and synthetic peptides together seem to serve as a good model for pursuing the BTV-induced IL-4 and IgE activity that precedes the development of an allergic reaction. This method may facilitate the study on pathogenesis of other viral proteins and the development of vaccine in the future.

Keywords: bluetongue virus, cytokines, pathogenesis, bioinformatics.

A32

Foot-and-mouth Disease Virus 3A Protein hijacks COPII factors for ER remodeling

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For viral RNA protection and resource gathering, all positive single stranded RNA viruses would modify host organelle for their own replication, which is termed “replication organelle”, RO. Enterovirus might provide the best understood examples of RO formation due to a large number of researches. Though, the core reason has yet to be worked out; it is believed that viral 3A protein should be an initiator and a marker for RO. However, foot-and-mouth disease virus (FMDV) is totally different from enterovirus, although they belong the same family, *Picoranviridae*. For example, enterovirus would hijack COPI factor, GBF1, by viral 3A protein to block secretory pathway, while FMDV was associated with COPII pathway instead. The C-terminus of FMDV 3A was much longer than that in other picornaviruses. FMDV doesn’t require PI4K or PI4P lipid that are essential for other picornaviral RO formation. Therefore, to better understand FMDV RO, deeply exploring FMDV 3A function is undoubtfully demanded. In our studies, we were the first to clearly proved that overexpression of FMDV 3A proteins would modify endoplasmic reticulum into vesicle-like structures, which were highly dynamic and moved along microtubules. Also, the ultrastructure was examined under electron tomography, together with APEX system. Furthermore, the vesicle formation was found to be associated with two unpublished 3A interaction partners, Sar1 and Sec12, both of which were COPII factors. After mapping out the binding sites on 3A for these two interactions and performing knockdown and re-expression assay, we established a theoretical model for answering how FMDV 3A protein modifies ER membrane into vesicles without COPII coat proteins. We presumed it might be the first, the most important, step for FMDV to build up the RO.

Keywords: Foot-and-mouth disease virus, 3A protein, COPII pathway

A33

A Survey of Bacteria Associated with Uterine Infection in Dairy Cows in Taiwan

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On dairy farms, cows and heifer reproductive performance target is to become pregnant in efficient interval period. The most important and frequently cause of prolong recovery after parturition is uterine bacterial infection. The disease has highly associate with high economic loss due to prolong days open, prolong interval from calving to first service, decrease first AI conception rate, decrease pregnancy rate and more cattle culled. Uterine bacterial infection has affected dairy cows in worldwide. The prevalence was range between 10-60%. Bacteria were categorized on the basis of expected pathogenic potential within the uterus. *Trueperella pyogenes* (*T. pyogenes*), *Escherichia coli* (*E. coli*), *Prevotella melaninogenica* (*P. melaninogenica*) and *Fusobacterium necrophorum* (*F. necrophorum*) are correlate with increase more severe clinical case and also known to cause endometrial lesions. In Taiwan, postpartum uterine infection is a common disease in dairy farms. Therefore, the aim of this study is to investigate bacteria associated with uterine infection in dairy cows in Taiwan. Twenty-two vaginal discharges were collected from cows with uterine infection in different counties in Taiwan. *T. pyogenes*, *E. coli*, *P. melaninogenica* and *F. necrophorum* were detected 54.5%, 59.1%, 63.6% and 22.7%, respectively using PCR. The risk for infection with these bacteria in uterine has affected by the time from the hypothesized that *E. coli* is likely among the first bacteria to colonize the intrauterine environment, potentially inducing changes that will favor future colonization by strict and facultative anaerobic bacteria.

Keywords: Uterine infection, dairy cows, Taiwan

A34

Preliminary Investigation of Filaria in *Cervus nippon taiouanus* in Kenting in 2020

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The *Cervus nippon taiouanus* is endemic to Taiwan and is mainly rebreeding bred in Kenting National Park. Filaria is a tiny, thread-like parasite that is spread mainly by the bites of mosquitoes and ticks. It spreads all over the world, causing heavy significant economic losses. The aims of the study is to investigate the infection rate of filaria infection in *Cervus nippon taiouanus*, the type of filaria infection, the value of serum biochemistry and complete blood count after infection. The sample source for this experiment is the *Cervus nippon taiouanus* rebreeding in Kenting National Park. A total of 77 blood samples of *Cervus nippon taiouanus* were collected. The blood smears and wet presses were initially tested and attempted to establish molecular detection and sequencing to confirm the species of blood filariasis. The positive rate of filaria by Blood smear and PCR detection was 0.025% (2/77). There was no significant difference between the value of serum biochemical and complete blood count in positive samples and the negative samples. The DNA sequencing results were compared with the National Center for Biotechnology Information (NCBI) database. The similarity of the comparison result of one positive sample was *Setaria digitata* 92.01%, and the similarity of the comparison result of the other positive sample was *Setaria digitata* 94.71%.

Keywords*: Cervus nippon taiouanus, Setaria digitate*, serum biochemistry

A35

Serological detection of the immune response using a quick dot blot assay

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Vaccination can trigger the immune response to defend against invading organisms and fight infection. Thus, it is important to know population-level exposure and the immunity to against the diseases. Nowadays, there are several assays could detect the antibody titer. Conventional methods such as Neutralization test and Enzyme-linked immunosorbent assay (ELISA). Although, the neutralization test is the more effective and accuracy, it is consuming and require days of waiting time. Hence, we used neutralization test's concept applied on dot blot assay system, which could fast and quick screening large numbers of samples. In this study, we already success serial diluted the BEFV N recombinant protein and optimized the antibody to get the linear regression between neutralization test of virus and signal intensity measurements. The future works will focus on BRSV N recombinant protein and optimize similar conditions. Hope this study could help farmers fast to know the antibody titer and achieve more economic profit in the future.

Keywords*: Antibody titer, Neutralization test, Dot blot*

A36

Gold Nanoparticles Enhance Antigen-Specific IgG_{2a} Production and Induce the Myeloid-Derived Suppressor Cells in Ovalbumin-Sensitized Mice

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Gold nanoparticles (AuNPs) have been extensively applied in drug delivery and cancer immunotherapy due to their biocompatibility as well as antineoplastic biological activity. Previous studies demonstrated that administration of mice with AuNPs mainly accumulated in the spleen and induced the production of inflammatory cytokines. Moreover, AuNPs are distributed widely in the spleen, including B cells, T cells, and myeloid-derived suppressor cells (MDSC). In particular, MDSC is one of the important cells uptake AuNPs. Although the mononuclear phagocytic system is known to play a critical role in the clearance of AuNPs, the interaction of AuNPs with the immune system has not been studied thoroughly. Therefore, my study aimed to investigate the effect of AuNPs on antigen-specific immune responses and the functionality of MDSC from splenocytes in mice sensitized with the T cell-dependent antigen ovalbumin (OVA). Mice were daily administered with AuNP (0.43 mg/kg) or vehicle by intravenous administration for 12 consecutive days. Except for the untreated group, the mice were sensitized with OVA by intraperitoneal injection on day 2 and day 12. After serum samples were collected, the mice were sacrificed on day 13. The serum and spleens were harvested for further experiments. The results revealed that AuNPs treatment significantly increased the spleen index and the population of $CD11b^+Gr1^+$ cells in mice, whereas the number of $CD4^+$, $CD8^+$, and $B220^+$ cells was not altered. Although the serum level of OVA-specific IgM and IgG_1 were not affected, the level of IgG_{2a} was augmented in AuNP-treated mice. AuNP treatment enhanced the production of TNF-α and IL-10 by splenocytes stimulated with lipopolysaccharide (LPS). The production of IFN-γ by splenocytes stimulated with concanavalin A (ConA) or OVA was also enhanced in AuNP-treated mice. The suppressive activity of MDSC isolated from AuNP treated mice on antigen-specific responses was further evaluated in vivo. Extracted splenocytes from AuNP treated mice were injected intravenously into mice with delayed-type hypersensitivity (DTH). DTH reactions represented by the swelling of footpads after the OVA challenge. The degree of footpad swelling in the mice received splenocytes from AuNP treated mice was significantly diminished. Collectively, these results suggested that systemic exposure to AuNPs induced inflammatory responses and enhanced Th1 immunity. In addition, AuNPs induced the elevated population of splenic $CD11b^+Gr\text{-}1^+$ cells with suppressive activity.

Keywords: gold nanoparticles, antigen-specific, myeloid-derived suppressor cells

A37

Molecular Chaperones Hsp90/Cdc37 and TRiC Stabilize Viral Proteins and p17-Modulated Hsp90/Cdc37 Chaperon Machinery to Recruit Nonstructural Protein σNS and Structural Proteins σA and σC and dsRNA to Viral Factory

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Viruses are molecular machines sustained through a life cycle that requires replication within host cells. Here, we first identified that two types of molecular chaperone, heat shock protein 90 (Hsp90) and the eukaryotic chaperonin T-complex protein-1 (TCP-1) ring complex (TRiC), are essential for avian reovirus (ARV) replication by stabilizing viral proteins (p17, σC, σA and σNS). The TRiC chaperonin controls reovirus replication through stabilization of outer and core proteins σC and σA as well as non-structural protein σNS. The non-structural protein p17 of ARV regulates phosphorylation of Cdc37 through casein kinase 2 (CK2), thereby enhancing the formation of Hsp90/Cdc37 complex that is required for stabilization of p17 protein. Furthermore, we discovered that p17 functions in reovirus replication through a mechanism that involves modulation of Hsp90/Cdc37 complex formation to recruit σNS, σC, and to viral factories. Inhibition of Hsp90/Cdc37 chaperon by inhibitors (17-AAG and celastrol) or shRNAs (CK2, Cdc37 and Hsp90) significantly reduced virus yield, suggesting that Hsp90/Cdc37 chaperon functions in virus replication and virus assembly. The expression levels of σC, σA and σNS proteins were increased while co-expression of p17 protein. Our data define a critical function for the ARV p17 protein in maturation of σA , σC and σNS proteins, and virus assembly.

Keywords: Heat sock protein 90, T-complex protein ring complex, and virus assembly

A38

Antiviral efficacy and immunological functions analysis of bovine interferon-α (BoIFN-α) recombinant protein

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Interferon-α (IFN-α), initially discovered in 1957, is a soluble glycoprotein with strong antiviral effects. Based on the cell surface receptors and features, interferons could be divided into different types. Among the type I interferons, IFN-α and IFN-β had the most significant antiviral effects, which include the inhibition of viral replication, and the mediation of the paracrine biological signaling process. Through this process, the neighboring cells were alerted of the viral infection causing the interferon-stimulated genes (ISGs) to be up-regulated and the uninfected cells to enter the antiviral state, thus limiting further viral transmission. In this study, the bovine interferon-α (BoIFN-α) gene was inserted into a vector for an *Escherichia coli* expression system. The production of the recombinant protein was induced using Isopropyl β-d-1-thiogalactopyranoside (IPTG), and the harvested protein was purified by Nickel-NTA chelating column. After purification, the recombinant protein was then analyzed by SDS-PAGE and Western blot. In addition, *in vitro* assays in MDBK cells were performed to determine the optimal dose and treatment time. The result showed that compared with the positive control, the amount of the viral nucleic acids of the group treated with BoIFN-α is significantly lower. Future works would focus on *in vivo* assays to test the effect of intramascular injection of the recombinant BoIFN-α in cattle. Cytokine level variations, including those of protein kinase R (*PKR*) and 2'-5'-oligoadenylate synthetase (*OAS*) in peripheral blood mononuclear spheres (PBMC), of the experimental animals shall be determined by real-time PCR to evaluate the immunological functions of the recombinant protein.

Keywords: Bovine Interferon-α, antiviral effect, *Escherichia coli* expression system

A39

Novel Cancer Vaccine with Induced Antigen Diversity Improves Antitumor Efficacy

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The immune editing theory discussing the interaction between cancer development and host immunity has been investigated for years. Though autologous cancer vaccine in veterinary oncology has been proposed as one of the anticancer strategies, the lack of immunogenicity of self-tumor cells indicates the significance of eliciting efficient immune responses. It is found that tumors could create a tumor microenvironment (TME) to escape from immune surveillance. Hence, in this study, we intend to increase the immunogenicity of cancer vaccine via promoting the diversity of tumor antigens. The murine colon carcinoma cell line (CT26) was inoculated into the severe combined immunodeficiency mice (NOD.CB17-Prkdcscid/NcrCrl). We hypothesize that, without immune attack from SCID mice, the tumors would grow into the more malignant counterparts with increasing expressions of tumor antigens. These tumor cells were then harvested and prepared for the treatment of parental CT26 bearing BALB/c mice. The results showed that this novel cancer vaccine can decline tumor growth and increase T cell infiltrating in tumors. IFN-*r* secretion and cytotoxic ability of immune cells enhanced significantly after stimulation. Together, our data provide evidence that novel cancer vaccine confers desired antitumor responses, which potentially result from its antigen diversity. Our study provides a model of an improved cancer vaccine in patients with colorectal cancers.

Keywords: autologous cancer vaccine, personalized therapy, tumor-associated antigens (TAAs)

A40

Characterization of the Genes That FimY of *Salmonella enterica* Serovar Typhimurium May Regulate

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Type 1 fimbriae are one of the most commonly found adhesive organelles in the *Enterobacteriaceae* family, including *Salmonella* species. This type of fimbriae possesses the ability to mediate mannose-sensitive binding to eukaryotic cells and is an important virulence factor. The *fim* gene cluster of *S.* Typhimurium contains all the genes required for type 1 fimbrial production. The structural genes *fimA*, *fimI*, *fimC*, *fimD*, *fimH*, and *fimF* are all expressed in one transcript from the *fimA* promoter. Expression of the structural genes is regulated by the interplay of three transcription factors, FimY, FimZ, and FimW, one phosphodiesterase Stm0551, and an arginine tRNA *fimU*. FimZ was shown to bind the *fimA* promoter and is the principal activator for type 1 fimbrial production. FimY is also an activator for *fimA* expression like FimZ and it was revealed that the binding region for FimY resides within the *fimZ* promoter. Fimbrial production may need to coordinate with other physiological functions for *S.* Typhimurium survival, for example, non-*fim* gene products Lrp and SirA were shown to affect *fimZ* expression. Since limited information is available regarding what other genes besides *fim* genes that FimY may interact, our study goal aims to focus on this issue. Our preliminary study using RNA sequencing analysis has demonstrated that a *fimY* deleted *S.* Typhimurium exhibited significantly different expressions in those genes that are involved in flagellar assembly, chemotaxis, biofilm formation as well as other types of fimbrial production as compared to its parental strain, which may imply FimY being a multifactorial activator. The *fimY* coding sequence was cloned into the pET101/D/*lacZ* vector and the FimY-His fusion protein was expressed in *E. coli* BL2 strain. This fusion protein was purified and will be used in the following electrophoretic mobility shift assay (EMSA) to confirm the binding ability of FimY to those DNA fragments that possess the genes found in the RNA sequencing. To delineate how FimY interact with other genes could shed some light on the multifactorial role of the this *fim* regulator.

Keywords: *Salmonella* Typhimurium, type 1 fimbriae, FimY, DNA binding protein

A41

Determination of the Genetic Elements and the Environmental Factors That Control the Expression of *fimY* in *Salmonella enterica* Serovar Typhimurium

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Salmonella is a Gram-negative facultatively anaerobic intracellular bacterium and the *subsp. Salmonella enterica* serovar Typhimurium remains one of the leading causes of foodborne illness in human. The ability to adhere to intestinal epithelium cells is considered as one of the complicated pathogenesis mechanisms conferred by *Salmonella* and is a prerequisite step for infection. Type 1 fimbriae (T1F), encoded by the *fim* gene cluster, is one of the most commonly seen adhesive organelles in *Salmonella*. In the *fim* gene cluster, *fimA* encodes for the major subunit of T1F, *fimZ* encodes for a DNA-binding protein which can directly bind to *fimA* promoter region and activate its expression. *fimY* acts as another T1F regulatory gene and FimY can directly active *fimZ* expression and indirectly upregulate *fimA* expression. Previous studies have shown that several genetic elements and environmental factors can regulate *fimZ* expression, while reports regarding the regulation of *fimY* are limited. Elucidation of the type 1 fimbriae regulatory network involving *fimY* could provide an insight to understand the pathogenesis of *Salmonella*. In order to clone the genetic elements that may upregulate the expression of *fimY*, a genomic library containing more than 8,000 colonies was constructed by ligating the partially digested genomic DNA fragments into pACYC184 vector and transformed into *S.* Typhimurium by electroporation. According to Carbon-Clarke equation, in order to increase the possibility of finding the target genetic elements to 90%, more than 5591 colonies were selected and tested for their ability to agglutinate to yeast cells when grown on agar medium. Any transformant that mediates agglutination of yeast cells may indicate the presence of the genetic determinants that promote the expression of type 1 fimbriae, possibly through activation of *fimY* within that cell. Nonetheless, this will be confirmed by RT-PCR. However, all the screened colonies turned out to be yeast agglutination negative so far. As to determine the environmental factors that may influence the expression of *fimY*, a *fimY* promoter-containing DNA fragment was cloned into the pMC1403 vector to construct a *fimY-lacZ* reporter molecule and transformed into *S.* Typhimurium. *β*-galactosidase activity assay of the transformant grown in different culture conditions was performed. Temperature was one factor that may affect *fimY* expression. It was revealed that the *fimY* promoter activity increased as the temperature increased from 25℃, 37℃, to 42℃. In addition, expression of T1F correlated with that of *fimY* expression. The *fimY* expression in an acid or an alkaline environment was also measured and its promoter activity was extremely low in pH=4 as compared to pH=7, while the promoter activity in pH=8 was higher than that in pH=7. Low Mg^{2+} concentration（10μM） environment could not up or down regulate *fimY* expression, the promoter activity was similar to that as observed when *Salmonella* was cultured in the rich media.

Keywords: genomic library, environmental factors, *Salmonella* Thyphimurium, type 1 fimbriae, *fimY*

A42

Development Inactivated vaccine against *Porcine epidemic diarrhea virus (PEDV)* with Flagellin protein adjuvant

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Porcine Epidemic Diarrhea Virus (PEDV) is a cause of highly contagious diseases in all ages of pigs. It was making an economic severe damage in the pig breed industry and meat market. The best way for prevention measure is vaccination. In this study, the inactivated PED virus is used as an antigen combined with the Flagellin protein adjuvant for oral vaccination. Besides any advantages, including low budget price, short research and development time requirement, and easy storage and vaccination. The inactivated vaccine against PED virus problem needed to be solved is low immune effective, it can be improved by adjuvant. Flagellin is a major component from the tail of Salmonella, its can activates Toll-like receptor 5 (TLR5) to activate pro-inflammatory immune responses. In this study, Flagellin protein was used as an adjuvant to enhance the immune effectiveness. Flagellin protein was expressed in *E.coli BL21* expression system and analyzed by SDS-PAGE and Western Blot, used His-taq Antibody. Furthermore, to protect the antigen and Flagellin adjuvant against the digestion on mice's intestine, they were coated with Alginate before vaccination. The immune evaluation to confirm the effective of adjuvant are direct ELISA for IgG and IgA evaluation and neutralized response.

Keywords: *PEDV*, Flagellin, adjuvant, inactivated vaccine, oral vaccination

A43

PED subunit vaccine based on COE fused with flagellin improved specific humoral and mucosal immunity in mice

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Porcine Epidemic Diarrhea (PED) virus is one of several important swine pathogens, which causes significant economic losses to the swine industry. Spike (S) protein, an important targeting antigen is composed of about 1386 amino acids and contains at least four neutralizing domains. Particularly, the ‘‘collagenase equivalent” (COE), is a highly conservative neutralizing domain, which could produce PEDV neutralizing antibodies. Subunit vaccine based-on COE domain becomes a promising candidate against PEDV. In addition, flagellin has emerged as a potent adjuvant. It provided a good adjuvanticity for N-terminal fusion targeting antigen. Therefore, we aim use the N-terminus as a vaccine adjuvant. In this study, the N-terminus of flagellin (nFliC) was linked to a modified core neutralizing epitope (mCOE) (amino acids 500-799). Then, the fusion protein antigen was used for developing subunit vaccine against PEDV.

Keywords*: Porcine epidemic diarrhea virus, COE, Flagellin*

A44

Cross-Sectional Study on the Viral-Dynamic and Genetic Characterization of Porcine Circovirus Type 3 in Taiwan

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Porcine circovirus type 3 (PCV3) is a newly emerging circovirus. In previous studies, PCV3 is related to porcine respiratory disease complex (PRDC). The impact of PCV3 on respiratory symptoms and the dynamic of viremia profiles of PCV3 had been reported in many countries, but there is limited relevant research of PCV3 in Taiwan. The purpose of the present study is to understand the detective rate of PCV3 in cross-sectional pigs, and to understand PCV3 detective rate of nursery and growing pigs with respiratory symptoms and the phylogenic relationship in Taiwan compared with different PCV3 isolates in neighbor countries. The serum samples from 3 to 31 weeks old and sows, and the serum samples from nursery and growing pigs with respiratory signs were collected in Animal Disease Diagnostic Center of National Pingtung University of Science and Technology. All serum samples were screened for PCV3 by using Real-time PCR. The entire sequences from high viral load samples would be analyzed and compared with different PCV3 isolates in different countries. The results of cross-sectional pigs showed PCV3 detective rate was significantly (p<0.05) higher in sow (45/70; 50%) than in pigs below 4 weeks old (9/100; 9%), pigs with 4-12 weeks old (25/260; 9.6%) and pigs over 12 weeks old (77/360; 16.7%). PCV3 virus titer was $10^{0.57}$-$10^{5.46}$ copies number/μL in sow and different aged pigs. No significant differences of PCV3 detective rate and viral load were observed among the cases with respiratory signs in nursery and growing pigs. Taiwanese PCV3 strains share 99.1-99.8% and 98.0-99.7% nucleotide identity in the complete genome and ORF2, respectively, compared with other already known PCV3 strains. PCV3 is widely spread and can infect all aged pigs in Taiwan. Phylogenetic analysis of two PCV3 strains shows a high nucleotide identity with the already known PCV3 strains. PCV3 has less detection rate on respiratory disease in Taiwan.

Keywords: Porcine circovirus type 3, genotype, porcine respiratory disease complex

A45

Possible Mechanism Governing Enhanced Florfenicol Antimicrobial Activity by Efflux Pump Inhibitor Against Resistant Swine *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*

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Previous studies have demonstrated the beneficial effect of efflux pump inhibitor, Carbonyl Cyanide Chlorophenylhydrazone (CCCP) on amphenicols antimicrobial activity against resistant but not susceptible *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* isolates; however, the mechanisms involved were not clear. The purpose of this study was to evaluate whether CCCP can enhance intracellular concentrations of florfenicol (FF) by antagonizing the drug-expelling effect of CCCP, and to explore the possible gene regulating such effect. The intracellular concentration of FF was evaluated by high–performance liquid chromatography on FF sensitive and resistant *A. pleuropneumoniae* and *P. multocida.* The presence of floR gene was detected on 40 *A. pleuropneumoniae* and 41 *P. multocida* swine isolates by Polymerase Chain Reaction. The results indicated that the intracellular concentration of FF increased by 70% and 150% when 4 and 2 μg/mL CCCP was presented for resistant *A. pleuropneumoniae* and *P. multocida,* respectively, but the increase was not observed for sensitive strains. 90% of resistant *A. pleuropneumoniae* isolates and 96% of resistant *P. multocida* isolates carried floR gene. The overall results suggested that the FloR efflux system mediated by the floR gene at least in part contributed to the FF resistance through alteration of intra-bacterial drug concentrations in both bacterial species. The development of combinational amphenicol treatment with efflux pump inhibitor may extend the clinical utility of amphenicols against bacteria developing respective resistance. The involvement of other efflux system is likely and warrants further study.

Keywords: Amphenicols, Mechanism, Carbonyl Cyanide Chlorophenylhydrazone (CCCP), *A. pleuropneumoniae, P. multocida*

A46

In silico typing of *Actinobacillus pleuropneumoniae* using whole-genome sequencing data

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Actinobacillus pleuropneumoniae (App), a causative agent of pleuropneumonia in all ages of pigs, is divided into 19 serovars based on capsular polysaccharides and lipopolysaccharides. Serovars of App isolates are commonly determined by multiplex polymerase chain reaction (mPCR) or serological tests; nevertheless, some clinical App isolates fail to be definitively designated into known serovars. In recent years, whole-genome sequencing (WGS) data of App were comparatively analyzed for sequence similarity of K locus (KL), including capsular polysaccharide synthesis *(cps*) and capsular polysaccharide export (*cpx*) genes. Consequently, App serovars 17 to 19 were proposed, and mPCR assays targeting serovar-specific *cps* genes were modified accordingly to classify serovars 1 to 19. However, current mPCR method is unable to identify new serovars, and to distinguish between serovars 9 and 11, which share high sequence similarities among *cps* gene clusters. As the need for a typing method with higher discriminatory power to detect known or potential new serovars remains, this study aims to develop an *in silico* analysis method for App molecular typing using WGS data generated by Illumina sequencing platforms. Firstly, a KL database was established by compilation of KL reference sequences covering 19 serovars, and optimized for compatibility with Kaptive, a tool for reporting target locus information in genome assembly. Nineteen KL types, KL1 to KL19, were designated to the corresponding serovars based on the reference sequences. Subsequently, WGS reads of 189 App isolates without serovar information were collected from the National Center for Biotechnology Information, USA. These WGS reads were trimmed, *de novo* assembled in to genome assemblies, and the quality of assemblies assessed. A total of 105 genome assemblies passed the quality assessment were subjected to *in silico* molecular typing using Kaptive against the KL database. The analytic results showed that 97 assemblies were classified into corresponding KL type which matched the serovar information recorded in the literature; KL9 and KL11 were assigned successfully to the assemblies of the serovars 9 and 11, respectively. Furthermore, 5 and 1 of the 6 assemblies from previously non-typable isolates were classified as KL17 and KL18, respectively. Collectively, an *in silico* typing method is established to determine accurately the serovars of App isolates based on WGS data. This method can be used to predict serovar and in retrospective analysis of App in the future.

Keywords: *Actinobacillus pleuropneumoniae*, molecular typing, genomic analysis

A47

Comparing Competitive Fitness and Transmissibility of Classical Swine Fever Viruses

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Classical swine fever (CSF) caused by classical swine fever virus (CSFV), is a highly contagious disease in pigs. Currently, the disease is controlled by stamping-out and systemic prophylactic policies. In Taiwan, the attenuated genotype 1.1 (G1.1) lapinized Philippine Coronel vaccine has been used to protect pigs against the CSFV since 1958. There were two genotypes of CSFVs reported in Taiwan. Before 1993, the historical G3.4 CSFV was predominant in Taiwan. However, the emerging G2.1 CSFV caused sporadic outbreaks in 1994 had become the major genotype affecting pigs after 1996. A shift in virus genotypes was also observed in several CSFV endemic countries. The previous studies have shown that the G2.1 CSFV had higher secreted(S)/cell-associated(C) ratio than the G3.4 CSFV in porcine cells and the G2.1 CSFV might secret and replicate more efficiently than that of G3.4 CSFV. For further study, animal experiments were performed for evaluating competitive fitness and viral shedding of G2.1 and G3.4 CSFVs in pigs. To investigate the viral transmissibility, the G2.1 and G3.4 CSFVs coinfected pigs were cohabitated with naïve animals. Our results showed that the serum viral load, peak viremia, oral and fecal viral shedding of G2.1 CSFV was higher than those of G3.4 CSFV in the same coinfected animal. In cohabitation groups, the viral shedding and transmission of G2.1 CSFV was higher than G3.4 CSFV. While no difference in viral load, shedding, and transmission of G2.1 CSFV was noted in all cohabitated animals, absent viral infection and transmission of G3.4 CSFV from the fifth passage G2.1 and G3.4 CSFVs coinfected animals to naïve animals was noted. The present study demonstrates that the replicability and transmissibility of G2.1 are superior to 3.4 CSFV in pigs. Investigation of the mechanism responsible for the genotype switching in the future should be important to plan the best interventions for controlling and eradicating the disease.

Keywords: Classical Swine Fever Virus, genotyping, competitive fitness

A48

Polymorphism Detection in Pigeons Based on Kompetitive Allele-Specific Polymerase Chain Reaction (KASP)

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SNPs that are associated with phenotype variability may be used as genetic markers for selection. In pigeons, SNPs are being detected in order identify individuals with genetic attributes associated with good racing ability. Detection of these SNPs are commonly performed using the polymerase chain reaction – restriction fragment length polymorphism technique (PCR-RFLP), which can be costly, laborious and time consuming. Among recent developments in SNP genotyping techniques, the use of Kompetitive Allele Specific PCR (KASP) is becoming more common. This technology utilizes two allele specific forward primers with additional unique tail sequences that corresponds with a universal FRET (fluorescence resonant energy transfer) cassette. Detection of fluorescent signals would identify the allele. This current research study refers to the development of KASP assays for the genetic polymorphisms in pigeon *MTCYB* genes, which were showed in the literature to be associated with flight ability. In this study, the entire sequence of each of the selected genes was used to design KASP primers. The aim of this research is to establish an accurate and stable KASP detection conditions by comparing the KASP assays results with PCR-RFLP. To date, the temperature of each step of KASP assays has been established, and 107 samples have been successfully classified. Comparison of the PCR-RFLP and KASP assays result showed 100% agreement between the two methods demonstrating the accuracy of the established KASP assay.

Keywords: *Columba livia*, Polymorphism, KASP

A49

A Toll-like Receptor 5 Ligand-Flagellin Combined with *Pasteurella multocida* Lipoprotein E Improved Cross-Protection against Heterologous Lethal Challenge in Mice

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Fowl cholera is a contagious, bacterial disease of domesticated and wild avian species caused by *Pasteurella multocida* (*P. multocida*) infection. The many serotypes of *P. multocida* complicate fowl cholera prevention efforts. Bacterins are currently widely used for vaccination against fowl cholera, but protection is limited to homologous strains. Live attenuated vaccines of *P. multocida* provide some heterologous protection, but side effects are considerable. More recently, a vaccine containing bacterin and recombinant lipoprotein E of *P. multocida* is on the market and affords some levels of cross-protection. As a pathogen-associated molecular pattern (PAMP), bacterial flagellin can activate the immune response and act as a vaccine adjuvant. This study aims to formulate a subunit vaccine that includes *P. multocida* lipoprotein E and flagellin (Toll-like receptor 5 agonist) to enhance cross-protection. We have successfully constructed and cloned the full-length flagellin of Salmonella typhi (fFliC) and lipoprotein E of *Pasterulla multocida* (fplpE) for expression in E. coli. The optimal dose of fplpE was determined by dose-dependent in mice at a low amount of purified rplpE (1 μg). Mice immunized with the fplpE containing fFliC developed high antibody titer (>1:8000 serum dilutions). The survival rates reached 100% by the vaccine group containing fFliC as an adjuvant for the homologous and heterologous challenge with *P. multocida* in mice. The study indicated the potential application of using flagellin as an adjuvant in developing a recombinant subunit vaccine against *P. multocida* infections in mice.

Keywords: *P. multocida*, lipoprotein E, cross-protection, TLR agonist, flagellin.

A50

Establishment of *Pigeon Circovirus* (PiCV) Cell Culture System in Cells Lines

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The *pigeon circovirus* (PiCV) belonging to the genus *Circovirus*, consists of single-stranded spherical DNA (ss-DNA) of approximately 2030 nucleotides (nt). PiCV can be transmitted horizontally and vertically (mainly horizontally). The clinical symptoms of PiCV infection were as follows: growth stagnation of 4 to 12 weeks old pigeons, anorexia, drowsiness and death. PiCV will destroy the immune system of pigeons, causing organ atrophy of the immune system, and can also lead to lymphocyte apoptosis. Pigeons are more vulnerable to other viruses, bacteria and parasites when their lymph system is damaged. PiCV infections are spread worldwide due to pigeons often travel to different country for competition or performance. PiCV is a threat to pigeon's squab and racing pigeons, until now, there is no effective vaccine available against PiCV. According to scientific literatures, there is no available experimental protocol developed to culture PiCV therefore the immunoprophylaxis of this disease is difficult. This study aims to establish an experimental protocol to culture PiCV by using pigeon cell lines, can be used to develop a PiCV vaccine experimental models. At present, we got virus from the diseased pigeon and used pigeon embryo fibroblasts (PEF) cells for virus culture. And then used real-time polymerase chain reaction (RT-PCR) to detect the amount of virus genome. The results show that PiCV can culture in PEF cells. After that, oncogenes have been transfected into PEF cell. The experimental results showed that the cell condition of the experimental group transfected with the oncogene was better than that of the control group.

Keywords: *Pigeon circovirus*、Pigeon Embryo Fibroblasts、PiCV Vaccine

A51

Oncolytic Activity and Immune Checkpoints of an Avian Reovirus in Several Cancer Cell Lines

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Animal viruses have the possibility to avoid pre-existing immunity in humans, while being safe and immunostimulatory. We used an avian reovirus (ARV) and tested it against a panel of carcinoma cells (AGS, B16F10, Hela and A549). We found that ARV replicated well and induced strong cytopathic effects (CPE). The direction of specific immunity is controlled by the specific cytokines. Therefore, the additive of specific cytokines in tumor immune microenvironment (TME) might help to induce an optimized specific immune reaction and enhance the efficacy of avian reovirus oncolytic activity. To prove this hypothesis, grouper TH1, TH2, and TH17 differentiation cytokines, Type I interferons, interleukin-2 and interleukin-6 were measured. Semiquantitative real time PCR was also employed to evaluate the effectiveness of immune checkpoints and Toll-like receptors (TLRs). Results showed that in groupers, Interleukin-12 were activated in all cancer cells. Furthermore, in AGS, an increased DR4 and DR5 were observed under the treatment of ARV. ARV-induced expression of functional TNF-related apoptosis-inducing ligand (TRAIL) on human PBMC. These results indicated that the direction of immune response can be controlled by ARV, further increasing oncolytic activity. It was determined that three mechanisms of cancer cell death are through syncytia formation, resulting in apoptosis, pro-inflammatory cytokines and induction of type I IFNs. We also investigated the effects of p17 and sigma C proteins of ARV against cancer cells. In all cancer cells, an increased TH1 (IFN-γ and interleukin-12) were observed under the transfection of ARV p17. In AGS, an increased DR4 and DR5 were observed under the transfection of ARV sigma C. Therefore, ARV can potentially expand the repertoire of oncolytic viruses for treatment of carcinoma and other malignancies.

Keywords: avian reovirus, p17, sigma C, oncolytic virus, cytokine, cancer, immune checkpoints

Running title : Oncolytic Avian reovirus for cancer therapy

A52

Study of Avian Reovirus p17 Protein-mediated Inhibition of Cancer Cell Migration by Regulating the PTEN/FAK/Src Pathway

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Our research team has demonstrated that the p17 protein of avian reovirus (ARV) can inhibit the nucleoporin Tpr, leading to activate p53 and PTEN and stabilize PTEN by promoting Rak binding to PTEN, thereby inhibiting the PI3K/Akt/mTORC1 pathway. This study further explored whether p17 protein inhibits the migration of different cancer cells (HeLa, A549 and B16-F10). In this study, we show that p17 negatively regulates the nucleoporin Tpr of HeLa, A549, and B16-F10 cancer cell lines, which causes p53 to accumulate in the nucleus and activates PTEN to inhibit cancer cell migration. The ARV p17 protein can inhibit the phosphorylation of FAK Y397. Co-transfection of p17 with PTEN or PTEN C124A mutant was performed to analyze whether the formation of the FAK/Src complex was affected. The results show that p17 protein inhibits the formation of the FAK/Src complex by activating the p53/PTEN pathway, thereby inhibiting cell migration. We further found that the ARV p17 protein can inhibit the expression of TKS5, Rab40b, and the downstream MMP9. The p17 protein was co-transfected with TKS5 or Rab40b shRNAs and the downstream signal was analyzed by Western blot. It was further found that p17 protein significantly inhibited the expression of NCK1 and downstream protein MMP9. Therefore, p17 protein negatively regulates the TKS5/Rab40b complex to affect the expression of MMP9 and inhibit cancer cell invadopodia. Immunofluorescence assay was further used to analyze the effect of p17 protein on invadopodia formation in A549 and HeLa cancer cells. The results confirmed that the ARV p17 protein can inhibit the formation of invadopodia. Co-transfection of pcDNA3.1-p17 and PTEN (C124A) mutant, TKS5 and Rab40b respectively in A549 and HeLa cancer cells, invadopodia production was restored. This study demonstrates that ARV p17 protein inhibits the formation of TKS5/Rab40b complex and the expression of MMP9 through the Src/FAK pathway, thereby inhibiting the formation of cancer cells invadopodia and cancer cell migration.

Keywords: avian reovirus (ARV), cell migration, invadopodia

A53

Characterization of NS1 Isoforms from H5N2 Avian Influenza Viruses

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Influenza virus type A (IAV) is an important pathogen of animals and humans. In particular, the highly pathogenic avian influenza viruses (HPAIVs) have received global attention owing to their public health threats. In the last decade, H6N1 and H5N2 AIVs are circulating in domestic chicken farms and become enzootic in Taiwan. In 2015, three subtypes of HPAIVs (i.e. H5N2, H5N3 and H5N8) devastated our local poultry farms. These outbreaks affected not only chicken, but also water fowls as well as wild birds, which caused severe economic losses. NS1 protein is a virulent factor of IAV by impairing host antiviral responses. According to our previous studies, the NS segments from different Taiwanese H5 viruses significantly influenced viral replication and host cytokine induction. Noticeably, NS1 protein, originated from a H5N2 HPAIV strain (i.e. NS1031), expressed a truncated NS1 isoform. To characterize this NS1 isoform, recombinant viruses expressing full-length only (RG-AIV-T375G), or the truncated isoform (RG-AIV-NS3) were initially generated. Results showed replication of the recombinant RG-AIV-NS3 virus in mammalian cells (e.g. A549 and M1 cells) is significantly efficient than that in chicken DF1 cells. Moreover, the yield of RG-AIV-NS3 progenies is higher than RG-AIV-NS1031 and RG-AIV-T375G in mammalian cells, indicating different NS1 isoforms influenced the viral replication in various host cells. Furthermore, infection of RG-AIV-NS3 virus in DF-1 cells induced a significant higher level of cytokines including type I interferons, MX and TNF-α, than viruses AIV-NS1031 and RG-AIV-T375G. Hence, stimulation of a higher (or failure in suppression of) the innate immune response partially explain the poor production of RG-AIV-NS3 in DF1 cells. In summary, we confirmed NS1 isoforms from avian influenza viruses could influence viral replication efficiency and host range in cell models.

Keywords: Avian influenza virus, H5N2, NS1, cytokines, replciation

A54

Immunohistochemical Identification of The Intestinal Nodule Induced by *Heterakis isolonche* in Golden Pheasants (*Chrysolophus pictus*)

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Heterakis isolonche is pathogen which induce intestinal nodules in golden pheasants (*Chrysolophus pictus*). It cause the birds to lose weight and die. The nature of the intestinal nodule in golden pheasants is controversial. To elucidate it, 10 cases submitted to the Fonghuanggu Bird and Ecology Park, National Museum of Natural Science, between 2018 and 2019, were collected. They were evaluated by immunohistochemistry for the expression of laminin, vimemtin, pan-actin, smoothelin, smooth muscle actin (SMA), periaxin, neuron-specific enolase (NSE), and nerve growth factor receptor (NGFR). In addition, the normal tissues, including epithelium, connective tissue, smooth muscle, myocardium, endothelium, skeletal muscle, peripheral nerve, lymphoid tissue (leukocyte), spinal cord, kidney, cerebrum, and cerebellum, were evaluated by immunohistochemistry for the markers as mentioned above. Vimentin was positive in all cases. Smoothelin, pan-actin, SMA were negative in all cases. 6 cases (6/10; 60.0%) were positive for NGFR. 2 cases (2/10; 20.0%) were positive for laminin. 8 cases (8/10; 80.0%) were positive for periaxin. Based on the result of immunohistochemistry in normal tissures, vimentin, smoothelin, SMA, pan-actin, NSE, and periaxin are well diagnostic markers for identification of the intestinal nodule in golden pheasants. The pathological features of the nodules were found in these collection cases, which were in whorls (10/10; 100.0%), staghorn vessels (4/10; 40.0%), storiform (4/10; 40.0%), bundles (10/10; 100.0%), solid (9/10; 90.0%), myxoid (1/10; 10.0%), and verocay-like (4/10; 40.0%). Tumor cells are well differentiated with low mitotic count. The results of parasite idnetification are all *Heterakis isolonche*. Based on the positive result of vimentin and NSE and negative result of smoothelin, SMA, pan-actin, the nature of the intestinal nodule in golden pheasants is neural origin. Moreover, based on the periaxin positive signals distribution, it is schwannoma or neurofibroma.

Keywords: *Heterakis isolonche*, the intestinal nodule in golden pheasants, immunohistochemistry

A55

Survey of the Genotypes of Parrot Bornavirus in 2020

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Parrot avian bornavirus （PaBV） is a neurotropic virus, which is single molecule of negative-sense, single-stranded RNA virus, about 8,900 bases in size that encode six protein：Helical nucleoside protein, Envelope glycoprotein, Viral polymerase, Phosphoprotein, Matrix protein and unknown protein. PaBV belongs to Famliy *Bornaviridae*, Genus *Orthobornavirus*, species *Psittaciform 1 orthobornavirus* and *Psittaciform 2 orthobornavirus*. There are eight genotypes from PaBV-1 to PaBV-8, of which PaBV-2 and PaBV-4 are the most common genotypes in the world. In Taiwan, PaBV accounts for 33% of parrot viral diseases, which has caused considerable economic losses to the parrot market. PaBV was also an agent of proventricular dilatation disease （PDD）, which causes malnutrition and death. So far, there is only palliative treatment for PDD and no commercially vaccines. In this study, parrot-related samples in 2020 were collected. The virus was purified and detected for the presence of Matrix protein gene by reverse transcription polymerase chain reaction （RT-PCR） detection. Followed by genome sequencing, 7 samples had been analyzed and all samples were PaBV-4. According to the phylogenetic analysis, countries with similar virus strains are divided into two categories, one of which includes Taiwan, Thailand, Canada, Germany, and Hungary; the other includes Taiwan, Japan, Germany, Canada, the United States, and Portugal. Further analysis of its nucleotide found 7 nucleotide mutations, but only the 311th pair of nucleotide mutation will cause amino acid changing, from the original AGA（arginine） to AAA（lysine）; In 7 results, 5 results caused the 311th pair of nucleotide mutations which made amino acid changing.

Keywords: Parrot avian bornavirus, proventricular dilatation disease, phylogenetic analysis

A56

Isolation and Characterization of Goose Tembusu Virus in Taiwan

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Since 2010, Tembusu virus (TMUV) has caused severe outbreaks with neurological signs and egg-drop diseases in ducks and geese in neighboring countries of Taiwan. In late 2020, an infectious disease outbreak characterized with white diarrhea, depression, lameness, prostrate and increased mortality occurred in a 45-day-old white Roman geese flock in southern Taiwan. TMUV infection was diagnosed from diseased geese by RT-PCR. TMUV was successfully isolated using minimal-pathogen-free duck embryos and designated as NTU/C225/20. The full-length genomic sequence of virus was determined by the next-generation sequencing. Genomic analysis revealed that the NTU/C225/20 shares approximately 87% nucleotide identity with recently reported TMUV strains in China, Malaysia and Thailand, and 91% with the prototype strain MM1775 identified back in 1955. In addition, TMUV NTU/C225/20 can be cultured and titrated in cells and embryos, where the virus was able to generate cytopathic effect (CPE) in the chicken DF-1 cell line and primary duck embryo fibroblasts, and cause death in specific-pathogen-free chicken embryos. Mammalian Vero cells also supported the viral growth in the absence of CPE, and the viral envelope protein can be visualized using anti-flavivirus antibodies in the immunofluorescence assay. In this study, for the first time, a novel TMUV strain from geese of Taiwan was isolated and characterized. Further studies on the infection prevalence and viral pathogenicity are warranted.

Keywords: Tembusu virus, geese, virus isolation, genomic analysis, embryos

A57

Phylogenetic Analysis of Avian Polyomavirus Taiwan Isolates

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Avian Polyomavirus (APV) is a non-enveloped virus with a circular double-stranded DNA (dsDNA) genome about 5000 bp in length. APV was first reported in fledgling budgerigars (*Melopsittacus undulatus*) as the etiologic agent of budgerigar fledgling disease (BFD) with high mortality rates in parrots in 1980s. This disease has been observed worldwide, and APV has a wide host range, which has been founded in budgerigar, cockatoos, lorikeets, lovebirds, and macaws. Twenty isolates of APV have been collected from diseased psittacine birds in Taiwan by polymerase chain reaction (PCR) in the past three years, and the full length genome of these isolates were sequenced. The full lengths of APV isolates in this study varied from 4971 bp—4982 bp. The sequences of the whole genome shared over 99% identities with other APV strains from the GenBank database. The genome of APV contains an early region which encodes two regulatory proteins, large tumor antigen (LT-Ag) and small tumor antigen (ST-Ag), and a late region which encodes the capsid proteins, VP1, VP2, VP3 and VP4. The nucleotide identity of the VP1 and VP4 ranged from 98.8% to 100%, while the sequences of the LT-Ag shared the lowest identities, 96.4—100%, with other APV strains from the GenBank database. The phylogenetic tree generated using the whole genome sequence showed that the APV Taiwan isolates were closely related to Japan and Portugal strains. Recombination events were analyzed using the Recombination Detection Program version 4 (RDP4), and APV Taiwan isolate TW-3 was identified as a minor parent of 10 potential APV recombinants. In this study, we first presented the whole genome sequences of APV Taiwan strains and compared with other APVs in GenBank database.

Keywords: Avian Polyomavirus, budgerigar fledgling disease, recombination

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B1

Use of TD-5472617A Prior to Veterinary Visits for Reducing Stress and Anxiety in Cats

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Objectives: Psychoactive medications have been commonly used in human and canine patients for treating stress and anxiety, and have been gaining popularity as pre-appointment anxiolytics in feline patients. However, the oral administration in cats often challenges the owners and limits the practicality. To find a more convenient route of administration, this study evaluated the efficacy and safety of TD-5472617A, which is a novel transdermal ointment compounded with serotonergic antidepressant.

Methods: This was a single-center and randomized prospective study. Enrolled cats were randomly assigned and administered the lower or the higher dose of TD-5472617A. Each cat served as its own controlled individual between two different visits (pre- and post-administration), and the behavior during transportation and examination was observed. According to feline behaviorology and previous studies, several assessment scoring systems were designed to help estimate the degree of stress and anxiety, including the cat stress score (CSS), global sedation score (GSS), behavioral response score (BRS), and owner-assessed overall experience score (OES). Theses scores along with the respiratory rate (RR), heart rate (HR), blood pressure (BP), pulse rate (PR) were compared between two visits respectively.

Results: The lower dose of TD-5472617A was applied to seven cats, and the higher dose was applied to thirteen cats. In the group with the lower dose, there was no significant decrease in BRS during the examination, and only the CSS during the transportation of departing from home was scored a marked reduction. In the group with the higher dose, there was significant decrease in both CSS and BRS during the transportation and the examination. There was no difference in GSS between two visits among all these cats. Neither of these two groups was identified significant changes in RR, HR, BP, and PR. Most of the owner's OES showed acceptable results and positive willingness to use TD-5472617A again.

Conclusion and clinical relevance: This study supports that using TD-5472617A at sufficient dose prior to veterinary visits can reduce stress and anxiety in cats, which is helpful for better patient and owner experience. TD-5472617A was well tolerated in this population of cats. Further studies are recommended to investigate the use of TD-5472617A in cats with the aim of decreasing fear and resistance associated with transportation and examination.

Keywords*:* TD-5472617A, serotonergic antidepressant, transdermal ointment, feline stress and anxiety

B2

Case Report: Iatrogenic Hypoadrenocorticism in a Dog

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Iatrogenic hypoadrenocorticism (HA) is uncommon in dogs and cats. It can be caused by three factors: (1) Treatment of hyperadrenocorticism using Trilostane or Mitotane; (2) Exogenous steroid medications stopped abruptly; (3) Hypophysectomy. The clinical signs of HA are not significant, which include hypotension, hypovolemia, hypothermia, weakness, vomiting, and diarrhea. A serum biochemical examination can support in the diagnosis of HA which shows evidence of hyponatremia, hyperkalemia, and hypochloremia. A 12-year-old, spayed female Maltese was diagnosed with hyperadrenocorticism (HAC) and was prescribed with Trilostane. It showed clinical signs of weakness and panting during a recheck 2 weeks later and had experienced Addisonian crisis on the same night. Immediate treatment was given to stabilize patient's condition. Despite history of HA, the patient no longer shows clinical sign of HA after treatment. Meanwhile, hypothyroidism was also suspected when dry, brittle hair coat was observed. Thyroid test was taken and thyroxine (T4) supplement was prescribed. Patient will be required to take biochemical examinations routinely to monitor its electrolytes balance.

Keywords: Hyperadrenocorticism, Iatrogenic hypoadrenocorticism, Trilostane

B3

Case Report: Canine Monocytic Ehrlichiosis in a Dog

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A ten years old, mix breed dog which had no parasite prevention record showed clinical signs of lethargy, abdominal pain, and anorexia for the past few weeks. Physical examination had no significant abnormalities. The complete blood count include mild normocytic normochromic nonregenerative anemia and moderate regenerative thrombocytopenia. Serum biochemistry findings include increased serum activity of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Blood smear examination showed morulae in the cytoplasm of monocytes. Antibody detection of *Ehrlichia canis* was done by CaniV-4 test kit using immunochromatographic assay, and the result was positive. Nucleic acid detection of *Ehrlichia spp*. was done by polymerase chain reaction which targets the *16S rRNA* gene of *Ehrlichia spp.*, and the result was positive. In the combination of regenerative thrombocytopenia, morulae in monocytes, positive antibody test, and positive nucleic test, the dog was diagnosed with canine monocytic ehrlichiosis. Doxycycline was given orally to the dog 5 mg/kg twice per day, and the length of treatment was 28 days. After doxycycline treatment, the clinical signs, anemia, thrombocytopenia, and elevated serum liver enzyme activity returned to reference intervals within a month. Nucleic acid detection was performed one month after doxycycline treatment, and the result was negative. In conclusion, the doxycycline treatment for the dog is effective and the prognosis is good.

Keywords: Canine monocytic ehrlichiosis, *Ehrlichia canis*, Doxycycline

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B4

Primary Keratinization Defects in Three Dogs

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Three dogs, one to two years of age, with small greasy follicular casts and waxy scale-like material adhering to the skin of ears, back, abdomen, tail and legs, were referred to National Chung Hsing University Veterinary Teaching Hospital. Before our treatment, these dogs had been misdiagnosed for ectoparasitic mites infestation or dermatophytosis in several veterinary clinics, and had been treated for several months with disappointment results. No bacteria, fungi and parasites were found on Scoot tape stripping. Based on the history, dog breeds and clinical diagnosis, we confirmed that this disease was primary keratinization defect. These dogs had well recovery after one to three months of treatment with oral essential fatty acids, prednisolone, vitamin B6 and topical therapy with medical shampoo, vitamin A, D and E cream.

Keywords: primary keratinization defects, dog

B5

The Practicality of Intranasal Administration of Dexmedetomidine for Premedication before Computed Tomography Examination

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Anesthesia is widely applied on companion animals for computed tomography (CT) examination and the utilizing of propofol and isoflurane for induction and maintenance are commonly used. However, hypotension and hypothermia during the procedure are expected. Previous studies indicated the intranasal administration of dexmedetomidine could improve hypothermia, hypotension and reduce adverse effects than intramuscular route. The aim of this study is to evaluate the practicality of intranasal administration of dexmedetomidine for premedication before CT examination. Two experiments were conducted. First, the effects by intranasal administration of dexmedetomidine at 125 μg/m^2 or 375 μg/m^2 on conscious dogs were evaluated. The results showed that the dose of 125 μg/m^2 which revealed similar levels of sedation and the effects on cardiovascular system with 375 μg/m^2, was selected for next experiment. Second, clinical canine patients which undergone CT examination were enrolled. The effects of intranasal administration of dexmedetomidine as sole premedication agent were compared with the patients that did not use any premedication. The same induction and maintenance protocols was performed by using propofol and isoflurane. The physiological parameters in two groups were recorded and compared. Results showed the significant decreased blood pressure was noted in non-intranasal group and intranasal dexmedetomidine group produced more stable blood pressure with less bradycardia episodes. In conclusion, the intranasal administration of dexmedetomidine at 125 μg/m^2 with the properties of maintaining the stable blood pressure levels and preventing the bradycardia is practical for premedication before CT.

Keywords: Computed tomography, Dexmedetomidine, Dog, Intranasal

B6

Recovery from Lameness after Femoral Head and Neck Ostectomy using Physical Therapies in a Dog

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A 2-year-old female Pembroke Welsh was admitted to National Chiayi University Animal Hospital on 2016/07/20 due to clear lameness of the left hindlimb (lameness score 4). A shallow socket, thickened femoral neck, formation of osteophyte around the joint and joint subluxation were observed at the left coxofemoral joint by the radiographic examination. Hip dysplasia of the left rear limb was diagnosed in the dog. Femoral head and neck ostectomy was performed for the joint disorder on 2016/08/02. The dog was presented again on 2016/08/16 due to the post-operative lameness (left hind limb lameness score 3) coupled with extreme reluctance to move, lasting for about 3 days. Physical therapies including transcutaneous electrical nerve stimulation (TENS), therapeutic ultrasound, manual therapies, assisted/active therapeutic exercise, gait evaluation and endurance training were sequentially and continuously conducted to manage the lameness, adjust the gait and counter muscle atrophy of the left rear limbs for approximately 3 months. Recurrence of the hindlimb lameness or a low willingness to move did not occur available for hospital follow-up by Jan 2021.

Keywords: post-operative lameness, therapeutic ultrasound, manual therapies, gait evaluation, endurance training

B7

Recovery from Severe Lameness using Physical Therapies after Closed Reduction for Hip Luxation in a Dog

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A 9-year-old female Poodle was admitted to National Chiayi University Animal Hospital on 2021/03/25 due to severe lameness (lameness score 5) of the right hindlimb. Dislocation of the right coxofemoral joint was suspected after the right pelvis was compressed by a 20 kg dog on 2021/02/17. Complete luxation of the right hip joint was observed and diagnosed by the radiographic examination in a local hospital on 2021/02/18. Ehmer sling application was firstly conducted from 2021/02 to 2021/03 to accomplish the closed reduction and manage the condition of the coxofemoral joint in the dog. Severe lameness (lameness score 5) of the right rear limb was present after removal of the sling. Physical therapies including Class IV therapeutic laser, manual therapies, neuromuscular electrical stimulation (NMES), assisted/active therapeutic exercise, and gait evaluation were sequentially executed to manage the lameness and adjust the gait for around 1 month. Recurrence of the right hindlimb lameness was not observed when walking and trotting available for hospital follow-up by Apr 2021.

Keywords: closed reduction, Class IV therapeutic laser, manual therapies, neuromuscular electrical stimulation, therapeutic exercise

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B8

Case Report: Intraocular Malignant Melanoma in a Dog

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Melanoma is the most common primary intraocular neoplasm in dogs and cats, and most melanomas originate from the iris or ciliary body. Occasionally, these tumors arise diffusely in the anterior uvea and are rarely seen in the choroid. The primary canine uveal melanoma has been thought to have lower risk of distant metastasis, but in feline has a greater tendency to metastasize and are more malignant. A 10-year-old male Cavalier King Charles Spaniel, weighing 10kg, showed clinical signs of left eye exophthalmic globe for the past two weeks. The ophthalmological examinations revealed no response to mean reaction and pupillary light reflex in the left eye. The treatment of this case was left eye enucleation, and the eye was histologically diagnosed as malignant melanoma. Prognosis for the animal's survival after enucleation is good. In this case, further monitoring of metastasis should be conducted because of the malignancy of the tumor.

Keywords: canine, intraocular, melanoma, enucleation

B9

Effects of Oral Gabapentin and Trazodone on Ocular Parameters in Healthy Dogs

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Hospital visit-related and/or ophthalmic examination-related anxiety is a common problem in dogs. Gabapentin and trazodone have been used in animals prior to transportation and hospital visits to reduce anxiety. However, some studies have shown that oral trazodone significantly decreases pupil diameter (PD) following administration in cats and that gabapentin could reduce the intraocular pressure in dogs. As far as we know, any factors that decrease tear production, alter intraocular pressure (IOP), alter pupil size or expedite tear break-up time (TBUT), may cause ocular pathology or worsen existing ocular diseases of canine patients. The purpose of the study was to determine the effects of oral gabapentin and trazodone on ocular parameters in normal canines with normotensive eyes during ophthalmic examinations. A randomized, blinded, case-crossover study with a 7-sequence, 4-treatment, and 3- washout period (7-day) design was performed in twelve healthy dogs. Treatments were randomized and the dogs were given either drug A (gabapentin 20 mg/kg), drug B (lactose powder), drug C (gabapentin 10mg/kg with trazodone 5mg/kg), or drug D (trazodone 10 mg/kg). Fear, anxiety, stress (FAS) score, noninvasive arterial pressure, heart rate, and respiratory rate were recorded. Measurements of tear production, IOP, PD, and TBUT were routinely monitored and recorded. No significance was found in blood pressure, heart rate, respiratory rate, tear production, IOP, PD, or TBUT, but a lower FAS score was noticed in drug C and drug D. Few adverse effects were detected during the present study. In conclusion, results of our study indicated that orally administered gabapentin and trazodone should have no clinically adverse effects on tear production, IOP, PD, or tear film stability in healthy dogs.

Keywords: Gabapentin, Trazodone, Ocular parameters, FAS, Dog

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B10

Identification and Antibiotic Susceptibility of Bacterial Isolates from Dogs with Pyoderma

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Canine Dermatitis has always been a common clinical disease, it has been proven that bacterial infection is the most common cause of dermatitis and could easily relapse. Thus, the present study focused on investigating the prevalence of pathogens of canine pyoderma and assessing the antibiotic pattern with a purpose of prudent use of antibiotics The sample were collected from veterinary clinics and animal shelters throughout Pingtung and Kauhsiung from June 2020 to April 2021. The bacterial culture was performed on Tryptone soy agar, Mannitol salt agar and identified by Gram Stain, standard biochemical tests, polymerase chain reaction using specific primers, also antimicrobial susceptibility tests were performed. The 34 bacteria were isolated from 30 samples. The result showed that the most common isolated bacteria were *Staphylococcus pseudintermedius(71%)* followed by *Staphylococcus aureus(11%)*, *Escherichia coli(9%)* ,etc. Most of the gram-positive isolates were sensitive to Enrofloxacin and Amikacin, while all of the gram positive isolates were resistant to Ampicillin. Meanwhile, most of the Gram-negative isolates were sensitive to Enrofloxacin, Ceftazidime , while other antibiotics appears resistant. Thus, microbiological culture combined with susceptibility testing is the best instrument for guiding treatment decisions in individual dogs.

Keywords: Pyoderma, bacterium, antibiotic, antimicrobial susceptibility test

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B11

Design of Canine transdermal carrier for Pets

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The drugs are delivered through the skin to ensure accurate delivery to the affected area and are easy to operate. For some drugs that are poorly absorbed through the GI tract or required prolonged administration in chronic pain, the patch can be made to achieve slow drug release. At present, there are many kinds of pet external medicine. But the drug absorption through the skin is relatively slow, it is not easy to penetrate the cuticle. Many humans use pasted topical medicines. However, as far as pets are concerned, it is easy to be bitten off using the pasted transdermal method. Therefore, it is worthwhile to develop a transdermal carrier for pets. In this study, the canine transdermal drug carrier, after moisturizing and the appropriate proportion of interfacial active agent (HLB 15), can assist the drug penetration into the canine skin, to achieve the effect of rapid penetration into the cuticle.

Keywords: Canine, transdermal carrier, HLB 15

B12

Study the Role of Nesfatin in Osteoarthritis Pathogenesis

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Synovial inflammation is the pivotal characteristic of osteoarthritis (OA) pathogenesis. Currently available medications for OA are restricted to nonsteroidal anti-inflammatory drugs (NSAIDs), which are accompanied by substantial side effects and are only temporarily effective. Thus, safer and more efficacious therapeutic agents are needed. Epidemiological data reveal that the incidence of OA increases with overuse of joints, older age, and higher body mass index (BMI >30). It is also recognized that adipocytes secrete and synthesize several adipokines such as Nesfatin, which promote proinflammatory factors in several diseases. Much evidence indicates that significantly higher amounts of Nesfatin are found in OA synovial fluids and serum than in samples from healthy volunteers, indicating that Nesfatin is a risk factor for OA. However, the role of Nesfatin in OA pathophysiology is unclear. Interleukin 1 beta (IL-1β) is one of the major proinflammatory cytokines secreted by synovial fibroblasts in OA pathogenesis. Up until now, little has been known about the relationship between Nesfatin and IL-1β in synovial fibroblasts. Our data demonstrate that significantly higher levels of Nesfatin and IL-1β expression in synovial fluid from OA patients compared with normal. Treatment with Nesfatin in OASFs significantly promotes IL-1β expression in a concentration-dependent manner, which was mitigated by blockade of the PI3K/AKT/AP-1 pathway. We also showed that Nesfatin inhibited the expression of miRNA-204-5p, which blocks IL-1β transcription; this suppression activity was reversed via blockade of the PI3K and AKT pathway. These results shed light on OA pathogenesis and suggest a novel treatment pathway.

Keywords: Nesfatin, osteoarthritis; miRNA-204-5p

B13

Application and Accuracy of a Magnetic Resonance Imaging-Guided Neuronavigation System in Brain Surgery of Small Animals

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Despite magnetic resonance imaging (MRI) characteristics are helpful for the diagnosis of brain tumors, the histopathological exam of the brain tissue is still essential for the definitive diagnosis. However, open and free-hand surgical brain biopsy carries out significant risks, which limits this diagnostic procedure to be conducted in veterinary clinics. The image-guided stereotactic technique can display the location of the surgical instrument and the biopsy target in real-time, allowing brain biopsy to be performed in a minimally invasive way with reduced risks. RETINA® is a frameless stereotactic navigation system. Its application and accuracy in dogs and cats have not been investigated. The purpose of this study is to describe the application of RETINA® in small animals and to evaluate the accuracy of this system. A prospective study, using canine and feline cadaver as studying subjects, was conducted. Phantom lesions were created at various depths and regions of the brain. Eight reference markers composed of titanium screws and plastic cylinders filled with diluted gadolinium were installed at the outer table of the frontal sinus and bilateral zygomatic arch in each cadaver. A T1-weighted turbo field echo three-dimensional sequence with 1-mm slide thickness was acquired in a 1.5 T MRI scanner and images were imported into the RETINA®. After placing the fiducial marker at teeth via dental bite block, the infrared camera was applied to register the head and the image set via detecting the spatial relationship between fiducial markers and the reference markers. RETINA® was used to determine the trajectory path to the targeted lesion in real-time and 0.2 μl of diluted gadolinium was injected at each target through the selected pathway. Coordinates of navigated target-point (X, Y, Z) and the center of gadolinium deposition (X', Y', Z') were established on postoperative MRI. The distance between these two coordinates was defined as targeting error and was used to evaluate the accuracy of RETINA®. Following the machine setting and method of registration described in this study, RETINA®could be applied to fulfill the purpose of neuro- navigation in brain surgery of small animals. Sixty-four lesions were targeted in this study and the mean targeting error for all targets was 2.87 mm ± 0.82 mm. The 95% confidence interval of the targeting error was 2.66 to 3.07 mm. Lesions from different brain lobe, length of trajectory path, or operator's experiences were not significantly affecting the accuracy. The feasibility and accuracy of this frameless stereotactic navigation system support its clinical application for brain lesions of diameter greater than 3.07 mm in dogs and cats.

Keywords: MRI-guided stereotactic device, neuro-navigation, brain biopsy

B14

Investigation of Canine papillomavirus infection of canine skin lesions in Taiwan over the last two decades

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Canine papillomaviruses (CPVs) are able to infect the squamous epithelial cells and cause various cutaneous lesions in canine with host specificity. The common papillomavirus-associated lesions in dogs include viral papillomas and viral pigmented plaques, yet the benign lesions may have a potential to transform to malignant squamous cell carcinomas (SCCs). There were a few case reports mentioning about other skin lesions including basal cell tumors (BCCs), transitional cell carcinomas(TCCs), and transmissible venereal tumors (TVTs). Additionally, CPVs often cause asymptomatic infection, which means the virus might also be detected in healthy or immunocompetent animals with little amount. However, except the detection of the viral genes of CPV in each lesion, little is known about the prevalence and the common genotypes of CPV infections in Taiwan. In order to understand the overview of CPV infection in Taiwan, 741 canine skin lesions, including papillomas, viral pigmented plaques, SCCs, TVTs, TCCs and BCCs from Animal Disease Diagnostic Center of National Chung Hsing University were collected for analysis of the morphology of lesions and genotypes of CPVs. The viral DNA in the formalin-fixed paraffin-embedded (FFPE) tissues were targeted by polymerase chain reaction (PCR) and the DNA quality of each sample was confirmed by the internal control primer set targeting canine GAPDH gene. The result demonstrated that 470 out of 741 cases showed positive (63.43%). Subsequently, these samples were amplified for CPV detection using PCR targeting pan PV E1 and L1(CP4/5 and CanPV/FAP64). CPV9 and CPV6 were successfully detected from 5 cases (1.06%), inclusive of 1 viral pigmented plaque and 4 viral papillomas, respectively. All of the positive cases in PCR result were reconfirmed by immunohistochemistry (IHC) stain, and *in situ* hybridization (ISH) will be applied for further locating the virus to verify the infection of CPVs in the lesions.

Keywords: canine papillomavirus, skin lesions, viral pigmented plaques

B15

Investigation of Anterior Chamber Parameters in Healthy and Glaucomatous Cats by Ultrasound Biomicroscopy

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By using ultrasound biomicroscopy (UBM), the cross-sectional structures of the entire iridocorneal angle (ICA) which are unable to assess with gonioscopic examination were evaluated objectively and quantitatively in live healthy and glaucomatous cat. However, nowadays, there haven't been a research focus on the cross-sectional structures of the entire iridocorneal angle of cats. The aim of this study was to evaluate the anterior chamber and ciliary cleft segments of healthy and glaucomatous cats objectively by analyzing dorsal and temporal position of limbus with ultrasound biomicroscopy. We collected glaucomatous and healthy cats presented to the Ophthalmology Department at the NCHU-VMTH for consultation between December 2020 and May 2021, and divided them into healthy group, and chronic glaucoma group. Results shows that Ciliary body thickness (CBT) are larger ($p<0.05$) at temporal limbus than dorsal limbus. However, there are not difference between dorsal limbus and temporal limbus in chronic glaucoma group. Anterior chamber depth and iridocorneal segments are smaller in chronic glaucoma group when compared with healthy group. The collapse of ciliary cleft is a character that who is suffered from chronic glaucoma.

Keywords: Glaucoma, UBM, Cat

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B16

Ultrasound Biomicroscopic Study of Anterior Segment Changes One Year after Phacoemulsification and Intraocular Lens Implantation in Dogs

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Ultrasound biomicroscopy (UBM) allows for qualitative evaluation in the structures of the anterior segment as well as for quantitative measurements of anterior chamber components. A research in humans showed that the capsular bag stretched horizontally with reduced vertical diameter as a result of adaptation to the implanted IOL after cataract surgery. However, another study showed that the relationship between iridocorneal angle opening distance measured by UBM is weakly associated with intraocular pressure elevations. Thus, the purpose of this study was to assess the changes of intraocular status before and after phacoemulsification and IOL implantation using UBM. A-mode, UBM and B-mode were performed on 40 eyes of 37 dogs before and at least one year after phacoemulsification surgery. The parameters, anterior chamber depth (ACD), axial length (AXL), capsular bag diameter (CBD), angle opening distance (AOD 500 and AOD 750), anterior chamber angle (ACA) and angle recess area (ARA) were obtained and further analyzed. In conclusion, this study measured several parameters of the anterior segment, and AXL, ACA, ACD showed statistically significant between pre-surgery and post-surgery in glaucoma and non-glaucoma group. Plenty of assumed risk factors of postoperative complications were evaluated, and the presence of trabecular meshwork was found significantly associated with glaucoma by means of chi-square test and logistic regression. Furthermore, the odds ratio was calculated as 0.007. Although the Degree of the cataract was not significantly associated with these parameters analyzed in this study, the decrease in median of AOD, ACA and ARA can be observed. Moreover, the differences between preoperative and postoperative parameters such as ACA, ACD, AOD and ARA showed significant results in hypermature cataract group. These findings may be beneficial to determine whether preventative treatment for glaucoma after phacoemulsification is necessary.

Keywords: Ultrasound biomicroscopy, phacoemulsification, iridocorneal angle, glaucoma, cataract, trabecular meshwork

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B17

Inflammatory Cytokine Profile in Canine Tears by Multiplex Analysis

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Cytokine profiling of body fluids has become key in the diagnosis, classification, and evaluation of inflammatory diseases, such research plays a significant role in achieving a deeper understanding of inflammatory disease states such as allergic reactions, inflammatory bowel disease (IBD), sepsis, and cancer. Monitoring cytokine changes and correlating with signs and symptoms will improve our understanding of the immune mechanisms. Ocular surface diseases are often associated with inflammation. In comparison to serum cytokine measurements, tear cytokine is expected to be more accurately reflect the inflammatory milieu associated with periocular disease. There is mounting evidence that inflammation plays roles in the pathogenesis of the ocular surface disease that develops in dry eye, which is significantly correlated between certain cytokines with ocular surface disease. Determination of tear cytokines above a certain threshold might provide an objective indicator of specific patients will benefit from anti-inflammatory therapy. Identification of cytokines expression could provide important information regarding the local inflammatory processes associated with the pathogenesis and perpetuation of these disorders. The purpose of this study was using a multiplexed assay to determine tear inflammatory cytokines including interleukin-2, 6, 8 and 10; (IL-2, IL-6, IL-8, IL-10), tumor necrosis factor-α, (TNF-α) and interferon-γ (IFN-γ) levels to determine basic profile in normal dogs. Dogs enrolled received basic ophthalmic exams including: Schmer's Tear Test (STT), corneal fluorescent stain, intraocular pressure measurement, pupillary reflex, slit-lamp examination of anterior chamber and fundus observation to exclude any significant ocular disorders. Other laboratory examinations were ELISA screen test for heart worm, *Anaplasma* infection, babesiosis and Lyme disease; regular blood works were complete blood count (CBC) and routine biochemistry panels. In this study, multiplex analysis successfully measured various cytokines in a small amount of sample of tear and serum at once, which is advantageous compare to ELISA technique. In past literature, similar experiment done by Martinez *et*, al. shown restrictive data due to sample dilution. Without dilution IL-6 and IL-8 varies amount asymptomatic dogs in both tear and sera, and proved that cytokines can be detected with regular clinic procedure. These differences need further analysis with clinical ocular parameters and laboratory data to explain such outcome.

Keywords: Multiplex, Cytokine, Ocular parameters, ELISA

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B18

The Relation and Prognosis of Serum Interferon Gamma in Dog Before and After Receive Cataract Surgery

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Lens is within the middle part of eye ball, that helps adjust diopter. The term of cataract is the change of opacity of lens, which can be congenital or degeneration along with age. Metabolic disorder in diabetes mellitus patients is one of the known reasons, but other factors and etiology is still unclear. Oxidative stress, UV exposure and local inflammation are assumed to contribute lens protein degeneration. Further lens induced uveitis (LIU) is concurrently progressed throughout all stage of disease. Such inflammation is continuing damage the intraocular tissues, like retina, and deteriorate vision abilities, also worsen the successfulness of surgical intervention. In human ophthalmologist, many studies were done to reveal the relation between pre- and post-cataract surgery, which including the acute and long-term complications; others had tried to investigate the inflammatory cytokines and chemokines in aqueous humor of different ocular disease entities. In 2006, Takase, *et*. al. had published that interferon gamma (INF-gamma) can be detected in aqueous humor and serum in both inflammatory and non-inflammatory uveitis patients. In our knowledge, there isn't similar studies designed prospectively and cohort in canine receives cataract surgery. That the purpose of our study is to investigate if serum INF-gamma present with post-cataract surgery induced uveitis, and the relation between the cytokine and complications occurs during short term recovery, also treatment successfulness. Animals were confirmed cataract dogs from out-patient department visited National Chung Hsing University Veterinary Teaching Hospital (CHUVTH), and appointed for cataract phacoemulcification. The sera sample were collected: 1, before surgery (without topical premedication given), 2, on the day of surgery (topical premedication applied), 3, six hours after operation completed, 4, and 5, twenty-four hours and forty-eight hours after surgery. The sample is further tested for INF-gamma concentration with ELISA kit, and the result is comparing with medical records for analysis. It is frustrated that in most of the cases, cytokine is below detective limits, which implies the itrogenic uveitis is not strong enough to induce adequate INF-gamma to peripheral circulation; and insinuated other intraoperative complications, patient conditions, or other post-operative cares shall be more influence to overall prognosis.

Keywords: Cytokine, Interferon-gamma, cataract, dog

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B19

Biomechanical Evaluation of Extracapsular Stabilization with Different Isometric Attachment Sites in Canine Cranial Cruciate Ligament-Deficient Stifles

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Cranial cruciate ligament (CCL) disease is one of the most common causes of lameness in dogs. The biomechanical outcome after surgical correction for CCL disease has been widely investigated. However, there were only a few studies that evaluated the stability and mobility of the reconstructed stifle. Therefore, the objectives of this study were to assess the stability of extracapsular stabilization (ECS) in cranial cruciate ligament-deficient (CCLD) dogs; and to compare the performance of ECS with bone anchors at two different pairs of isometric attachment sites *in vitro*, using a custom-made jig and a motion capture system. Six cadaveric pelvic limbs were harvested from three adult dogs without history of musculoskeletal diseases and free from abnormality on orthopedic examination and under radiography. Each specimen was tested under different CCL conditions, namely intact, CCLD, and repaired with ECS using bone anchors. The ECS group was divided into two subgroups, each with a unique pair of isometric attachment sites of sutures (i.e., F2-T1 and F2-T3). For CCL reconstruction with ECS, two 3.5-mm stainless steel anchors were placed at different isometric attachment sites and connected with 80-lb nylon leader line (NLL). Under the tension that cranial drawer test became negative in stifle angle of 135°, the NLL was fixed with 80-lb crimp clamp. The craniocaudal drawer test and tibial internal rotation test were conducted for each CCL conditions in all samples. The results indicated that both ECS subgroups tend to diminish the tibial cranial thrust caused by CCL deficiency, but lead to over-constraint tibial internal rotation comparing to both intact and CCLD groups, in agreement with previous studies. While the two chosen pairs of isometric points in this study were shown to effectively decrease the cranial tibial displacement, the excessive tibial external rotation in comparison to those of intact state should be concerned.

Keywords: canine cranial cruciate ligament disease, extracapsular stabilization, bone anchor, isometric attachment sites

B20

Effect of Bacillus *licheniformis* on clinical and laboratory parameters in cats with chronic diarrhea

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Chronic diarrhea is a common clinical presentation in companion animals in different ages and breeds, while the exact diagnosis was difficult to be made in the clinical practice. Symptomatic therapy such as modification of the intestinal microbiome by probiotics is an alternative treatment option. The compound of Bacillus *licheniformis* with its fermented products which has been used to against porcine epidemic diarrhea virus was evaluated in this prospective study for its effect on clinical and laboratory parameters in cats with chronic diarrhea. 24 cats from the same household were included in the first phase pilot study, and 9 cats were so far recruited in the second phase clinical study. All cats in the first phase were divided into 3 groups: the probiotic was prescribed to the 8 diarrhea cats in the first group; the probiotic was also prescribed to the 8 non-diarrhea cats in the second group; the other 8 non-diarrhea cats without treatment were categorized into the control group. The probiotic was administered for 7 days in the first phase, and fecal examination, blood examination as well as clinical evaluation by owner questionnaires were performed before the initiation of the probiotic, and after 1 and 12 weeks, respectively. After taking the probiotic product for a week, the result of fecal occult exam turned into negative from positive in one of the diarrhea cat. The overall clinical activity and fecal consistency were assessed by feline chronic enteropathy activity index (FCEAI) and modified Purina fecal score, respectively. For the 8 diarrhea cats who took the probiotic, the mean FCEAI and fecal score decreased from 3.13 to 2.38 and from 5.69 to 4.81, respectively. Clinical improvement of fecal condition with varying degrees in 8 diarrhea cats was noted by the owner, but these differences were not statistically significant. No obvious ill clinical effects or significant impact to the hematological or biochemistry examinations was noticed in the following-up period for12 weeks. Although there was an improvement observed by majority of the owners in fecal score of the diarrhea group, no significant difference was found. These may relate to the limited case numbers and the short administering duration. Thus the second phase experiment with prescribing probiotic for 2 weeks as well as more studies with a larger number of cats and dogs are needed to further assess the effects of B. *licheniformis* as probiotic in companion animals with chronic diarrhea.

Keywords: Bacillus *licheniformis*, chronic diarrhea, Purina fecal score

B21

Extended Delivery of Doxycycline by Silicone-Hydrogel Contact Lenses

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Spontaneous chronic corneal epithelial defect (SCCED) in dogs are chronic epithelial erosions that fail to resolve through normal wound-healing processes. There is a theory of rising in Matrix metalloproteinase (MMP)-2, MMP-9 as the same disease pattern in human recurrent corneal erosion (RCE). The therapeutic value of doxycycline has been ascribed to an ability to inhibit MMPs activity. However, the doxycycline peak concentration in the normal dogs' tear film was 4.32ng/mg, which is unable to inhibit MMPs. Studies in recent years have shown that the use of commercial silicone hydrogel contact lenses as a drug carrier can extend the release time of the drug and also can be worn for a long time. The purpose of this study was to ensure that the commercial silicone hydrogel contact lenses can release doxycycline stably and have a higher drug release concentration in the eye than oral doxycycline. Three commercial contact lenses were used in this study, including ACUVUE® OASYS™, clariti™, and ilens®. The commercial contact lenses were soaked in 15μg/ml doxycycline solution for seven days. The concentration of doxycycline released in 1,2,4,8,12,24,48 hours was measured by high-performance liquid chromatography. Vitamin E was loaded into contact lenses in the other group. The results of this study are that all three contact lenses can release doxycycline stably, and gradually reach equilibrium after 12 hours of release. In addition, contact lenses with added vitamin E have a higher drug release concentration. In conclusion, the silicone hydrogel contact lens can be used as a carrier for doxycycline, and the drug concentration released is higher than oral administration.

Keywords: silicone hydrogel contact lenses, doxycycline, spontaneous chronic corneal epithelial defect (SCCED)

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B22

Mouse Quiescin Sulfhydryl Oxidases Exhibit Complementary Epididymal Distribution with Distinct Sperm Membrane Surface Associations

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Spermatozoa are infertile and genetically inactive after exiting from the testis; thus, it is required for spermatozoa to undergo maturation process occurring in the epididymis. Sulfhydryl oxidation is one essential sperm maturation process engaging in the acquisition of sperm fertilization competency and structural stabilization; however, the specific sulfhydryl oxidases that fulfill these roles have yet to be identified. In this study, we investigate the potential involvement of one atypical thiol oxidase family called quiescin Q6/sulfhydryl oxidase (QSOX) using the mouse epididymis as our model system. With multidisciplinary approaches, we show that QSOX isoform 1 and 2 not only exhibit complementary distribution throughout the epididymal duct, but also possesses distinct subcellular localization within the epididymal principal cells. While QSOX2 was exclusively present in the Golgi apparatus in the proximal epididymal segment, QSOX1c, the most profusely express QSOX1 variant, was abundantly present in the cauda luminal fluids. Moreover, immunohistochemistry studies together with proteomic identification in isolated epididymosomes provided evidence substantiating the release of QSOX2, but not QSOX1c, via an apocrine secretory pathway. Furthermore, we demonstrate for the first time, distinct association of QSOX1c and QSOX2 with the sperm acrosome and implantation fossa, during sperm transition across the epididymis. In conclusion, our study provides the first comprehensive comparisons between QSOX1 and QSOX2 in the mouse epididymis, revealing their distinct epididymal distribution, cellular localization, mechanisms of secretion and sperm membrane as- sociation. Together, these data suggest that QSOX1 and QSOX2 have discrete biological functions in male germ cell development.

Keywords: Epididymis, sulfhydryl oxidation, spermatozoa

B23

Therapeutic Application of Ge-Gen-Tang for Hyperglycemia on an Obese Mice Model

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Diabetes is a diverse metabolic syndrome and caused by insulin deficiency or insulin resistance. Like human, pets such as dogs and cats eat too much carbohydrates and fats to cause nutritional imbalances in recent years, In addition, most pets are kept indoors and do not exercise enough, which has led to pets take obese and diabetes. Generally, clinical treatment for diabetes are using insulin injections and oral hypoglycemic drugs. Compared with western medicines, traditional Chinese medicines have fewer side effects and decrease hyperglycemia, like pueraria lobata, white peony root, coptis, astragalus, and pueraria lobata decoction. Here, we used Ge-Gen-Tang to explore protect against high-fat died induced obesity and hyperglycemia. Firstly, we developed obese diabetic mice, and then the mice were randomly divided into a normal saline control group and a traditional Chinese medicine Ge-gen-Tang group, and they were fed with normal saline and Ge-gen-Tang (700 mg/kg BW/day) daily for 4 weeks. The results showed that mice treatment with Ge-gen-Tang had decreases in body weight, food intake, weekly weight gain, calorie intake, daily food efficiency, organ and fat pads weights, fasting blood glucose levels, serum insulin levels, insulin resistance index, and serum and liver triglyceride levels, Fatty Acid Synthase (FASN) expression and fatty liver infiltration than those of the control group. Insulin sensitivity index, the expression of phospho-AKT, AKT, glucose transporter 4 of insulin signaling proteins and hepatic adiponectin in Ge-Gen-Tang-treated obese mice were significantly higher than the control group. This study confirms that Ge-gen-Tang have the effect of lowering blood glucose levels and improving obesity. Finally, this study can provide clinical applications for the treatment of canine and cat diabetes in the future.

Keywords: Ge-Gen-Tang, obesity, diabetes, mice, insulin

B24

Use Mouse Knee Osteoarthritis Model to Investigate the Effect of Carprofen and Duhuojisi Decoction on Osteoarthritis

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Degenerative joint disease is common in an older population. It causes disability in the elderly in spite of the increasing sophistication in medical cares. The aim of therapy is the relief of pain and preservation of joint function. Of the commonly available analgesic drugs, carprofen are widely used in the treatment of osteoarthritis. Here, we conducted four week, Duhuojisi Decoction controlled study to evaluate the efficacy of anti-arthritic inflammation in the therapy of papain induced osteoarthritis. Osteoarthritis was induced in mice by intraarticular injection of 1.0 mg of monosodium iodoacetate. Carprofen, Duhuojisi Decoction, and Carprofen combined with Duhuojisi Decoction was orally administered to the osteoarthritis mice. Saline was used as the reference control group. The joint activity was assessed by wire hang test and measurement of the knee joint diameter. At the end of 28 days blood samples were collected for the analysis cytokines of IL-1, IL-10 and TNF-α. The animals were sacrificed and the knee of the each animal was collected for the histological studies of the joint. In carprofen, Duhuojisi Decoction, and carprofen combined with Duhuojisi Decoction-treated mice produced a significant decrease in joint diameter and significantly increase joint strength as depicted by increase in wire hanging time. Additionally, there was a significant reduction levels of IL-1β, IL-10 and TNF-α in the mice treated with the three groups. Histopathological revealed that the reversal of damages in the cartilage induced by monosodium iodoacetate after the administration of carprofen, Duhuojisi Decoction, and carprofen combined with Duhuojisi Decoction. Sections were analyzed by immunohistochemistry showed that carprofen, Duhuojisi Decoction, and carprofen combined with Duhuojisi Decoction reduced IL-1β, and increased collagen II expression in those osteoarthritis mice. These results suggest that carprofen combined with Duhuojisi Decoction had the effectively reduced experimental degenerative osteoarthritis.

Keywords: carprofen, Duhuojisi Decoction, osteoarthritis, inflammation, cytokine, mice

B25

Establishment and Investigation of Dry Eye Models in Rats

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Dry eye disease (DED) is a multifactorial and complex disease commonly seen in human and veterinary ophthalmology practices. It can be attributed to one or more than one of the following factors, including dysfunction of lacrimal gland, dysfunction of meibomian gland or goblet cells, pathological changes to the extraocular structures, disturbances in neurotransmitters, hormones, and immunological processes, that ultimately causing qualitative or quantitative changes of tear film. In order to simulate different etiologies and severity of dry eye, several methods have been reported for the creation of animal models of dry eye, such as placement in a desiccating environment, application of a muscarinic receptor antagonist, injection of atropine and botulinum B into lacrimal gland, surgical removal of the lacrimal gland and topical administration of benzalkonium chloride or mucolytic agent. In this study, we established two types of dry eye models in rats; one is aqueous deficiency model through surgical removal of extraorbital lacrimal gland, or both extraorbital and infraorbital glands; the other one is mucin deficiency model through administrating topical N-acetylcysteine(NAC) with or without applying trichloroacetic acid (TCA) on conjunctiva. The results revealed that no obvious dry eye symptoms were noted in all mucin deficiency dry eye models. There were decreases in tear production in both aqueous deficiency dry eye models. Among these, the dry eye model by surgical excision of both extraorbital and infraorbital lacrimal glands demonstrated the most substantial and severe dry eye symptoms. The results of the study indicated that the methods to induce mucin deficiency failed to establish stable and successful dry eye models, while other two aqueous deficiency dry eye models were more stable and reliable models. Besides, surgical removal of both lacrimal glands induced most severe dry eye symptoms and no compensation was noted till the end of the study. The successful dry eye models provide an important basis for further investigations of experimental therapy for dry eye disease.

Keywords: dry eye model, N-acetylcysteine, lacrimal gland excision

B26

The Comparative Study of Using Western Blotting, Enzyme-linked Immunosorbant Assay (ELISA) and Immuno-Fluorescent Assay for Chlonid Herpesvirus 5 (ChHV5) Infection Detection in Sea Turtles.

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Fibropapillomatosis (FP) of green turtles is a neoplastic disease associated with infection by Chelonid alphaherpesvirus 5(ChHV5), characterized by the presence of external epidermal fibrophapillomas and visceral fibromas. FP have been clearly reported to consist of high concentrations of ChHV5 DNA, but high proportions of asymptomatic green turtles are also carriers of ChHV5. FP is currently lack of robust serological assays to monitor previous exposure to ChHV5. Our previous study has successfully established various fragments of glycoprotein B (gB) expression encoded by UL27 gene in E. coli system. The sub-fragements of these various gB proteins were then used for monoclonal antibody production. In this study, using UL27-transfected cells, it was identified that the viral protein is distributed in cytoplasmic regions by indirect immunofluorescence analysis. After analysis of ROC curves, it indicates that ELISA test has low discriminatory ability for seropositivity comparing to western blotting method. The main reason is unknown and possibly due to antigenic conformation. The antigenic conformation is more likely to be linear in western blotting analysis, compared to the presentation of secondary conformation in ELISA. Therefore, such a difference may be related to easiness of antibody bindings for detection. The results of this study are of major importance and strongly suggest that the indirect ELISA might not be useful for serological diagnosis. Further modification for ELISA needs to be studied for sero-epidemiological tracking of ChHV5 infection.

Keywords: enzyme-linked immunosorbent assay, Immuno-Fluorescent Assay, Chlonid Herpesvirus 5

B27

Resistance to Antimicrobial Agents Among *Enterococcus* Species from Stranding Sea Turtles in Taiwan

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Sea turtles play an important role in marine ecosystems, and now are facing threats from pathogenic bacteria and marine pollution. This study investigated *Enterococcus* species and their antimicrobial resistance in sea turtles admitted to the sea turtle rehabilitation center at the National Museum of Marine Biology and Aquarium (NMMBA). Cloacal swabs were taken from 12 green turtles between 2016 and 2020. A total of 14 *Enterococcus* isolates were collected and identified using by rapid ID 32 STREP system, polymerase chain reaction or 16S rRNA sequence analysis. The most *Enterococcus* sp. isolates in study samples were identified to the species level as *E faecalis* (78.5%). Antimicrobial susceptibility testing was carried out by a standard disc diffusion method and the minimum inhibitory concentration (MIC) of amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, and vancomycin for resistant *Enterococcus* isolates were determinate by broth dilution method. In disc diffusion method, ceftiofur had the highest antimicrobial resistance (85%). Other antibiotics antibiotics that had higher antimicrobial resistance (66.7%) were azithromycin, and oxytetracycline. Additionally, there were 4 out of 7 antibiotics that were detected had resistance, ciprofloxacin (50%), doxycycline (42.8%), chloramphenicol (42.8%), erythromycin (35.7%), and amoxicillin (7.1%) in MIC test. The data of resistance patterns in our study could help veterinarians in sea turtle rehabilitation facilities in treating marine lives.

Keywords: sea turtles, *Enterococcus,* antimicrobial resistance, minimum inhibitory concentration, disc diffusion method

B28

Hand-rearing Techniques and Medical Intervention of a Common Bottlenose Dolphin (*Tursiops truncatus*) Calf at Farglory Ocean park

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Establishing a neonatal care associated with hand-reared dolphins such as bottlenose dolphin (*Tursiops truncatus*) calf is a crucial reproduction component for marine facilities and marine animal preservation. Although the highest mortality rate presents within the first 30 days following birth, there are limited neonatal interventions existing. Here we describe a hand-reared 21 kg, 4.1 Ft (125 cm) female common bottlenose dolphin named Ponyo, she was born on Oct 22, 2019 that has been hand rearing right after birth till present (now one and a half years old) at the Farglory Ocean park in Taiwan. While the maternal rejection occurs when Ponyo was delivered, she was fed hourly, 20 mL formula milk per time via a bottle or an orogastric tube as 180 Kcal/kg intake per day at the first stage (week 0 to week 4). The intake volume increased gradually to 1500 mL for 3-5 meals a day and calories requirement down to 80 Kcal/kg per day from 10 month after birth till weaning at 10 month of age (second stage). Additionally, 42 substitute formulas we had ever blended from her born to her weaning. Ponyo gained weight at 0~0.4 kg/day in each growing stage she was 81kg, 200 cm at the age of 1 year old, and shifted diet from milk to fish successfully. Currently, she is 94 kg and 206 cm in size. Furthermore, we reveal nutrient requirements, calories calculating, body weight monitoring, diet shift between each stage, signs of behavioral change as well as medical examinations to ensure the survivability and development. This case report describes neonatal critical care of a hand-reared dolphin calf, and will provide insights to the field of marine mammal veterinarian.

Keywords: *Tursiops truncates,* common bottlenose dolphin, hand-rearing, nutrient, formulate milk

B29

Established the platform of antimicrobial resistance gene multiplex PCR detected method in aquaculture

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Bacterial diseases cause serious death of aquatic animals and cause economic losses. Nowadays, the prevention and treatment of bacterial diseases is mainly based on the administration of antibiotics. However, due to its cheapness and convenience, antibiotics are often overuse. Improper use not only causes drug residual problems, but also causes drug resistance. Antibiotic resistance gene is diverse and complex. It can be passed on between bacteria. This research aims to design a process for resistance gene screening and to established a platform for aquaculture bacterial drug resistance. We have isolated 70 of bacteria strains that collected from aquatic animals in Pingtung. Testing antibiotic susceptibility includes amoxicillin, erythromycin, doxycycline, florfenicol and oxolinic acid. The resistance genes of the above five antibiotics were chosen from Comprehensive Antibiotic Resistance Database (CARD). We established the gene screening process, sequence arrangement and multiple primer evaluation. Analysis statistical model will be performed by comparing the results of polymerase chain reaction and antibiotic resistance test. It is expected that a rapid detection method of polymerase chain reaction which will replace traditional antibiotic susceptibility test. This will save time more than 24hours and treat the animal more efficiently.

Keywords: Antibiotic, Antibiotic resistant gene, multiple primer

B30

The Postpartum Oral Calcium Supplementation Decreased the Risk of Negative Energy Balance in Third and Greater Parity Holstein Dairy Cows.

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Hypocalcemia was a common problem of postpartum dairy cows. The main reason was secreting colostrum to feed calves after parturition. The huge calcium outflow induced parathyroid hormone (PTH) secreted and activated calcitriol to switch on the mechanism of increasing blood calcium. Hypocalcemia could result to skeletal muscle contraction, smooth muscle contraction and immune function decreased. It would increase the prevalence of ketosis, displaced abomasum and metritis, and all of these disease resulted to decrease appetite. Decreased food intake might increase the negative energy balance period. The prevalence of clinical hypocalcemia was 5%, the prevalence of subclinical hypocalcemia of third and greater parity dairy cows was 47% and resulted 4 times greater economic loss than clinical hypocalcemia. We hypothesized the postpartum oral calcium supplementation could decreased the incidence of subclinical hypocalcemia in third and greater parity Holstein dairy cows. The research animals were the third and greater parity Holstein dairy cows and randomly assigned to control group (0.9% NaCl 1000ml, orally, n=5), powder group (calcium gluconate powder 400g containing 25.93g calcium dissolved in 1000 ml warm water, orally, n=5) or liquid group (calcium gluconate solution 600ml containing 8.05g calcium, orally, n=5). All group gave treatment in postpartum 0 hour and 24 hours, and collected blood sample to analyze total blood calcium in postpartum 3 hours (p3), 6 hours (p6) and 12 (p12) hours, and collected blood sample to analyze biochemistry in postpartum 0 hour (D0), 24 hours (D1), 72 hours (D3) and 168 hours (D7). The result showed no significant difference in serum calcium concentration between all groups. The subclinical hypocalcemia incidence was 40% in all group in D0, and in D7 was 40% (control), 0% (powder) and 0% (liquid). The NEFA concentration of control group in D7 was higher than two experiment groups. (control=562.4±102.9, powder =279.8±122.3, liquid = 314 ±103.2, p=0.003) In conclusion, postpartum oral calcium supplementation might decrease the risk of negative energy balance in third and greater parity Holstein dairy cows..

Keywords: oral calcium supplement, hypocalcemia, dairy cow

B31

Using Hazard Analysis and Critical Control Points to Reduce the Dairy Cow Mastitis in a Farm

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Mastitis is a highly important disease for dairy cattle farmers. The pathogens can be categorized into environmental and contagious bacteria based on the primary reservoir of infections and modes of transmissions. In order to find a good way to manage the occurrence of mastitis in a farm, hazard analysis and critical control points (HACCP) was used to measure the risk factors related to the occurrence of mastitis. From February 2020 to January 2021, milk samples (*n*=62, population=204) and milking procedures (*n*=2) were analyzed and then potential interventions were adopted to evaluate the somatic cell count and the prevalence of mastitis. In addition, the minimum bactericidal concentrations (MBC) of pre-dipping and post-dipping disinfectants were recalculated based on the pathogens identified in this farm. The results showed that to modify the concentrations of disinfectants (MBC_{90} = 250 ppm of chlorhexidine), to adjust the milking procedures can successfully turn contagious mastitis (10/26, 38% = *mycoplasma* spp.) into environmental (8/28, 29% = coagulase-negative staphylococci) and, moreover, into opportunistic (100%, *n*=8). The incidence decreased from 11.2% to 3.9%. In January 2021, the somatic cell count of bulk milk was as low as 156,800 cells/mL. In conclusion, except the traditional methods of re-arrangement of the milking procedure, we believe to adjust the concentrations of disinfectants are also important in helping reduce clinical mastitis in dairy cattle farms.

Keywords: mastitis, dairy cattle, hazard analysis and critical control points, minimum bactericidal concentrations, somatic cell count, milking procedure

B32

Alterations of Coronavirus Genome Structure Caused by Antivirals Affect Viral Gene Expression, Pathogenicity and Adaptation

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With the global threat of coronaviruses, much effort has been focused on treatments and prevention for the disease; however, how coronaviruses react to the treatments and whether the surviving viruses have altered their characteristics are also unanswered questions with medical importance. To this end, bovine coronavirus (BCoV), which is in the same genus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was used as a test model and the findings were as follows. Under the treatment of antiviral remdesivir (GS-5734), the selected BCoV variant with an altered genome structure developed resistance, but its pathogenicity was not increased in comparison to that of wild type (wt) BCoV. Under the selection pressure of innate immunity, the genome structure was also altered; however, neither resistance developed nor pathogenicity increased for the selected BCoV variant. Furthermore, both selected BCoV variants showed a better efficiency in adapting to alternative host cells than wt BCoV. In addition, the previously unidentified feature that the spike protein was a common target for mutations under different antiviral treatments might pose a problem for vaccine development because spike protein is a common target for antibody and vaccine designs. We anticipate that the findings derived from this fundamental research can contribute to the development of antivirals and therapeutic strategies against coronaviruses.

Keywords: coronavirus, remdesivir, innate immunity, spike protein

B33

Dysregulation of the Antigen-Specific Th1/Th2 Immune Balance by Fipronil in OVA-Sensitized BALB/c Mice

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Fipronil (FPN), which belongs to the phenylpyrazole chemical family, is a broad-spectrum insecticide. FPN is widely used in agriculture and veterinary fields. FPN has been revealed as one of the most exceed-standard used pesticides in Taiwan that increases the risk of non-target species exposure including humans. Accidental exposure to FPN could cause a variety of toxic effects on vertebrates including neurotoxic, reproductive, and cytotoxic effects. FPN is characterized as an endocrine disruptor that may affect several non-reproductive tissues, notably the immune system. Besides, prediction by the ChemDIS system, FPN is inferred to be potentially associated with immune-related diseases (DOID:2914). To date, FPN has not carefully examined its impact on immune responses. This study investigated the immunotoxicity of FPN in ovalbumin (OVA)-sensitized mice. Six-week-old male mice were orally given FPN (0.2, 1, 5, 10 mg/kg/day) for 11 days and sensitized by OVA twice. Splenocytes were isolated to evaluate the viability and functionality of antigen-specific T cells ex vivo. Only the high dosage group (10 mg/kg of FPN) demonstrated a slight decrease in cell viability in the presence of concanavalin A (ConA), a T cell mitogen, ex vivo. Notably, there were no significant differences in the production of IL-2, IL-4, and IFN-□ by FPN-treated groups after ConA stimulation. Interestingly, the production of OVA-specificTh1/Th2 cytokines and antigen-specific antibody were significantly dysregulated in FPN-treated groups. The production of IL-2 by OVA-specific T cells was slightly decreased while IFN-□ was significantly increased. In addition, the value of OVA-specific IgG_{2a} was increased in the 10 mg/kg FPN group. The level of mRNA expressions of Th1/Th2 cytokine and related transcription factors was also imbalanced in the FPN-treated group. Collectively, FPN didn't strongly impaired total T cell responses but did affect antigen-specific immune responses. These data suggest that FPN causes non-target species immunotoxicity which induces dysregulation of the antigen-specific Th1/Th2 immune balance in adult mice.

Keywords: Fipronil, antibody, immunotoxicity, concanavalin A, ovalbumin

B34

Establishment of Analytical Methods Validation for Phenol Content in Preservatives for Animal Vaccines

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In order to prevent microbial contamination during the manufacturing process and multi-dose injection. According to the Test Standards for Veterinary Drugs, the preservative content limits for phenol in different types of animal vaccines are 0.5-0.1% or less. In the current methods for testing the preservatives thimerosal and phenol mainly include titration and ultraviolet–visible spectrophotometry. However, these methods lack specificity for complex matrix vaccines. By using high performance liquid chromatography (HPLC) to effectively separate the matrix from the analyte, the accuracy of the test can be improved. The analytical methods developed and established in this study were validated in accordance with the GL02 Analytical Validation Guideline published by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). For the pretreatment condition, Tetrahydrofuran was used as the extraction solution for phenol extraction. Acetonitrile-deionized water (55:45, v/v) was used as the mobile phase at a flow rate of 0.7 mL/min and a Waters Symmetry® C18 (4.6 × 250 mm, 5 μm) column with a detection wavelength of 217 nm was used for analysis. The items required for validation of analytical methods include accuracy, precision, selectivity, robustness, limit of detection (LOD), limit of quantification (LOQ), linearity, and range. Under the optimal conditions, the linear range was 0.005-0.2 mg/mL, the results showed a good linear relationship (R^2= 0.9997), the LOD was 0.001 mg/mL and the LOQ was 0.005 mg/mL. The spiked levels were 0.08, 0.1, 0.12 mg/mL and the average recoveries were 103.20-103.92 %. For precision part, the relative standard deviation (RSD) of day precision was 0.33-0.60 %. The results are in accordance with the standard requirements of the guideline.

Keywords: Animal Vaccines, Phenol, HPLC

B35

Using Filter Papers for Storing Samples and Different Storing Conditions Test

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To find convenient containers to store liquid samples is worth of study, although it is believed samples can be stored at -20ºC or -80ºC. A sterilized filter paper, which can absorb liquid, is considered as a good storage for researchers to transport liquid samples at/under room temperature. However, samples may be decayed. In this study, we used canine core vaccine as the sample to detect the filter paper (Whatman™ No. 1, 90mm filter circle paper) can be used as a good container to store samples at different temperatures. Polymerase chain reaction was used to detect the presence of canine adenovirus type 2 DNA (10^7TCID$_{50}$/mL) in the paper. We dropped different volumes of canine core vaccine on filter papers and then the papers were put in a 2 mL tube at/under room temperature (30, 4 and -20℃) for 1 day, 7 days and 30 days. After DNA was extracted, 100 μL of vaccine (put into 300 μL DDW) can be detected after 24 hours. The limit of detection volume was 20 μL, equivalent to 10^5TCID$_{50}$, and the storage time can up to 30 days at the three different temperature. In conclusion, it seems that liquid samples can be stored at the filter paper at the three temperatures more than 30 days. Further investigations are required to identify whether the results can be used to store animal blood samples.

Keywords: storing, vaccine, canine adenovirus type 2

B36

An Evaluation Study Between PRRSV Infection and Sow Reproduction Performance in A Farrow-to-finish Pig farm in Yunlin County

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Porcine reproductive and respiratory syndrome(PRRS) is a common disease in many pig farms. Serum samples of weaning pigs were examined PRRS virus (PRRSV) is usually determined herd PRRSV infection status. A farrow-to-finish pig farm in Yunlin county is implemented a three-week production system. All pigs are vaccinated with PRRSV vaccine. A total of 9 batches pigs were sampled. Each sampling batch, we collect 3 placentas, 1 pooling testicular tissue sample and 3 oral fluid samples. The PRRSV genome were quantitated by qPCR and M gene was sequenced. The results showed that 2 placenta samples, 2 pooling testicular tissue samples and 4 oral fluid samples were PRRSV positive. The Kappa correlation of detection rates between placenta and tissue fluid samples, between tissue fluid and oral fluid, between placenta and oral fluid are 0.357 (fair), 0.053 (slightly), 0.526 (moderate), respectively. In this study, 4 North American strains and 2 European strains of PRRSV were detected. And 5 samples were detected both European strains and North American strains of PRRSV. The correlation between reproduction performance and infection of PRRSV is poor in this farm. It shows that PRRSV infection is not the only one factor influence reproduction performance in this pig farm.

Keywords: PRRS, reproduction performance, farrow-to-finish pig farm

B37

Investigation of Infection Status of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* in Nursery and Finishing Pigs

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Mycoplasmal pneumonia, also known as swine enzootic pneumonia (SEP), induce clinical signs such as dry cough and reducing growth rate in pigs, with high morbidity and low mortality and common in nursery and finishing pigs. Mortality will rise when secondary bacterial infection and cause economic losses of the swine industry. SEP is mainly caused by *Mycoplasma hyopneumoniae* (MHP). However, *Mycoplasma hyorhinis* (MHR) also can induce gross and microscopic lesions of SEP. In Taiwan, there is currently limited information about the detection rate of MHP and MHR. Therefore, the aims of the present study were to investigate the detection rate of MHP and MHR in nursery and finishing pigs in Taiwan. Two hundred and eight lung samples of nursery pigs and 189 lung samples of finishing pigs were collected from Animal Disease Diagnosis Center in National Pingtung University of Science and Technology and slaughterhouse, respectively. Real-time polymerase chain reaction was used to detect MHP, MHR and GAPDH gene, and relative quantification had been analyzed. In nursery pigs, detection rates of MHP and MHR were 26.4% and 88.0%, respectively. Detection rate of MHR was significantly higher than MHP. In finishing pigs, detection rates of MHP and MHR were 94.7% and 69.3%, respectively. Detection rate of MHP was significantly higher than MHR. Relative quantification ratio of MHR was significantly higher than MHP in nursery pigs and opposite in finishing pigs. It suggested that susceptible age of MHP and MHR are different. The present study showed that MHP and MHR were more often existed in finishing and nursery pigs, respectively.

Keywords: *Mycoplasmal pneumonia, Mycoplasma hyopneumoniae, Mycoplasma hyorhinis*

B38

ApxI Induces Cell Death Involving Akt Attenuation through LFA-1

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Actinobacillus pleuropneumonia causes porcine pleuropneumonia, leading to severe economic loss in swine industry. ApxI exotoxin, a virulence factor of *Actinobacillus pleuropneumoniae*, elicits cell death in leukocytes. Lymphocyte function-associated antigen 1 (LFA-1) consisting of CD 18 and CD11a is expressed mainly on cell surface of leukocytes. Our previous studies showed that ApxI-caused cell death was attenuated by an antibody specific to porcine CD18 (pCD18). Moreover, ApxI reduced the activity of pro-survival signaling proteins, e.g. Akt and FAK. The aim of this study was to investigate the roles of porcine LFA-1 (pLFA-1) and Akt in ApxI-induced cell death. To demonstrate the interaction between pLFA-1 and ApxI, PAM lysate was incubated with ApxI and subjected to pCD18 immunoprecipitation and Western blot analysis. The results showed that bioactive ApxI and porcine CD11a (pCD11a) were co-immunoprecipitated with pCD18. To further investigate the role of each subunit of pLFA-1 in ApxI cytotoxicity, ApxI-insensitive human embryonic kidney 293 (HEK293) cells were transfected with plasmids encoding pCD18 and/or pCD11a, and susceptibility toward ApxI was analyzed. The results showed that cells co-transfected with pCD18/pCD11a were the most sensitive to ApxI, followed by those transfected with pCD18 only. Cells transfected with pCD11a only exhibited similar susceptibility toward ApxI as comparing to control vector transfected cells. Subsequently, to study the effect of ApxI on Akt activity in pLFA-1-transfectants, cells were treated with ApxI, and cell lysates were collected and subjected to Western blot analysis for phosphorylated Akt^{S473}. The results showed that the phosphorylation level of Akt^{S473} decreased in response to ApxI, indicating that ApxI downregulated Akt activity in pLFA-1 transfectants. To investigate the role of Akt on ApxI cytotoxicity, HEK293 cells were transfected with plasmids encoding pLFA-1 along with or without a plasmid encoding Akt, and susceptibility toward ApxI was assessed. The data demonstrated that Akt/pLFA-1 transfected cells were less sensitive to ApxI than pLFA-1 transfected cells. In summary, this study demonstrates an interaction between ApxI and pLFA-1. ApxI cytotoxicity is mainly mediated through pCD18, while pCD11a might have a synergistic role in this effect. ApxI-induced cell death is in part through the attenuation of Akt activity.

Keywords: *Actinobacillus pleuropneumoniae*, ApxI, lymphocyte function-associated antigen 1, Akt

B39

Isolation and Genetic Characterization of A/H1N2 Influenza Viral Reassortants from Taiwanese Swine Populations

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A continued active surveillance programme for influenza activities in pig herds has been launched since July 1998. We collected nasal swabs from pigs in swine farms and auction markets. We performed virus isolation in Madin-Darby canine kidney cells and chicken embryonated eggs, respectively. There are 125 A/H1N2 virus isolates until the end of 2020. We determined the type and subtype by immunofluorescence antibody staining, hemagglutination inhibition and multiplex reverse transcription-polymerase chain reaction (RT-PCR). We used the DNASTAR software to compile and compare the nucleotide sequences to the reference viruses retrieved from the public available sequence database (GenBank and GISAID), followed by the direct sequencing of RT-PCR products. We used the Molecular Evolutionary Genetics Analysis (MEGA) software to estimate the phylogenetic relationships among several related viruses with the method of maximum parsimony. Our results indicated that before 2009, the human-swine influenza double reassortants of A/H1N2 subtypes dominated the majority of influenza outbreaks in Taiwanese swineherds. The nucleotide sequence identity of the hemagglutinin (HA) and neuraminidase (NA) genes are best similar with the 1980s' North American classical swine H1N1 virus and the 1980s' human H3N2 virus, about 92%, and 92%, respectively. It implies that the older version of classical swine H1 HA gene and human influenza N2 NA genes may persist in the current swine influenza viruses among swine populations more than 20 years. In case a human is infected with this kind of virus, the cross-protection of the current seasonal influenza vaccine would not be enough for the human being and especially the young children in Taiwan. After the invasion of the pandemic (pdm) H1N1/2009 influenza virus into swine populations in Taiwan followed by major human outbreaks since early 2009, two different constellations of genes from the human-swine double reassortants of A/H1N2 and the pdm/2009 H1N1 virus were further reassorted to generate several kinds of the viral progeny of human-avian-swine influenza triple reassortants. One is the 2+6 constellation that H1 HA and N2 NA genes from the human-swine influenza double reassortants of A/H1N2 virus and the other 6 internal protein genes from the pdm/2009 H1N1 virus. The other is the 1+7 constellation that the N2 NA gene comes from the human-swine influenza double reassortants of A/H1N2 virus and the other 7 genes are originated from the pdm/2009 H1N1 virus. Once again, the older version of classical swine H1 HA gene and human influenza N2 NA genes have been passed into the genome of new emerging viral progeny. In addition, the triple reassortant internal protein gene cassettes of the pandemic H1N1/2009 influenza virus had contributed to the emergence of several swine influenza viral variants in Taiwan. Therefore, it may potentiate the high risks of zoonotic infections for humans. In conclusion, the possible origins of genes in those new reassorted A/H1N2 viruses are included the 1980s' North American classical swine H1N1 virus, 1980s' Taiwanese human H3N2 virus, and the pandemic H1N1/2009 influenza virus. The results suggested the urgent need for monitoring the future activities of those A/H1N2 influenza viral reassortants in Taiwanese pigs.

Keywords: Reassortment, Zoonoses, Emergence

B40

Evaluation of the Adjuvancy of Flagellin N Terminal When Fused to An Infectious Bursal Disease Virus Antigen

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Receptors of the innate immune system, Toll-like receptor 5, and neuronal apoptosis inhibitory protein 5, signal in response to bacterial flagellins. Flagellin has two conserve domains D0 and D1, two variable D2 and D3. N terminal conserve domains D0 and D1 was found to have more role in eliciting TLR5 immune activity. And provide substantial support to primary and secondary dimerization compare to the C-terminal domain. Structural studies explain that N -terminal domain almost 70% support TLR5 binding and signaling, while the C-terminal(D1, D0) only support primary binding with TLR5. Flagellin domains are studied extensively regarding their structure and function. Yet domains(D0, D1) are not applied individually to antigen as the chimeric product. This study put light on the N-terminal role in immune activation and protection from lethal viral challenges when applied with IBDV antigen. In this study, our focus will combine the conserved domains from the N terminus (1-176) and hypervariable domain (contain neutralizing antibody epitopes) from VP2 of infectious bursal disease virus. PCR cloning performed to construct a chimeric vaccine, followed by the expression of the protein by the E. coli system. Lastly, we evaluated the immune efficacy and protection offered by these constructs. Immune response verified by the cytokine analysis specific to TLR5 and NLRC4/NAIP5 activation. Meantime, cytokines related to infectious bursal diseases were evaluated and compared with the antigen-only and control groups. Analysis of antibody response and cell proliferation assay elaborate on the humoral and cellular nature of immune response characteristic to TLR5. In vitro neutralization assay help to understand the capability of our product to cope with a lethal virus. Lastly, a challenge test will be performed to detect the protection offered by these constructs in natural field conditions.

Keywords: Infectious Bursal Disease Virus, Immunosuppression, flagellin, adjuvant, N-terminus, chimeric protein.

B41

Development of subunit vaccine against Infectious Bursal Disease Virus (IBDV) using flagellin as adjuvant

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Infectious bursal disease virus (IBDV) is a highly pathogenic virus that causes immunosuppressive disease in the chicken. IBDV targets the bursa of Fabricius and depletes the B cell, leading to bursal atrophy and immunosuppression. IBDV compromises both humoral and cellular immunity making chickens prone to other secondary infections. The disease-causing higher morbidity and mortality in the young chickens. Flagellin is the part of the bacterial flagella used for locomotion and also served as an important component to initiate an innate immune response in the host. Flagellin protein from *Salmonella* Typhimurium is recognized by the Toll-like receptor 5 (TLR5) of the host and activates the signaling pathway to stimulate the activation of the Toll-like receptor 5 which in turn activate the immune system, so it may act as a vaccine adjuvant. In this study, we evaluated the adjuvant effect of the N-terminus of flagellin (residues 1–99) when linked to an antigen (truncated VP2 protein of the infectious bursal disease virus residues 1-158). The chimeric antigen-adjuvant was used to immunize the chicken and evaluation of the immune response was done using different immunological assays. The chimeric antigen-adjuvant group showed an increased level of the cellular immune response and the antibody response was also higher. In this study, we concluded that the N-terminus of the flagellin act as an immune activator and significantly raise the level of protection against the pathogen.

Keywords: Infectious Bursal Disease Virus, Immunosuppression, flagellin, adjuvant, N-terminus, chimeric protein.

B42

Discussion on the Detection of Mycotoxins in Turkey Feed

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Mycotoxins are commonly found in animal feeds. If animals eat feed contaminated with mycotoxins, they will decrease appetite and affect growth. This experiment mainly monitored a turkey farm that implementing HACCP, and we collected each batch of feed for detecting deoxynivalenol (DON, also known as vomitin) or zearalenone (ZEA, also known as F2). The sample collection period was from May 2020 to April 2021, with a total of 41 samples. The samples were collected once a week and stored in the refrigerator at -20 °C. After detecting by DON (1000 ppb) and F2 (500 ppb) rapid test kit, high performance liquid chromatography (HPLC) or liquid chromatography with tandem mass spectrometry (LC-MS/MS) would be performed for further testing to quantify the mycotoxins in feeds. There were 7 positive samples and 2 suspected positive samples. After HPLC analysis, the highest concentration of F2 was 2464.79 ppb and the mean concentration was 1634.56 ppb. Another suspected positive sample after LC-MS/MS analyzing was found that the feed contained Fumonisin B1, 51 ppb; Fumonisin B2, 24 ppb; Zearalenone, 9 ppb; Vomitin, 93 ppb. The Rapid test Kit could be completed within 10 minutes, which is very suitable for on-site workers.

Keywords: Mycotoxins, deoxynivalenol, zearalenone, Rapid Test Kit, HPLC, LC-MS/MS

B43

The Sequences at the N-terminal Region of the Hemagglutinin Play A Rey Role in Determining the Serotype of *Avibacterium paragallinarum*

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Avibacterium paragallinarum has been subtyped into three serogroups (A, B and C) and nine serovars (A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3 and C-4) according to the Page and Kume schemes. Both schemes use the hemagglutination inhibition (HI) test for serotyping. However, the relationship between the hemagglutinin gene (*HMTp210*) sequences and serotypes of *A. paragallinarum* is still unclear. This problem is partly due to the lack of information on the complete *HMTp210* sequence from the formal reference strain of Page serogroup B (strain 0222 or Spross). In this study, we determined the complete *HMTp210* sequence of strain Spross. The sequence of Spross and those of other *HMTp210* sequences retrieved from GenBank were used to conduct phylogenetic analyses to investigate the relationship between the serotypes and *HMTp210* sequences of *A. paragallinarum*. Four phylogenetic clusters, designated clusters A-1, A-2, B and C, were identified. Clustering based on complete *HMTp210* sequences correlates with serotyping based on HI tests. Serovar A-2 was found to contain a chimeric *HMTp210* gene that might have resulted from recombination between serovar A-1 and serovar C-1. In addition, phylogenetic analysis based on partial sequences (approximately nucleotides 1–1200) of *Hmtp210* was sufficient to discriminate among serogroups A, B and C. These findings could be valuable for developing a molecular method for serotyping of *A. paragallinarum*.

Keywords: *Avibacterium paragallinarum*, infectious coryza, serotype, hemagglutinin

B44

Identification and Antibiotic Sensitivity Tests of Bacterial Isolates from Cloacal Swabs of Domestic Turkey

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The present study was to focus on the isolation, identification, of bacteria from cloacal swabs of turkeys during the period from June 2020 to April 2021. The samples were sent immediately and carefully under 4℃ for the incubation, identification and antibiotic susceptibility test of the pathogenic bacteria. The samples were immediately and carefully sent to lab under 4℃ for the incubation. By the basis on the colony's morphology, Gram's stain, biochemical tests, it came out that among the 29 isolates from 21samples, 10(34.5%) were *E. coli*, followed by 8(27.5%)*Providencia staurri*, 6(20.6%)*Proteus mirabilus* and 4(13.8%)*Salmonella spp*. In addition, there was 1(3.4%) *Pasteurella multocida* detected, which further research is needed. Antibiogram profiles showed that all isolates of *E. coli* were susceptive to Gentamicin, and 9(90%) were susceptive to Ceftiofur. Meanwhile, Ceftiofur was susceptive to all isolated of *Providencia staurri*, followed by Gentamicin, Kanamycin, and Enrofloxacin which were 87.5, 87.5 and 67.5%, respectively. On the other hand, all isolates of *Proteus mirabilus* were susceptive to Ceftiofur, while 4(66%) isolates were susceptive to Gentamicin, and 3(50%) were susceptive to Neomycin. Additionally, it was found that all the 29 isolates appeared resistant to 3 or more kinds of antibiotics. To sum up, this study provides a baseline data on the antibiogram of the bacteria in turkeys' intestine, which may help veterinarians in bio-safety protocols and the rational use of antibacterial drugs for the treatment in turkey farm.

Keywords: Turkey, bacteria, antimicrobial drug, cloacal swab, antimicrobial susceptibility test.

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B45

Prevalence Investigation of Viral Pathogens in the Reproductive System of Spent Hens in Yunlin-Chiayi-Tainan Region of Taiwan

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Avian Metapneumovirus (aMPV), Egg drop syndrome virus (EDSV), and Infectious bronchitis virus (IBV), are viral pathogens which affect eggs production and quality of layers. This study has detected prevalence of aMPV, EDSV and IBV in spent hens among commercial layers and breeders of colored broiler from 35 farms, which located in Yunlin-Chiayi-Tainan region of Taiwan. During the period from March 2018 to April 2019, we collected the blood samples and the reproductive system samples to detect virus nucleic acid and antibody against of aMPV, EDSV and IBV. In addition to comparing the positive rate between difference ages, regions and species of poultry, the reproductive system had been divided into five segments: ovary, infundibulum, magnum, isthmus and shell gland. The flock's positive rates of aMPV, EDSV, and IBV were 28.6%, 94.2%, and 14.3%, respectively. No significant effect of age, regions, or seasons was identified on aMPV, EDSV and IBV infection. Comparing the five segments of the reproductive system, there was no significant difference in the prevalence rate of aMPV, EDSV, and IBV ($p=0.3422$, $p=0.6918$, $p=0.8239$). However, the positive rate of IBV had a significant correlation with bird species ($p < 0.05$). The serum antibody titers (GMT) of aMPV and IBV were 4524 and 5251, and the HI titers (log2) of EDSV were 5.2, respectively.

Keywords: *aMPV, EDSV, IBV, PCR, antibody, prevalence, reproductive system*

B46

Effects of Plant Extracts in Feed Supplementation on the Growth Performance and Coccidiosis of Chickens

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In commercial broiler production, the birds are exposed to various stress factors which induces immune-suppression. This leads to a higher chance of infection with opportunistic pathogens, bacteria, virus, as well as coccidiosis. The purpose of this study is to investigate the effect of plant extracts supplementation on the growth performance and coccidiosis in chickens. A total of one hundred and twenty black native broilers was randomly assigned into three dietary groups: control, polyether ionophores group (Nicarbazin, 125 ppm or Salinomycin, 66 ppm) or plant extracts group (citrus fruits and saponin-containing plants, 400 ppm) for twelve weeks. Water and feed were provided *ad libitum*. Chicken in early stage (3-6 weeks old) are susceptible to chicken coccidiosis. Thus, *Eimeria* challenge on chickens was performed on day 38. Growth performance, fecal oocyst excretion, microscopic oocyst count in intestinal tissues and lesion score were assessed. Statistical analysis was performed in groups between treatments and controls. The results showed that supplementing plant extracts in basal diets with slaughter weight of 2,094.50±190.96 g, daily weight gain of 24.54 g, and feed conversion ratio of 2.83. The supplementation groups were on par with coccidiostats (polyether ionophores) treatment. Fecal oocysts in plant extracts group on day 44 were fewer than control and polyether ionophores group. Although secondary infection was observed on day 59 in polyether ionophores group and plant extracts group with oocysts detectable in the feces, the number of excreted oocysts were higher in polyether ionophores group than in plant extracts group and there was no mortality in the flocks. In conclusion, supplementing plant extracts in chicken diets help prevent the outbreak of coccidiosis and the effects were on par with coccidiostats (polyether ionophores). Furthermore, addition of plant extracts improves growth performance in chickens. With proper animal management and biosecurity control, applying plant extracts benefit chicken industry in improving growth performance and preventing coccidiosis.

Keywords: polyether ionophores, plant extracts, coccidiosis, native chickens

B47

The Influences of Recombinant Avian Influenza Viruses NS Segment on Viral Replication and Host Immune Response.

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Influenza A viruses (IAV) are important pathogens with worldwide prevalence and possesses the potential of pandemic. Influenza virus triggers both intracellular and extracellular receptors during infection to elicit host innate immune responses. Cytokines, which fight against infection of influenza virus, are triggered to recruit immune effector cells and facilitate intracellular control of viral replication through induction of interferons, interferon mediated antiviral signaling, and interferon-stimulated genes (ISGs). The influenza virus non-structural protein 1 (NS1) is a common factor, by which influenza A viruses antagonize host immune responses and contribute towards efficient virus replication and virulence. According to previous studies, the functions of NS segments from different virus strains emerged in Taiwan played crucial roles in affecting virulence and host adaptation of avian influenza viruses, independent from the HA and NA proteins. To investigate the influences of NS genes on viral replication and innate immune responses of hosts, two virus strains, A/chicken/Taiwan/0702/2013 (H6N1) and A/Taiwan/1/2017 (H7N9), were selected to generate reassortant viruses under the backbone of A/chicken/Taiwan/0702/2013. H6N1 virus is the enzootic subtype in Taiwanese chicken farms which can be isolated frequently in poultry, while the H7N9 virus causes human cases of acute lower respiratory tract infection and deaths from 2013 to 2017. Moreover, two recombinant viruses containing different portions of the NS segments were rescued to investigate the functional changes caused by the replacement of different parts in NS gene segments. The results showed that the replacement of the whole part of NS1 effector domain of A/Taiwan/1/2017 into parental A/chicken/Taiwan/0702/2013 restored the abilities for viral replication and enhanced the RNA-dependent-RNA polymerase (RdRp) activity. Intriguingly, the replacement of the N-terminus of NS1 of A/Taiwan/1/2017 into parental A/chicken/Taiwan/0702/2013 restored the abilities for viral replication but did not enhanced the RdRp activity. The inhibition of host innate immune responses of the NS1 proteins did not show significant differences, and high cytokine induction was detected in the recombinant viruses infected cells. The precise mechanism that contributes to the viral replication of these recombinant NS1 proteins and the interactions of host cytokine induction require further investigation.

Keywords: Avian influenza virus, NS1 protein, Immune response

B48

Erythromycin, Tetracycline Resistance and Transposon Genes in the Indigenous Plasmid pTE15 of *Lactobacillus reuteri* Isolated from Gastrointestinal Tracts of Native Chicken in Taiwan

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Lactobacillus reuteri was the dominant lactic acid bacteria in the gastrointestinal (GI) tracts of native chickens in Taiwan. It could produce a board antimicrobial agent – reuterin, which was the intermediate from glycerol metabolism in anaerobic fermentation. The antimicrobials susceptibility test shown the isolated *L. reuteri* had 75.0% erythromycin resistance and 98.1% tetracycline resistance that was a serious public health problem concerned with the dissemination of antimicrobials resistant gene. Therefore, the goal of this study I identify a theta-type replication plasmid pTE15 from isolated strain *L. reuteri* N16 and to analyze the 15.3-kb full-length DNA sequence. The results were shown a total of 17 open reading frames (ORF) in pTE15 by ORF prediction software. In seventeen ORF had 7 unknown functional proteins, ORF4 was transposase for genetic elements movement, ORF10 was rRNA methylase for acquired erythromycin-resistant activity, ORF13 was tetW for acquired tetracycline-resistant activity, ORF17 was integrase/recombinase for genetic elements recombination. Otherwise, the ORF6 and ORF7 were RepA and RepB but they were not essential to pTE15 replication that was identified by replication region assay. From the genetic structure of pTE15 we conclude, the *L. reuteri* isolated from GI tracts of chicken had highly antimicrobials resistant especially to erythromycin and tetracycline because the indigenous plasmid carried the antimicrobial resistance gene and genetic elements movement transposon.

Keywords: *Lactobacillus reuteri*, antimicrobials resistance genes, transposon

B49

Using infrared thermography on early post hatch in chicks with pathogens-infection

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It's a high opportunity that chick born in coccidiosis-infected farms have worse performance with increased cost in the feeding of poultry. It's an important issue to efficiently cull serious *Eimeria tenella*-infected early post hatch chicks and ignite anti-coccidial therapy base on techniques or methods of early diagnosis in order to reduce coccidiosis pollution range. In our experiment of *E. tenella*-infected early post hatch chicks, *Eimeria tenella* infected (ET) group showed occult blood response, bloody cecum stool and decreased packed cell volume compared to non-infected group (CTR) on the same duration. Hypothermia occurred before these above former lesions lesion (3 days post-infection) and final diagnosis with oocyst release (4~5 days post-infection). As previous mention, linked early diagnosis and treatment on avian coccidiosis are a key technique on chicken farms management control. *E. tenella*-infected young chick with significant lower body temperature induced by above ongoing course of sick bird syndrome and related lower food intake ability can be early detection with infrared thermography (IRT) at first day after *E. tenella* infection. To sum up, using IRT, an artificial intelligence technique, on early post hatch chicks with *E. tenella* infection can provide one effective reference indicator on avian disease control to maintain precision agriculture.

Keywords: artificial intelligence, avian disease, early diagnosis, infrared thermography

B50

Cloning and characterization of *Agapornis fischeri* IFN gamma (IFN-γ) and its anti-beak and feather disease virus activity

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The interferon-γ (IFN-γ) gene of *Agapornis fischeri* was cloned and then subjected to the sequencing analysis. The coding sequence of the IFN-γ gene was composed of an open reading frame of 498 bps which encoded the IFN-γ protein of 165 amino acids. All IFN-γ genes of parrots were clustered as an independent clade in the phylogenetic tree. The IFN-γ gene of *Agapornis fischeri* was split from that of *Melopsittacus undulates*. The IFN-γ gene of *Columba livia* was the closet to those of parrots among avian species and the IFN-γ gene of *Mus muscullus* had the closest genetic distance with those of parrots among mammalian species. The IFN-γ protein of *Agapornis fischeri* shared the SDVA motif, the KRKRS motif, and the histidine at the residue position 140 with those of some species. The Raw264.7 cells treated with the expressed IFN-γ protein led to the up-regulation of the iNOS protein expression and NO production, the promotion of the phagocytosis and pinocytosis, and the induction of the interferon-stimulated genes through the activation of the NF-κB factor which represented the innate immune responses of the activated macrophages. Similar to the IFN-γ protein of other species, the activity for the NO production of the parrot IFN-γ protein reduced 80% after the exposure at 60 °C for 4 min. Besides, only a half of the activity for the NO production of the parrot IFN-γ protein remained after the exposure to HCl for 30 min. These suggested that the parrot IFN-γ protein was heat labile and sensitive to the acidic condition. Thus, all these effects contributed to the blockage of the uptake of BFDV (virus-like particle) VLP by cells, the nuclear entry of the Cap protein of BFDV VLP, and the clearance of virus from BFDV-infected parrots by the IFN-γ of *Agapornis fischeri*. This report firstly described the cloning of IFN-γ gene of *Agapornis fischeri*, characterization of the activity of the IFN-γ protein *in vitro*, and anti-beak and feather disease virus activity of the IFN-γ protein of *Agapornis fischeri*.

Keywords: Interferon, parrot, virus live partide

Index (presenting author)

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