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目 錄

口頭論文競賽組與非競賽組(OA and OB).....	4
壁報論文競賽組(PA).....	18
壁報論文非競賽組(PB).....	35



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口頭論文競賽組與非競賽組 (OA and OB)



口頭論文競賽組

編號	篇名與作者	頁碼
OA1	Constructing STR Profiles and Genetic Polymorphism Analysis of Pitbull-type Dogs in Taiwan Using Commercialized Canine Genotype Kits <u>Ann Nee Lee</u> ¹ , Wei-Hsiang Huang ^{1*}	6
OA2	Application and Limitation of a Blocking ELISA based on FMD VLP and Neutralizing Monoclonal Antibodies <u>Heng-Wei Lee</u> ¹ , Ivan-Chen Cheng ^{1**}	7
OA3	Detection, Isolation, and Molecular Characterization of the First Detected Local Case of Pigeon Rotavirus A Infection in Taiwan <u>Benji Brayon Ilagan Silva</u> ¹ , Jaymee R. Encabo ² , Kuo-Pin Chuang ^{1,3*}	8
OA4	Transplantation of Allogeneous Mesenchymal Stem Cells in Renal Subcapsular Space via a CompanionPort™ Device is Feasible to Treat Feline Chronic Kidney Diseases <u>Chuan-Te Kuo</u> ¹ , Lee-Shuan Lin ¹ , Cheng-Shu Chung ¹	9
OA5	Biomarker Characterization of Three <i>Eimeria</i> spp. Isolated from Domestic Chickens in Taiwan by MALDI-TOF MS <u>Hsyang-Hsun Chung</u> ¹ , Ming-Chu Cheng ¹ , Yi-Lun Tsai ¹ , Jentaie Shiea ² , Yi-Yang Lien ^{1*}	10
OA6	Efficacy of Herb-TF Against <i>Eimeria tenella</i> in Layer Chickens <u>Hsyang-Hsun Chung</u> ¹ , Ming-Chu Cheng ¹ , Yi-Lun Tsai ¹ , Meng-Shiou Lee ² , Yi-Yang Lien ^{1*}	11

OAI

Constructing STR Profiles and Genetic Polymorphism Analysis of Pitbull-type Dogs in Taiwan Using Commercialized Canine Genotype Kits

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Due to the rising cases regarding the aggression of pitbull-type dogs in Taiwan, the Council of Agriculture, Executive Yuan, has banned American Pit Bull Terrier and American Staffordshire Terrier from being owned, imported or exported under Animal Protection Law on the 26th of October. Standards of breed identification must be formulated to aid the implementation of the legislation in the future. Pitbull-type dogs encompass purebred dogs of various breeds and dogs presumed to be mixes of those breeds. Visual breed identification among breeds is often shown to be inaccurate. Thus, a more objective method, such as molecular markers, should be considered. Single Nucleotide Polymorphism (SNP) and Short Tandem Repeat (STR) of nuclear DNA have been described in animal breed-related studies and have revealed promising results. To identify a breed, a reference population is required, and there is little knowledge of the pitbull-type dog population in Taiwan. Therefore, this study aims to assess visual breed identification via body measurement ratio, determine the population structure and relatedness, and construct STR profiles of pitbull-type dogs in Taiwan for future molecular technique establishment for canine breed identification. A total of 81 dogs were included in the study, and saliva swabs were taken from each dog. The breeds of dogs in the current study are confirmed via SNP breed identification by Embark Veterinary Inc. Based on guidelines of The Dangerous Dogs Act (DDA) 1991 (UK), body measurement ratios of 55/81 dogs are taken. Results show that 4 to 5 out of 6 parameters are not specific to pitbull-type dogs, indicating that visual identification of breeds is unreliable, which corresponds to previous studies. 22% of American pitbull terriers and 50% of mixed pitbull-type dogs are related by one generation and are omitted from the following analyses. Genetic polymorphism analysis showed that the observed heterozygosity (H_o) of American pitbull terriers is higher than longer-established breeds such as Bedlington terrier and lower than Korean dog breeds such as Poongsan and Formosan Mountain dogs. Using individual assignment tests, 45% and 52% of individuals are successfully assigned to the right population by Bayesian statistical method and allele frequency method, respectively. In conclusion, there is high relatedness among the pitbull-type dog population in Taiwan. An STR profile and basic pitbull-type dog breed identification system can be established using the commercial Canine Genotype kits.

Keywords: Pitbull-type dogs, STR profile, genetic polymorphism analysis, veterinary forensic science

OA2

Application and Limitation of a Blocking ELISA based on FMD VLP and Neutralizing Monoclonal Antibodies

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Foot-and-mouth disease virus (FMDV) is a notorious infectious agent composed of 60 repeat units of protomer (including VP1, VP2, VP3, and VP4). The serum neutralization (SN) test, the "gold standard" for seroconversion following FMDV vaccination, requires a high-level biosafety laboratory. Therefore, several scientists aimed to develop an ELISA system as an alternative. Since there are five neutralizing sites (sites 1-5) on serotype O FMD virions, our initial goal is to develop a blocking ELISA (bELISA) without viruses and detect specific neutralizing antibodies in animal serum. Given authentic antigenicity, diagnostic antigens were virus-like particles (VLPs) and unprocessed protomers (polyproteins, P1) in our bELISA format. We chose four self-produced monoclonal antibodies (MAbs) for tracers: site 1 Q10E, site 2 P11A, site 3 S11B, and a non-neutralizing MAb, TSG. After evaluation, VLP pairing with S11B showed the highest correlation toward SN titers ($R^2 = 0.8071$, $n=63$), and P1 pairing with Q10E also showed a high correlation ($R^2 = 0.768$). However, S11B as a site 3 antibody is the best tracer does not mean the concentration of site 3 antibodies in vaccinated animals can reflect SN titer. We found that the bulk of MAb is too large compared with a protomeric unit, indicating that it is impossible to precisely detect neutralizing antibodies when using MAb as a tracer. However, our results still provide convincing support for the application of two pairs of bELISA systems, VLP: S11B and P1: Q10E. In conclusion, in this talk, I would like to share the process of developing a bELISA considering the "authentic antigenicity" of antigens and the "steric effect" of antibodies.

Keywords: foot-and-mouth disease virus, neutralizing monoclonal antibody, blocking ELISA

OA3

Detection, Isolation, and Molecular Characterization of the First Detected Local Case of Pigeon Rotavirus A Infection in Taiwan

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Since the first identification of the novel pigeon Rotavirus A genotype G18P[17] infections in 2016 in Australia, increasing number of epidemic outbreaks and case reports highlighted the importance of this previously undetected pathogen. The disease is characterized as an acute illness associated with hepatic necrosis, and a clinical presentation consistent with the young pigeon disease syndrome. The virus has been reportedly detected in Germany, Belgium, Denmark, Great Britain, and the United States. Cases of infections were described to lead to high morbidity and mortality rates of up to 50%. Aware of the local cultural and economic significance of pigeons and pigeon racing in Taiwan, locally archived liver samples from submitted pigeons and fecal samples from local lofts collected from 2018 to present were also tested for the presence of Rotavirus A infection. Detection by polymerase chain reaction revealed a positive case for the virus infection. Isolation of the virus from the organ sample was conducted using different cell lines (QT35, MDBK, Vero, MARC-145). A SYBR Green-based quantitative real-time polymerase chain reaction protocol was optimized to monitor the viral titer during the serial passage for the isolation. Ct values (RNA copies) obtained from the supernatant of the cell cultures confirmed replication of the virus in MARC-145 cell line. Electron microscopy of the supernatant revealed organized structures resembling rotaviral particles. Sequencing of several viral genome segments confirmed the identity of the isolate, and therefore the local presence of the pigeon Rotavirus A. Phylogenetic analyses showed high similarity of the sequence of the local isolate with previously reported epidemic strains.

Keywords: hepatic necrosis, pigeons, Rotavirus A, viral epidemiology, young pigeon disease syndrome

OA4

Transplantation of Allogeneous Mesenchymal Stem Cells in Renal Subcapsular Space via a CompanionPort™ Device is Feasible to Treat Feline Chronic Kidney Diseases

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Cats are prone to suffer from chronic kidney disease (CKD) among companion animals and the prevalence increases with age. However, it is a progressive disease and incurable by current therapies. Mesenchymal stem cells (MSCs) have been shown the potency to repair injured kidneys in recent studies and the renal subcapsular space with an abundant blood network may provide an appropriate transplantation site. Further, multiple treatments may contribute better outcomes. Therefore, a subcutaneous vascular access port, CompanionPort™, was applied in this study for evaluating its feasibility of multiple deliveries of MSCs on a cat with CKD. A 10-year-old male intact mixed-cat infected with feline immunodeficiency virus and stage 4 CKD (IRIS) was diagnosed. A CompanionPort™ Device with a subcutaneous titanium access port was implanted into subcapsular space of left kidney by lateral flank approach. Then, 5×10^6 allogeneous omentum adipose-derived MSCs suspended in 1ml normal saline was injected through the access port right after the surgery and followed by the 2nd day, 4th week, and 8th week. Results showed the BUN and creatinine levels were decreased and the stage 3 CKD was attained after treatment. Besides, the cat tolerated the implants well and related side effects were not seen. The cat was euthanized 1 year later due to acute-on-chronic renal failure. Although a focal, chronic, moderate granuloma formation on transplantation site was noted through necropsy, the severity of interstitial nephritis was similar in both kidneys. This study indicated multiple transplantation of MSCs in renal subcapsular space through the CompanionPort™ device is feasible to treat feline CKD.

Keywords: Chronic kidney disease, CompanionPort™, Feline, MSCs, Renal subcapsular space

OA5

Biomarker Characterization of Three *Eimeria* spp. Isolated from Domestic Chickens in Taiwan by MALDI-TOF MS

Hsyang-Hsun Chung¹, Ming-Chu Cheng¹, Yi-Lun Tsai¹, Jentaie Shiea², Yi-Yang Lien^{1*}

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The high temperature and humidity in Taiwan can easily lead to the outbreak of chicken coccidiosis. There are a total of seven species of coccidiosis, all of which have been found and isolated strains have been purified. Matrix-assisted laser desorption free time-of-flight mass spectrometry (MALDI-TOF MS) is a technique that has been mostly used in microbial identification and research in recent years. Based on the establishment of the bacterial species biological database, it is determined by combining the data of morphology and molecular biology, which is a powerful tool for the rapid identification of microorganisms. In this study, MALDI-TOF MS was used to analyze the oocyst whole protein of chicken coccidia isolates (*E. tenella*, *E. maxima* and *E. acervulina*), and try to find out the distinctive peaks. In this study, the ITS1 primers and appearance morphology were used to identify the coccidia isolates confirm the isolates were not contaminated. Continue to compare the matrix type used in MALDI-MS with the ratio of matrix to sample mixture, and find the most suitable matrix type and mixing ratio. The results show that when matrix 2,5-DHB and dilution ratio 1 : 20, the best mass spectral signals can be obtained. The results showed that the major peaks of the three coccidial oocysts were mostly concentrated between 3000 (m/z) and 11000 (m/z), and most common peaks appeared between 3500 (m/z) and 5500 (m/z). Most common peaks occur between 3500 (m/z) and 5500 (m/z). Some species-specific peaks were also found in the results. For example, peak 3278 (m/z) was only found in *E. acervulina*, while peak 3751 (m/z) was only found in *E. tenella*. These specific peaks have the potential to be biomarkers for identifying this species of coccidia.

Keyword: Biomarker 、coccidiosis 、MALDI-TOF MS

OA6

Efficacy of Herb-TF Against *Eimeria tenella* in Layer Chickens
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Chicken coccidiosis is an important disease with great impact in the poultry industry, among which *E. tenella* has the greatest impact. The methods of preventing the development of coccidiosis include the use of anticoccidial drugs, vaccines and natural anticoccidial plants extracts. Anticoccidial drugs are the cheapest and most commonly used methods for treating chicken coccidiosis, but they also often lead to the emergence of drug resistance. Herb-TF is a natural organically grown edible herb, traditionally used for antibacterial and antiviral use. In previous experiments in our laboratory, the scientific Chinese medicine of herb-TF has been used as feed addition, and it has been proved that it can alleviate the infection symptoms after challenge with *E. tenella*. In the in vivo experiment, the natural organically grown herb-TF whole grass extract was used as a feed additive to observe its control effect on *E. tenella*. The main observation indicators in the test are: cecal segment lesion score, relative weight gain rate, oocysts per gram of feces and other items to be evaluated. The results showed that compared with the competitive products, each group using herb-TF had obvious improvement, indicating that herb-TF has anti-coccidial potential. Continue to extract herb-TF with organic solvent, and observe the effects of various extractions of different polarities on the oocysts of *E. tenella* and *E. acervulina*, and found that the herb-TF extract with high polarity solvent has a better effect on the oocysts, indicating that the extraction of herb-TF has high anticoccidial potential. The extract was analyzed by gas chromatography-mass spectrometry (GC-MS) and compared with the built-in database. The results showed that dozens of obvious peaks could be identified, which may have anti-coccidial potential substances.

Keyword: chicken coccidiosis, herb, oocyst

口頭論文非競賽組

編號	篇名與作者	頁碼
OB1	Developing the Safety Assessment and Risk Management Techniques of Genetically Modified Animal Biologics <u>I-Ting Ko</u> ^{1*} , Chun-Ta Lin ¹ , Chia-Chen Chang ¹	13
OB2	Development of an Oil Emulsion Vaccine against Bovine Ephemeral Fever <u>Chiu-Hui Lin</u> ^{1*} , Yen-Lin Lee ¹ , I-Hsiang Lai ² , Chia-Yi Chang ¹ , Chwei-Jang Chiou ¹	14
OB3	Emergency Surveillance on Coronaviruses and Pathogenic Microorganisms in Wildlife in Taiwan <u>Yen-Wen Chen</u> ¹ , Wei-Cheng Hsu ¹ , Shu-Chia Hu ¹ Yang-Chang Tu ¹ , Yu-Liang Huang ² , Ming-Chung Deng ² , Fan Lee ^{1*}	15
OB4	The Emergence of Decapod Iridescent Virus 1 in Cultured Shrimp from Taiwan in 2020 <u>Yi-ping Lu</u> ^{1,2} , Chien Tu ^{1*} , Chieh-hao Wu ¹ , I-wen Chen ¹ , Ming-chu Cheng ^{2*}	16

OBI

Developing the Safety Assessment and Risk Management Techniques of Genetically Modified Animal Biologics

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To complete the management of genetically modified (GM) animal biologics, the establishment of safety assessment techniques is urgent. Moreover, strengthening international information collection can ensure that inspection technology can keep pace with international. In this study, we collected European and United State regulations of safety assessment of GM animal biologics, and inventoried the documents and tests of the live GM animal biologics reviewed in recent years, to check the inspection technical gap. According to the results of the inventory, we established the safety assessment model such as the tissue tropism test, the transmission route test, virulence regression *in vivo/in vitro* test, and environmental survival/spread test. During this study, we also set up basic test technology and trained technical staff in the team by standard procedure establishment and education. We can effectively solve the current risk assessment technology gap of GM animal biologics, and enhance the international competitiveness of biopharmaceutical industries for veterinary GM products in our country. The related research achievements can also be used as references when authorities elaborate on regulations.

Keywords: genetically modified animal biologics, safety assessment, target animal safety, environmental safety

OB2

Development of an Oil Emulsion Vaccine against Bovine Ephemeral Fever

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The adjuvant of bovine epidemic fever inactivated vaccine manufactured by AHRI was changed from aluminum-phosphate gel to oil. The bovine epidemic fever inactivated vaccines manufactured by AHRI in 2020 were all oil based. Oil-adjuvant vaccines have induced good antibody responses in previous laboratory and field experiments, with good efficacy and safety, significantly high titers were detected in every cattle after the third immunization. However, because farmers generally have doubts about oil-based vaccine, there were several suspecting adverse events after the use of the vaccine in 2020. Investigation showed that adverse effects were due to incorrect use of vaccines. For the sake of prudence, AHRI enhanced the formula of vaccine, conducting efficacy test and single-dose multiple immunization test in 2020. Due to the change of vaccine formula, field experiments have to be conducted to evaluate efficacy and safety and the stability of vaccine new formula have to be recheck. The experimental design is that after choosing 2 field farms and selecting negative cattle without bovine epidemic fever virus antibody, the basic immunization, intramuscular injection 2 doses at 2 weeks interval is administered. After completing basic immunization, withdraw bovine blood regularly every 2 weeks in the first month. After that, blood samples were collected every 4 weeks to evaluate the fluctuation in antibody titers for half a year. The booster dose was administrated and blood samples were collected every 4 weeks to evaluate the fluctuation in antibody titers for another half a year to complete the test. It is expected to select cattle with negative bovine epidemic fever antibody titer in 2 field farms, in order to conduct efficacy and safety tests. In terms of stability test, trial vaccines will be stored to check whether its characteristics, sterility and efficacy still meet the standards. The data collected so far shows that in the second week after completing basic immunization, antibody titers high above recommended protected titer. In the fourth week after boosting, antibody titers are high above 900. In the 16th week after boosting, antibody titers are high above 1024.

Keywords: bovine ephemeral fever, vaccine, oil emulsion

OB3

Emergency Surveillance on Coronaviruses and Pathogenic Microorganisms in Wildlife in Taiwan

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A nationwide surveillance on pathogens of wildlife in Taiwan was carried out in reacting to the COVID-19 pandemic and the concern of introducing SARS coronavirus type 2 into Taiwan's wildlife population. The period of surveillance was from May of 2020 to June of 2021. Twelve research groups composed of university scholars, zoo, non-governmental wildlife preservation organizations, and national veterinary laboratory were collaborating and the surveillance project was funded by Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture. As to June 2021, more than one thousand animals were sampled and tested. Viral RNA of alphacoronaviruses and betacoronaviruses were detected in various species of bats and rats. Fortunately, all these coronaviruses were similar to those previously identified in bats and the SARS coronavirus type 2 was not detected in all the samples, supporting a freedom status in Taiwan's wildlife population. Some other microorganisms, including canine parvoviruses, canine distemper virus, Salmonella, Bartonella, Leptospira, avian paramyxoviruses, mammalian orthoreoviruses, calicivirus, and a few endoparasites, were also identified during this surveillance. These findings may be the basis for further characterization of the found microorganisms and organization of targeting surveillances.

Keywords: coronavirus, wildlife, Taiwan

OB4

The Emergence of Decapod Iridescent Virus 1 in Cultured Shrimp from Taiwan in 2020

Yi-ping Lu^{1,2}, Chien Tu^{1*}, Chieh-hao Wu¹, I-wen Chen¹, Ming-chu Cheng^{2*}

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The infection with decapod iridescent virus 1 (DIV1) causes severe losses of farmed redclaw crayfish (*Cherax quadricarinatus*), whiteleg shrimp (*Litopenaeus vannamei*), and giant freshwater prawn (*Macrobrachium rosenbergii*). DIV1 is responsible for atrophied hepatopancreas, necrosis of hematopoietic tissue, hemocytes and lymphoid organ; mass mortality. The aim of this study was to identify and characterize the DIV1 in the outbreak reported in shrimp farms in Taiwan. In 2020, three cases of decapod iridescent virus 1 (DIV1) infection occurred in cultured penaeid shrimp in the northern region of Taiwan. Specimens from both *Litopenaeus vannamei* and *Penaeus monodon* gave positive PCR and identical sequencing results for DIV1. Moreover, cultured *Penaeus monodon* was first found to be infected with DIV1 through natural pathways. Additionally, we obtained positive PCR test for two specimens from two asymptomatic *Cherax quadricarinatus* farms close to the affected whiteleg shrimp farms, respectively, which means giant freshwater prawn may play a reservoir to transmit the virus. Nucleotide sequence analysis of the outbreak confirmed their close relation to the Chinese strain of the virus. This is the first report of DIV1 in cultured shrimp in Taiwan. The emergence of DIV1 signals a warning to shrimp aquaculture industry worldwide.

Keywords: *Cherax quadricarinatus*, Decapod iridescent virus 1, *Litopenaeus vannamei*, *Penaeus monodon*.



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壁報論文競賽組 (PA)



壁報論文競賽組

編號	篇名與作者	頁碼
PA1	Investigate the Combined Application of Huang-Lian-Jie-Du-Tang with Doxorubicin in the Treatment of Canine Mammary Gland Tumors in Nude Mice Model <u>Chen-Yung Kao</u> ¹ , Jui-Te Wu ^{1*} , Geng-Ruei Chang ^{1*}	21
PA2	Therapeutic Effect of Bao-Yuan-Tang in a Chronic Kidney Disease Mice Model <u>Shun-Nung Yang</u> ¹ , Jui-Te Wu ^{1*} , Geng-Ruei Chang ^{1*}	22
PA3	Comparison of Conventional Molecular and Whole-Genome Sequencing Methods for Differentiating <i>Salmonella</i> <u>I-Chen Li</u> ¹ , Rayean Wu ¹ , Chung-Wen Hu ¹ , Keh-Ming Wu ² , Zeng-Weng Chen ^{3*} , Chung-Hsi Chou ^{1*}	23
PA4	Comparison of Reference-Based Assembly and De Novo Assembly for Bacterial Plasmid Reconstruction and AMR Gene Localization <u>I-Chen Li</u> ¹ , Gine-Ye Yu ² , Jing-Fang Huang ² , Zeng-Weng Chen ^{2*} , Chung-Hsi Chou ^{1*}	24
PA5	Antioxidant Properties and Antimutagenicity of <i>Antrodia cinnamomea</i> and Other Extracts in Ames Test <u>Jia-Shuan Wu</u> ¹ , Tun-Tschu Chang ² , Chun-Lin Lee ³ , Yew-Min Tzeng ³ , Jiunn-Wang Liao ^{1,*}	25
PA6	Evaluation of efficacy and safety chlorine dioxide in <i>Litopenaeus vannamei</i> <u>Jessintya Palupi</u> ² , Shih-Chu Chen ^{1,2} and Chin-En Tsai ^{1,2*}	26
PA7	Feline mammary carcinoma cell-derived extracellular vesicle promoted tumor liver metastasis <u>Bing-Ing Lin</u> ¹ , Yi-Fei Chen ¹ , Yi-Ting Huang ¹ , Li-En Chen ² , Jiunn-Wang Liao ¹ , Yu-Chih Wang ¹	27
PA8	The Investigation of Canine Babesiosis from Animal Shelters in Central and Southern Taiwan <u>Hao-Wei Hsu</u> ¹ , Kun-Wei Chan ^{2*}	28
PA9	Paclitaxel Encapsulated Autologous Tumor Cell-derived Extracellular Vesicles for Feline Mammary Carcinoma Treatment <u>Yi-Fei Chen</u> ¹ , Bing-Ing Lin ¹ , Yi-Ting Huang ¹ , Li-En Chen ² , Hsun-Lung Chan ^{1,3} , Pei-Ling Kao ¹ , Jiunn-Wang Liao ¹ , Cheng-Yao Yang ¹ , Yu-Chih Wang ¹	29
PA10	Urinary Malondialdehyde-Creatinine Ratio Predict Feline Chronic Kidney Disease Progression <u>Hua-Chen Huang</u> ¹ , Chou, Chi-Chung ² , Ya-Jane Lee ^{1,3* *}	30

PA11	Surveillance on Immune Efficacy of Classical Swine Fever vaccine in Biomedical Lanyu Pig <u>Yu-Ling Huang</u> ^{1,2} , Han-Sheng Wang ² , Yi-Long Chen ² , Shih-Sin Li ² , Chia-Chieh Chang ² , Chao-Nan Lin ¹ , Wei-Hao Lin ¹ , Ming-Tang Chiou ^{1*}	31
PA12	Study on the analysis of composition of <i>Artemisia capillaris</i> (Yin Chen herb) and its pharmaceutics by HPLC method <u>Hoi-Yan Man</u> ¹ , Chin-En Tsai ^{2*}	32
PA13	The nucleocapsid protein-regulated molecular switch between <i>cis</i> -acting RNA structures in the coronavirus 3' untranslated region is important for coronavirus gene expression <u>Feng-Cheng Hsieh</u> ¹ , Yu-Chia Chang ¹ , Ching-Hung Lin ¹ , and Hung-Yi Wu ^{1*}	33

PA1

Investigate the Combined Application of Huang-Lian-Jie-Du-Tang with Doxorubicin in the Treatment of Canine Mammary Gland Tumors in Nude Mice Model

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Mammary gland tumor is the most common spontaneous tumor in intact female dogs, and its poor prognosis remains a clinical challenge. Huang-Lian-Jie-Du-Tang (HLJDT), a traditional Chinese medicine formulation containing *Coptidis Rhizoma*, *Scutellariae Radix*, *Phellodendric Cortex* and *Gardeniae Fluctus*, are widely used in the treatment of bacillary dysentery and jaundice. However, there are no reports evaluating the antitumor effects of HLJDT in canine mammary tumor. Here, we investigated whether HLJDT was able to inhibit canine mammary tumor development and explored the related mechanisms. We established canine mammary gland tumors xenografts in nude mice, following which we randomly divided the mice into four groups: control, doxorubicin, HLJDT, and HLJDT combined with doxorubicin. Compared the results with those for control mice, doxorubicin, HLJDT, and HLJDT combined with doxorubicin inhibited the growth of canine mammary tumor in BALB/c nude mice. The antitumor effects induced were associated with increased tumor apoptosis through the enhanced expression of cleaved caspase 3 and p53; reduced anti-apoptosis Bcl-2 protein; decreased inflammation cytokines levels (e.g., IL-1 β , and TNF- α); reduced inflammation-related factors such as cyclooxygenase-2 (COX-2) protein; and inhibited metastasis-related factors such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9). Consistent with the results in vivo, a significant suppression of tumor growth by doxorubicin, HLJDT, or HLJDT combined with doxorubicin was observed in canine mammary tumor xenografts. Notably, mice treated with HLJDT combined with doxorubicin had significantly retarded tumor growth, reduced tumor size, and increased tumor inhibition compared with the groups of mice treated with doxorubicin or HLJDT alone. Therefore, both HLJDT alone and HLJDT combined with doxorubicin may be potential anti-tumor agents for treatment of canine mammary gland tumors.

Keywords: *Huang-Lian-Jie-Du-Tang, doxorubicin, Canine Mammary Gland Tumor, mice*

PA2

Therapeutic Effect of Bao-Yuan-Tang in a Chronic Kidney Disease Mice Model

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Chronic kidney disease (CKD) is a worldwide public health issue with poor outcomes and high cost. Chinese herbal medicine is frequently applied in conjunction with western pharmacotherapy to relieve symptoms in patients with CKD. However, evidence-based research into the effectiveness of Chinese herbal medicine use as applied to treat CKD is limited and warrants further investigation in animals. The aim of this study is to study the protective effects of Bao-Yuan-Tang on 5/6 nephrectomy induced model mice with inflammatory renal injury. The traditional Chinese medicinal herbal extract Bao-Yuan-Tang had been used for many years to treat renal disease in China, however it is limited to explore the protective mechanism in the experimental studies. Mice were equally randomized into control group and Bao-Yuan-Tang group. Models were established by performing 5/6 nephrectomy on the mice in all groups through the left renal artery branch ligation and right nephrectomy were performed in the model group at the age of 8 weeks. Compared to the control group, gavage administration of Bao-Yuan-Tang for 28 days significantly increased the body weight, food intake, anti-inflammation cytokine IL-10, anti-inflammation factor NF- κ B inhibitor α (I κ B α) and anti-oxidant enzyme such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase. 5/6 nephrectomy mice treated with Bao-Yuan-Tang reduced inflammation-related cytokine such as serum TNF- α , IL-1 β , IL-6 and CRP levels; serum blood urea nitrogen, creatinine, creatinine, potassium, chloride; and inflammation-related factor like nuclear factor kappa light chain enhancer of activated B cells (NF- κ B). Moreover, Bao-Yuan-Tang can alleviate renal pathologic changes and reduce inflammatory injury of the kidneys of 5/6 nephrectomy induced model mice. Collectively, these findings indicate that Bao-Yuan-Tang is effective in protecting the function of remnant kidneys from progressive injury, which may be mediated by ameliorating inflammation development and oxidative stress injury in remnant kidney tissue. Therefore, Bao-Yuan-Tang may be potential anti-CKD agents for treatment of renal disease.

Keywords: *Bao-Yuan-Tang, chronic kidney disease, mice, nephrectomy, inflammation*

PA3

Comparison of Conventional Molecular and Whole-Genome Sequencing Methods for Differentiating *Salmonella*

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Over the last decade, *Salmonella enterica* serovar Schwarzengrund has become more prevalent in Asia, Europe, and the US with the simultaneous emergence of multidrug-resistant isolates. As these pathogens are responsible for many sporadic illnesses and chronic complications, as well as outbreaks over many countries, improved surveillance is urgently needed. For 20 years, pulsedfield gel electrophoresis (PFGE) has been the gold standard for determining bacterial relatedness by targeting genome-wide restriction enzyme polymorphisms. Despite its utility, recent studies have reported that PFGE results correlate poorly with that of closely related outbreak strains and clonally dominant endemic strains. Due to these concerns, alternative amplification-based molecular methods for bacterial strain typing have been developed, including clustered regular interspaced short palindromic repeats (CRISPR) and multilocus sequence typing (MLST). Furthermore, as the cost of sequencing continues to decrease, whole genome sequencing (WGS) is poised to replace other molecular strain typing methods. In this study, we assessed the discriminatory power of PFGE, CRISPR, MLST, and WGS methods to differentiate between 23 epidemiologically unrelated *S. enterica* serovar Schwarzengrund isolates collected over an 18-year period from distinct locations in Taiwan. The discriminatory index (DI) of each method for different isolates was calculated, resulting in values between 0 (not discriminatory) and 1 (highly discriminatory). Our results showed that WGS has the greatest resolution (DI = 0.982) compared to PFGE (DI = 0.938), CRISPR (DI = 0.906), and MLST (DI = 0.463) methods. In conclusion, the WGS typing approach was shown to be the most sensitive for *S. enterica* serovar Schwarzengrund fingerprinting.

Keywords: *Salmonella*; subtyping; PFGE; MLST; CRISPR; WGS

PA4

Comparison of Reference-Based Assembly and De Novo Assembly for Bacterial Plasmid Reconstruction and AMR Gene Localization

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It is well established that plasmids carrying multiple antimicrobial resistance (AMR) genes can be easily transferred among bacterial isolates by horizontal gene transfer. Previous studies have shown that a combination of short- and long-read approaches is effective in reconstructing accurate plasmids. However, high-quality Illumina short reads mapped onto the long reads in the context of an AMR hybrid monitoring strategy have not yet been explored. Hence, this study aimed to improve the reconstruction of plasmids, including the localization of AMR genes, using the above-described parameters on whole-genome sequencing (WGS) results. To the best of our knowledge, this study is the first to use S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) to confirm the number and sizes of plasmids detected by in silico-based predictions in *Salmonella* strains. Our results showed that de novo assembly did not detect the number of bacterial plasmids more accurately than reference-based assembly did. As this new hybrid mapping strategy surpassed de novo assembly in plasmid reconstruction, it was further used to identify the presence and genomic location of AMR genes among three *Salmonella enterica* serovar Schwarzengrund isolates. The AMR genes identified in the bacterial chromosome among the three *Salmonella enterica* serovar Schwarzengrund isolates included: AAC(3)-IV, AAC(60)-Iy, aadA2, APH(4)-Ia, cmlA1, golS, mdsA, mdsB, mdsC, mdtK, qacH, sdiA, sul2, sul3, and TEM-1 genes. Moreover, the presence of TEM-1, AAC(3)-IV, aadA2, APH(4)-Ia, cmlA1, dfrA12, floR, sull, sul3, and tet(A) genes found within three IncFIB plasmids and one IncX1 plasmid highlight their possible transmission into the environment, which is a public health risk. In conclusion, the generated data using this new hybrid mapping strategy will contribute to the improvement of AMR monitoring and support the risk assessment of AMR dissemination.

Keywords: *Salmonella enterica* serovar Schwarzengrund; antimicrobial resistance genes; reference-based assembly; de novo assembly; plasmids

PA5

Antioxidant Properties and Antimutagenicity of *Antrodia cinnamomea* and Other Extracts in Ames Test

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The aim of this study was to evaluate the antioxidative capacity of the extract and whether the extract could reduce the biological activity of mutagenic agents, and to evaluate whether it could be used as a preventive agent for the development of chemical carcinogens. In this study, 5 samples of *Antrodia cinnamomea* (AC) mycelium or fruiting body alcoholic extracts, ovatodiolide, antrocin and antrocinol were studied. Results revealed that the total phenol content of the alcohol extract of AC dish-cultured fruiting body which was the highest at 10 mg/mL. The ferric reducing ability of plasma of the alcohol extract of AC fruit body was the best at 2 mg/mL. The total antioxidant capacity of the alcohol extract of AC fruit body was the best at 0.4 mg/mL. In this study, the Ames test used *Salmonella typhimurium* TA98 and TA1535 as the test strain to test the samples of ovatodiolide and different extracts of AC. For the toxicity of the sample to bacteria, the non-toxic concentration of bacteria was selected as the highest concentration for subsequent experiments. Then a functional experiment of antimutagenicity effects, using the direct mutagenic agent 4-Nitroquinoline-N-oxide (without S9 mixture), Sodium azide (without S9 mixture), and the indirect mutagenesis agent 2-Aminoanthracene (with S9 mixture) was to explore the antimutagenicity effects of each extract. In Ames test, the anti-mutation test against the Frame-shift mutation strain of TA98 found that in the group without the addition of the S9 mixture, the alcohol extract of AC dish-cultured fruiting body which antimutagenicity effect was the best. At 0.625 mg/plate, the maximum antimutagenicity effect of the direct mutagen 4-Nitroquinoline-N-oxide was 54.4%, which was a strong anti-mutagenic agent. In the other group with the addition of S9 mixture, the alcohol extract of AC fruiting body which antimutagenicity effect was the best. At 1.25 mg/plate, the maximum antimutagenicity effect of the indirect mutagen 2-Aminoanthracene was 51.73%, which was a strong anti-mutagenic agent. In addition, the anti-mutation test against the Base-pair substitution strain of TA1535 found that in the group without the addition of the S9 mixture, ovatodiolide which antimutagenicity effect was the best. At 2.5 mg/plate, the maximum antimutagenicity effect of the direct mutagen Sodium azide was 41.15%, indicating a strong anti-mutagenic agent. In the other group with the addition of S9 mixture, the alcohol extract of AC fruiting body which antimutagenicity effect was the best. At 2.5 mg/plate, the maximum antimutagenicity effect of the indirect mutagen 2-Aminoanthracene was 97.07%, which was a strong anti-mutagenic agent. The antimutagenicity effect of the extract showed a dose-response relationship. According to the experimental results, the alcohol extract of AC fruiting body is estimated that a good antimutagenic effect in the human body after ingestion and metabolic activation. Therefore, the extract could be as a potential pharmaceutical for inhibiting cancer development.

Keywords: *Antrodia cinnamomea*, ovatodiolide, antioxidative and antimutagenicity effects

PA6

Evaluation of efficacy and safety chlorine dioxide in *Litopenaeus vannamei*

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Generally, female *Litopenaeus vannamei* (*L. vannamei*) are larger than males due to a greater gain in mass per moult cycle, resulting in a clear size dimorphism in adults. It is unknown whether this dimorphism is driven by differences in behaviours between male and female *L. vannamei*, which may be important in deciding whether to treatment with same concentration of pharmaceutical for cultures during diseases outbreak. In the other point, one of the limiting factors is the maintenance of sufficient water quality and disinfectant like chlorine dioxide (ClO₂) was added to the water either as prophylactic or treatment measure with this aim in mind, physical and chemical methods are used to reduce the total amount of bacteria in the water. In this study, a ClO₂ was determined to contain 2000 ppm in stock solution and divide into different concentrations of 0, 1, 5, 10, 20, 30, 40 and 50 ppm was applied in *L. vannamei*. The efficacy, safety and immerse toxicity were evaluated. The aerobic bacteria activity by petrifilm was more than 90% reduction when ClO₂ concentrations were applied 50 ppm in 12 hours, respectively. A concentration of 40 ppm led to significant changes in the chemical water parameters already after 4 hours and to a significant decrease level of nitrite and nitrate for 24 hours. Moreover, up to 10 ppm ClO₂ showed significant symptoms. In a LC (lethal concentration) 50 test for 48 hours 17.5 mg/L with R² 0.93, treatment with 1 ppm ClO₂ for showed no abnormality and total of mortality 68.75% female. Using 1 ppm ClO₂ for prophylactic use is therefore also might be an alternative option for short-term treatment in cases of disease outbreaks and sex might plays an important role in *L. vannamei* behaviour male shrimp active while females were mostly inactive to survive.

Keywords: behaviour, disinfectant, LC50

PA7

Feline mammary carcinoma cell-derived extracellular vesicle promoted tumor liver metastasis

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Extracellular vesicles (EVs) can mediate long-distance communication between cells by delivering biomolecular cargo in physiology and pathology. EVs uptake through fusion, phagocytosis, endocytosis or macropinocytosis at multivesicular bodies in recipient cells and release their functional cargo. Feline mammary carcinoma (FMC) is a common feline neoplasm in female old cats. Most are hormone-independent carcinomas with an aggressive biological behavior. Reported high rate of metastases and most frequently affect the lungs (83%) and liver (25%). Metastasis occurs through a series of key steps: epithelial-mesenchymal transition underlie local invasion, angiogenesis and intravasation, survival in the circulation and attachment to the endothelium, extravasation, and colonization. Pre-metastatic niche (PMN) created a fertile environment for the colonization of disseminated cancer cells in selected organs by primary tumor through tumor secreted EV. To investigate the correlation between FMC-EV and FMC distant metastasis, we isolated the FMC-EV from FMC cell (FMT-1807) conditioned medium by differential ultracentrifugation (DUC), polyethylene glycol precipitation or size exclusion chromatography (SEC). These methods vary in the resulting FMC-EV recovery purity and yield. High recovery rate of FMC-EV was isolated by SEC and high recovery purity was purified by DUC. To gain insight into FMC-EV uptake at future metastatic sites, we intraperitoneally injected FMC-EV labelled with CD63-GFP into FMT-1807 patient derived xenograft mice, then used *de vivo* IVIS imaging to unveiling the biodistribution of FMC-EV. FMC-EV accumulated mainly in primary tumor and liver. To test the uptake by a recipient cell in liver is cell-specific, we incubated the CD63-EGFP-labeled FMC-EV with hepatocyte, hepatic stellate cell (HSC), macrophage, and vascular endothelial cell. Flow cytometry analysis showed that FMC-EV was accumulated most in HSC (LX-2 cells) than other cells in liver. To investigate the correlation between FMC-EV uptake by liver and liver metastasis, liver metastasis model after FMC-EV education was performed. RFP-labelled FMT-1807 cells or MDAMB-231 cells were intra-splenic injected after three weeks FMC-EV treatment. Mice sacrificed post three weeks cell implantation for *de vivo* detection of liver metastasis. Three weeks FMC-EV education significantly increased both the hepatic metastatic nodules of FMT-1807 cell and MDAMB-231 cell. This result suggested that FMC-EV promoted liver PMN formation. Further studies will try to find out which substances in FMC-EV contribute to PMN establishment and how stellate cell uptake of FMC-EV affects hepatic PMN.

Keywords: Feline mammary carcinoma, pre-metastatic niche, extracellular vesicle

PA8

The Investigation of Canine Babesiosis from Animal Shelters in Central and Southern Taiwan

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Canine babesiosis is a tick-borne protozoal infection caused by different species of *Babesia*, causing a series of clinical symptoms in dogs like anemia, lethargy, and pale mucous membranes. The traditional method for diagnosing canine babesiosis is to observe the types of *Babesia* in erythrocytes through a blood smear under the optical microscopy. However, the result of this diagnostic method may differ due to the operator's inspection skills, experience, or the concentration of parasitemia in the blood samples. In recent years, the usage of polymerase chain reaction (PCR) to detect specific *Babesia* gene fragments in blood samples can greatly improve the accuracy of the positive rate of the canine babesiosis. Nonetheless, limited studies were conducted on the positive rate of canine babesiosis on dogs in animal shelters in Taiwan. Thus, the purpose of the present study aimed to collect blood samples from dogs in animal shelters in six counties and cities in central and southern Taiwan (Nantou, Yunlin, Chiayi, Tainan, Kaohsiung, and Pingtung regions) through random sampling to investigate and compare the positive rate of canine babesiosis in each regions. So far, 40 samples were collected in Nantou area with 11 positive PCR results; 30 samples were collected in Chiayi area with 15 positive PCR results. It should be noted that the positive rate of canine babesiosis in sheltered dogs varied, and more rigorous preventive measures or formulate better treatment methods should be taken for canine babesiosis. As canine babesiosis is mostly asymptomatic or mild infection, more awareness on the infection of blood parasites for owners and veterinarians should be raised. Furthermore, action should be taken to prepare for more examination to make a diagnosis about the disease of blood parasites.

Keywords: Canine babesiosis, animal shelters, *Babesia* spp.

PA9

Paclitaxel Encapsulated Autologous Tumor Cell-derived Extracellular Vesicles for Feline Mammary Carcinoma Treatment

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Feline mammary carcinoma (FMC) is the third most common tumor diagnosed in female cat. Most FMCs are malignant. The main treatment of FMC is surgery, but the recurrence and metastasis after surgery happens common. Traditional chemotherapy drug, such as paclitaxel (PTX), kill not only tumor cells but also rapidly differentiated host cells. Novel treatment with specificity to cancer is needed. Extracellular vesicles (EV) are nano-vesicular structure which secreted as signal transduction carrier for cell-cell communication. Autologous tumor cell-derived EV showed the higher cell specificity to cancer cell than liposome or other nanoparticles. In addition, PTX within EV may prolong the half-life of drug and decrease the dosage of drug during therapy. Therefore, we explored the feasibility of autologous EV from FMC cells (FMC-EV) as a specific carrier to introduce PTX into FMC. FMC-EV was isolated from conditioned medium of FMT-1807 cells by ultracentrifugation. The half maximal inhibitory concentration (IC₅₀) of PTX encapsulated FMC-EV (EV-PTX) determined on FMC cells or human breast cancer cells compared with PTX. Significantly higher IC₅₀ value of PTX on FMT-1807 cells compared with IC₅₀ value of EV-PTX (10 fold), but not on MCF-7 cells or MDA-MB-231 cells, were observed. This result suggested that FMC-EV targeted delivery PTX to FMC cells showed an efficient accumulation in FMC cells, and induced cell apoptosis. Red fluorescent protein (RFP) labeled FMC cells were orthotopic xenografted into NPG mice to determine the antitumor effect of EV-PTX. Insufficient dose of PTX or EV-PTX did not significantly reduce the tumor growth and promoted the liver metastasis. In order to increase the loading capacity of PTX for *in vivo* study, PTX was loaded into liposome (L-PTX) and then fused with FMC-EV by sonication (EV-L-PTX). Combination of liposome with FMC-EV increased the loading capacity of PTX. PTX (10 mg/kg body weight) or EV-L-PTX (2.1 mg/kg body weight) showed the significant tumor-suppressing effect compared to control group by caliper or IVIS measurement. In conclusion, FMC cell-derived EVs showed the specificity to FMC. EV-PTX exhibited similar anticancer efficacy of 5 times dose of PTX in an orthotopic mouse model of FMC.

Keywords: feline mammary carcinoma, extracellular vesicles, treatment

PA10

Urinary Malondialdehyde-Creatinine Ratio Predict Feline Chronic Kidney Disease Progression

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Chronic kidney disease (CKD) is one of the most common disorders found in cat. In veterinary practice, evaluation of CKD has mainly relied on indirect glomerular filtration rate (GFR) markers, with serum creatinine serve as the most commonly used indicator. However, this marker is insensitive and can be influenced by extrarenal factors. Search for a more sensitive, reliable, and direct indicator of renal injury in CKD is therefore warrant in veterinary practice. Oxidative stress has been found to play a direct role in changes of multiples renal structures, and associated with human renal function decline especially at the early stage. Malondialdehyde, an end-product of lipid peroxidation, is one of the most widely recognized biomarker for oxidation stress. Urinary MDA has been found to increase following experimental ischemia-hypoxia insult, which is suggested as one of the main mechanisms behind feline CKD. Also, the concentration of urinary MDA has been reported to predict the occurrence of kidney injury in human ICU patients. Hence, we hypothesized that feline urinary MDA may serve as a non-invasive, sensitive indicator for kidney function decline. To prove this hypothesis, we evaluated urinary malondialdehyde concentration of client-owned cats with chronic kidney disease. Batch thiobarbituric acid-reactive substances assays, together with high performance liquid chromatography, was used to evaluated MDA concentration. The results showed that urinary MDA concentration, after calibrated with urinary creatinine, can predict the elevation of serum creatinine within 30, 60 and 90 days. This result indicated that urinary MDA-to-creatinine ratio may be a more sensitive biomarker for feline renal function than serum creatinine.

Keywords: chronic kidney disease, biomarker, oxidative stress

PA11

Surveillance on Immune Efficacy of Classical Swine Fever vaccine in Biomedical Lanyu Pig

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As one of the vital laboratory animal species, miniature pigs gradually became more and more important in biomedical research. Nowadays, the studies of immunization and disease transmission on swine focus on commercial pigs and there are few researches on the Lanyu pig. However, the researches of immune status on the Lanyu pig are necessary for monitoring health status and establishing vaccine program. Therefore, the objective of this study was to investigate the dynamics of the classical swine fever (CSF) maternal-derived antibodies on Lanyu pigs. Twenty four pregnant sows with vaccination and three offsprings from each parity were randomly selected to the trial. Blood samples were collected from sows at three weeks after farrowing and piglets without CSF vaccination at the age of 3, 6, 9, 12 and 15 weeks to analyze the CSFV antibody. The serum concentration of CSFV antibody was evaluated and analysed. The relationship of CSF ELISA blocking percentage between sows and their piglets was analyzed by linear regression. The results showed that the average CSF ELISA blocking percentage of piglets at the age of 3, 6, 9, 12 and 15 weeks were $65.0 \pm 20.4\%$, $48.6 \pm 22.9\%$, $34.6 \pm 21.4\%$, $24.5 \pm 18.3\%$ and $10.4 \pm 13.8\%$, respectively. The average CSF ELISA blocking percentage of sows was $80.8 \pm 17.5\%$ and there were highly significant positive correlation between the sows and their piglets at the age of three weeks ($P < 0.0001$). It indicated that the CSF antibody condition of piglets at the age of three weeks might predict from their mother. In conclusion, the average maternal-derived antibodies of piglets declined into negative after 6 weeks of the age and it revealed that the vaccination should be implemented after 6 weeks of the age under this experimental condition.

Keywords: Lanyu pig, classical swine fever vaccine, maternal-derived antibodies

PA12

Study on the analysis of composition of *Artemisia capillaris* (Yin Chen herb) and its pharmaceuticals by HPLC method

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The Yin Chen Hao Tang and Yin Chen Wu Ling San are often used as the prescription of jaundice constantly. The bioactive ingredients of Yin Chen, which is *Artemisia capillaris*, are highly valued. Scoparone and chlorogenic acid are considered as the vital natural compounds of Yin Chen in treating liver disease. The scoparone and chlorogenic acid were used as the reference standards to develop the high performance liquid chromatography (HPLC) method for qualitative and quantitative among the commercial products included Yin Chen in the market. Chromatographic separation was conducted on Cosmosil packed Column C18 (250 mm × 4.6 mm). The mobile phase consisted of 0.1% phosphoric acid aqueous (A) and acetonitrile (B). The gradient program were as follows: 1 - 10 min, linear gradient 5 - 25% B; 10 - 20 min, linear gradient 25 - 80% B. Chromatography was performed at 30 °C. The UV detection wavelength was set at 327 nm. The flow rate was 1.0 mL/min and aliquots of 50 µL were injected. Samples are collected from six commercial brands which are selling the Yin Chen and Yin Chen Hao Tang concentrated scoparone and chlorogenic acid. The results showed that the regression equation and linearity for scoparone and chlorogenic acid are $y = 142.19x + 8.6526$, $R^2 = 1$ and $y = 128.4x + 3.2764$, $R^2 = 0.9996$ respectively. The commercial products showed different concentration for scoparone and chlorogenic acid.

Keywords: high performance liquid chromatography, Yin Chen, scoparone, chlorogenic acid

PA13

The nucleocapsid protein-regulated molecular switch between *cis*-acting RNA structures in the coronavirus 3' untranslated region is important for coronavirus gene expression

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The two RNA structures, bulged stem-loop (BSL) and pseudoknot (PK), in coronavirus 3' untranslated region (UTR) overlap each other and thus are mutually exclusive despite their indispensability for coronavirus viability. Therefore, the molecular switch must occur between BSL and PK for the regulation of viral gene expression. However, the role of BSL and PK in viral gene expression and the factors regulating the molecular switch remain largely unknown. Since viral translation is an imperative step prior to viral replication, it is hypothesized that the base pairings within BSL and/or PK may affect viral replication through modulating viral translation. For this, various BSL-PK mutants in both bovine coronavirus (BCoV) defective interfering (DI) RNA and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) DI RNA were constructed to determine the interactions between viral proteins and the molecular switch, and how BSL and PK function in viral replication and translation. The results suggest that (i) both BSL and PK are crucial for SARS-CoV-2 replication, (ii) BSL is important for the regulation of BCoV and SARS-CoV-2 translation, (iii) the tertiary structure of PK is absent *in vitro* and exists only with the competing BSL disrupted, and (iv) coronavirus nucleocapsid (N) protein facilitates the formation of PK tertiary structure. In conclusion, BSL is essential for viral translation, PK functions in viral replication, and coronavirus N protein regulates the molecular switch between BSL and PK, thus the translation and replication, respectively.

Keywords: coronavirus, 3' untranslated region, molecular switch, replication, translation, nucleocapsid protein



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壁報論文非競賽組(PB)



壁報論文非競賽組

編號	篇名與作者	頁碼
PB1	Investigate the Combined Application of Ping-Wei Decoction with Dexamethasone for Inflammatory Bowel Disease in Mice Model <u>Chia-Ling Li</u> ¹ , Jui-Te Wu ^{1*} , Geng-Ruei Chang ^{1*}	39
PB2	The Relationship between Arterial and Intraosseous Pressure in Pigeons (<i>Columba rupestris</i>) in Hypovolemic Model <u>Po-Hsiang Wang</u> ¹ , Pin-Huan Yu ^{1*}	40
PB3	Establishment of immortalization of fish cell lines <u>Huei-Ming Shih</u> ^{1,2} , Guan-Ming Ke ^{1,2}	41
PB4	Survey of Pathogenic <i>Leptospira</i> in The Environment of Different Seasons in Southern Taiwan <u>Yu-Chi Lee</u> ¹ , Yu-Sheng Chang ¹ , Shin-Ji Hsu ¹ and Chuen-Fu Lin ^{1,2*}	42
PB5	The Impact of Different Urine Sources on <i>Leptospira</i> <u>Yu-Chi Lee</u> ¹ , Yu-Sheng Chang ¹ , Shin-Ji Hsu ¹ and Chuen-Fu Lin ^{1,2*}	43
PB6	Detection of cat-scratch disease (<i>B. henselae</i>) of blood and saliva in cat by using iiPCR and Nested PCR methods <u>Kuan-Ying Lee</u> , and Chin-En Tsai*	44
PB7	Establishment of Deep Corneal Stromal Ulcer Animal Model in Rats and Investigation of Features in Optical Coherence Tomography and Histopathology. <u>Hao Lee</u> ¹ , Wei-Hsiang Huang ² , Chung-Tien Lin ^{1,3*}	45
PB8	Estimation of Origins and Insertions of Canine Stifle Ligaments <u>Yu-Ying Lin</u> ¹ , Cheng-Chung Lin ² and Ching-Ho Wu ^{1*}	46
PB9	Protective efficacy against <i>Vibrio harveyi</i> in shrimp induced by feed containing anti- <i>V. harveyi</i> IgY encapsulated in PLG microparticles <u>Yi-Chen Lin</u> ¹ , Chung-Da Yang ^{1*}	47
PB10	Effects of Oral Supplementation on Dry Eye Disease in a Rat Model <u>Yi-Shan Chiang</u> ¹ , Chung-Tien Lin ^{1**}	48
PB11	Development of Skin Damage Model Induced by Ultraviolet B Irradiation in Mice <u>Shao-Wen Hung</u> ^{1,*} , Guan-Hong Chen ¹ , Chia-Chi Chen ¹ , Ying-Ching Hung ¹ , Tzu-Yun Chi ¹ , Yun-Xuan Chang ¹ , Wen-Der Fang ²	49
PB12	Establishment of a Mouse Model with Colitis via Dextran Sulfate Sodium Administration <u>Shao-Wen Hung</u> ^{1,*} , Tzu-Yun Chi ¹ , Yun-Xuan Chang ¹ , Chia-Chi Chen ¹ , Ying-Ching Hung ¹ , Guan-Hong Chen ¹ , Wen-Der Fang ²	50
PB13	Establishment of a Mouse Model with Retinal Lesion via Light-induction <u>Shao-Wen Hung</u> ^{1,*} , Yun-Xuan Chang ¹ , Chia-Chi Chen ¹ , Tzu-Yun Chi ¹ ,	51

PB14	Epidemiology of Porcine Circovirus Type 3 in Central Taiwan from 2020 to 2021 <u>Yu-Fan Hung</u> ¹ , Yi-Fan Chao ² , Chung-Ping Huang ² , Tsung-Ting Chuang ² , Cheng-Yao, Yang ^{1*} *	52
PB15	Comparison of Leptospiral Serovars in Dogs and Wild Small Mammals in Taiwan <u>Shin-ji Hsu</u> ¹ , Chuen-fu Lin ^{1,2*}	53
PB16	Monitoring of bacterial pathogens in poultry farms with Hazard Analysis and Critical Control Points (HACCP) system <u>Ching-Ching Hsu</u> ¹ , Chin-En Tsai ^{1*}	54
PB17	A Dog with Infected Prostatic Cyst and Severe Electrolytes Imbalance <u>Ching-Jung Lien</u> ^{1,2} , Shang-Lin Wang ^{2,3*}	55
PB18	The Impact of Preservation Methods of Dog and Cat Urine Samples on the Result of Quantitative Bacterial Culture <u>Ching-Jung Lien</u> ^{1,2} , Shang-Lin Wang ^{2,3*}	56
PB19	Investigation and Comparison of the Efficacy of Gelatin-Epigallocatechin gallate Nanoparticles with Hyaluronic Acid and Commercial Artificial Tears on Keratoconjunctivitis Sicca Associated Keratopathy in Rats <u>Man-Ha Chan</u> ¹ , Ching-Li Tseng ² , Wei-Hsiang Huang ³ , Chung-Tien Lin ^{1*}	57
PB20	Development of Recombinant Protein Adjuvants Based on <i>Vibrio harveyi</i> Flagellin Gene in <i>Epinephelus coioides</i> <u>Andre Giovanni</u> ¹ , Shun Maekawa ^{1,6*} , Pei-Chi Wang ^{2,3,5} , Shih-Chu Chen ^{1,2,3,4,5**}	58
PB21	Development of a Novel Scoring System for the Assessment of Neurological Function in Dogs <u>Wenyi Wong</u> , Ya-Pei Chang*	59
PB22	The Effects of Equilibrative Nucleoside Transporter 1 (ENT1) Inhibitor on Insomnia <u>Yu-Cheng Huang</u> ¹ , Pei-Lu Yi ² , Fang-Chia Chang ^{1,2*}	60
PB23	Treatment of Acute Pancreatitis in One Dog with Traditional Chinese Veterinary Medicine <u>Chen-Yin Liao</u> ¹ , Cheng-Hung Lai ^{1*}	61
PB24	Chemo-Drug Loaded Hydroxyapatite-Gelatin Composite Microspheres for Local Treatment in a Subcutaneous Xenograft Model <u>Wan-Ru Hsieh</u> ¹ , Meng-Ying Wu ² , I-Fang Koa ² , Shio-Kang Yen ^{2*} , Jiunn-Wang Liao ^{1*}	62
PB25	Multiple Prolonged Stress Disrupts Behavioral and Neuronal Activities in Rats <u>Yun Lo</u> ¹ , Pei-Lu Yi ² , Yi-Tse Hsiao ¹ , Fang-Chia Chang ^{1*}	63

PB26	Synovial Fluid Analysis of Rescued Sea Turtles in Taiwan (2019-2021) <u>Hsiang-Pei Su</u> ^{1*} , Chau-Hwa Chi ¹ , Pin-Huan Yu ¹	64
PB27	Application of HACCP to Monitor the Hazard Factor in Horse Farm — the Impact of Mycotoxins in Concentrations of Fecal Bacteria_ <u>Chih-Chun Lu</u> ¹ , Chin-En Tsai	65
PB28	Development of a Colloidal Gold-Based Immunochromatographic Strip for Rapid Detection of Classical Swine Fever virus <u>Shen-Pang Tsai</u> ¹ , Kuo-Pin Chuang ^{1,2,3,4,5}	66
PB29	Case Report: Nasal Adenocarcinoma in A Canine <u>Yi-En Tay</u> ¹ , Sheng-Kai Tsai ^{1,2} , Kun-Wei Chan ^{1,2*}	67
PB30	The Feasibility of Diffusion-weighted Magnetic Resonance Imaging for the Diagnosis and Prognosis Prediction in Dogs with Meningoencephalitis of Unknown Etiology <u>Yi-Cheng Chuang</u> ¹ , Ya-Pei Chang ^{1,2*}	68
PB31	Diagnosis of Thymoma and Thymic Lymphoma in Rabbits (<i>Oryctolagus cuniculus</i>) <u>Pin-Yu Chen</u> ¹ , Pin-Huan Yu ^{1*}	69
PB32	Expression of Androgen Receptor in Canine Hepatoid Gland Tumors <u>Shang-Lun Chang</u> ¹ , Shih-Chieh Chang ^{2,3} , Jiunn-Wang Liao ^{1*}	70

PB1

Investigate the Combined Application of Ping-Wei Decoction with Dexamethasone for Inflammatory Bowel Disease in Mice Model

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Inflammatory Bowel Disease (IBD) involves chronic inflammation in the patient's digestive track, leading to the development of ulcerative colitis and Crohn's disease. IBD has a multifactorial pathogenesis whereby environmental and genetic factors contribute to disease onset. Ping-Wei Decoction, a Chinese medical decoction, was used to improve digestive absorption and used to treat gastrointestinal disorders. However, there are no reports evaluating the treatment of Ping-Wei Decoction in IBD. Here, this study was aimed to determine whether treatment with Ping-Wei Decoction reduce IBD induced by dextran sulphate sodium (DSS) and influencing its anti-inflammatory effect. Mice were treated with DSS (5%) by oral administration for 9 days and then mice were divided into control group, Ping-Wei Decoction group, dexamethasone group and Ping-Wei Decoction combined with dexamethasone group. Gavage administration of Ping-Wei Decoction, dexamethasone, or Ping-Wei Decoction combined with dexamethasone for 28 days with significantly inhibited the severity of ulcerative colitis compared to the colitis control group. The body weight, anti-inflammation cytokine IL-10, and anti-oxidant enzyme such as glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase in Ping-Wei Decoction, dexamethasone, or Ping-Wei Decoction combined with dexamethasone group were significantly higher than control group. Compared to control group, inflammation-related cytokine factors like serum TNF- α levels, inflammation-related factors such as cyclooxygenase-2 (COX-2) protein, reactive oxygen species (ROS), and myeloperoxidase, (MPO) were significantly lower in those groups. Taken together, gavage with Ping-Wei Decoction, dexamethasone, or Ping-Wei Decoction resulted in marked inhibitory effects on IBD after DSS treatment and less extensive colonic shortening in mice. Notably, mice treated with Ping-Wei Decoction combined with dexamethasone had significantly improved the histological scores of the colons compared with the groups of mice treated with Ping-Wei Decoction or dexamethasone alone. Therefore, both Ping-Wei Decoction alone and Ping-Wei Decoction combined with dexamethasone may be potential anti-inflammatory properties for treatment of in DSS-induced colitis.

Keywords: *Ping-Wei Decoction, dexamethasone, Inflammatory Bowel Disease, mice*

PB2

The Relationship between Arterial and Intraosseous Pressure in Pigeons (*Columba rupestris*) in Hypovolemic Model

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Blood pressure is an important vital sign in veterinary medicine. However, both direct and indirect blood pressure measurement methods are not practical due to the invasiveness and inaccuracy respectively. Studies in African grey parrots showed that the inflow arterioles likely account for the retained arterial waveform radiating into the intraosseous space. To further investigate if the routinely used intraosseous route could serve as a blood pressure assessing method, the aim of the research is to find out the relationship between intraosseous pressure (IOP) and arterial pressure (AP) in pigeons (*Columba rupestris*) using a hypovolemic model and whether needle placement sites affect the results; this research was approved by the IACUC of National Taiwan University. Twenty-six healthy pigeons were included in the study and divided into two groups randomly. The intraosseous route was set up in the distal ulna in one group and in proximal tibiotarsus in another using a 21-gauge 1-1/4 inch hypodermic needle. At the same time, a 24-gauge 3/4 inch catheter was placed into the deep radial artery. AP and IOP were recorded simultaneously under 4 stages for 10 minutes: 0%, 20%, 30% and 60% of blood loss. Pressure data are descriptively summarized, with the percent of change of IOP at each stage compared to change of AP using a multilevel mixed effects linear model. The result showed mean intraosseous pressure (MOP) and mean arterial pressure (MAP) were normally distributed ($p < 0.05$), except tibiotarsus MOP and MAP in 20% of blood loss. Significant difference ($p < 0.05$) was found in MAP of each stage of both groups, except it between 20% and 30% of blood loss. Significant difference ($p < 0.05$) was founded between 0% and 30%, 0% and 60%, 20% and 60% of blood loss in IOP of ulnar group. In contrast, this was only found between 0% and 30%, 0% and 60% of blood loss IOP of tibia group. According to the result, the decreasing tendency of both MAP and MOP is noted, especially compare to the normal stage (0% blood loss), in the hypovolemic model in pigeons. Results of this study provide a potential alternative option for blood pressure assessment in hypovolemic pigeons, but more studies have to be conducted before practical use.

Key words: Intraosseous pressure, arterial pressure, hypovolemic model

PB3

Establishment of immortalization of fish cell lines

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Cells are the main biological material for virological research and the production of viral vaccines. Based on the wide variety of fish species and the species-specificity of viruses, and very few types of fish cells for research in biological resource conservation centers, there are many restrictions on the development of virus and fish vaccines. Therefore, this study hopes to establish immortalized cell of fishes including *Acipenser sinensis* and *Epinephelus fuscoguttatus* for virology research and vaccine development. Currently, the liver, brain, fin, kidney, gill, and spleen of *Acipenser sinensis* and grouper are selected for cell line establishment. For confirming the stability of primary cells by successive subcultures by using *Nervous necrosis virus* (NNV) and *Koi herpes virus* (KHV) infection and observing virus proliferation, cytopathic effect determined the growth rate of virus. NNV and KHV were used to infect *Acipenser sinensis* fins, gills, brain, liver cells, and brain, fin, gill cells of grouper, and CPE were observed daily. qPCR and TCID₅₀ are used to calculate the titer of the virus to understand the growth of the virus in the cell. Under daily microscope observation, only the brain cells of *Acipenser sinensis* had CPE on NNV infection; the brain, fin, and gill cells of the grouper had CPE on NNV infection. The results of cycle threshold (CT) value was detected by qPCR. Results shows that the initial NNV infection of *Acipenser sinensis* brain and spleen cell is not as good as grouper cells, but results are similar. After multiple infections and domestication, the virus are expected to improve the adaptability on *Acipenser sinensis* cells and elevate titer.

Keywords: *Acipenser sinensis*, *Epinephelus fuscoguttatus*(grouper), Nerve necrosis virus (NNV), Koi herpes virus (KHV)

PB4

Survey of Pathogenic *Leptospira* in The Environment of Different Seasons in Southern Taiwan

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Leptospira can infect from trauma or mucosa in almost all mammals in the world. Humans and animals could be infected by contacting direct or indirect with urine, water, or wet soil that contaminated *Leptospira*. The purpose of the study was to investigate the potential risks of water when containing the pathogenic *Leptospira* in different seasons from 2021 to 2022. We used the flotation-like method to concentrate the *Leptospira* in the water samples, which were the Yingxia lake, wastewater treatment center, goat houses in NPUST, and the drainage outlet after sewage treatment in pig farms of Pingtung County and Kaohsiung City. We extract the leptospiral DNA and a real-time polymerase chain reaction assay using a TaqMan probe targeting lipL32 was applied to detect pathogenic *Leptospira*. Results were *Leptospira* in the water of drainage outlet of pig farms and goat houses in the rainy season (summer and early autumn, 2021). We did not get *Leptospira* in the water samples during the dry season (winter and early spring, 2022). Otherwise, we measured the *Leptospira* species by the OmpL1 PCR-RFLP that the *Leptospira interrogans* 1b had been detected in the pig farms. However, we could not isolate the *Leptospira* from the water samples directly. The study indicated that the water from the drainage outlet of animal farms is a significant source of pathogenic *Leptospira* to contaminate the environment, especially in the rainy season in Southern Taiwan.

Keywords: *Leptospira*, water, lipL32

PB5

The Impact of Different Urine Sources on *Leptospira*

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Leptospira can infect from trauma or mucosa in almost all mammals in the world and the bacteria were shedding from the animal urine. The aim of the study was to find out the different animal's urine impacts leptospiral transmission. We selected the non-pathogenic *L. biflexa*, intermediate-pathogenic *L. inadai*, and pathogenic *L. interrogans* to place in the different animal's urine for different periods. After the urine treatment, we took the *Leptospira* every interval into the EMJH medium and checked the *Leptospira* growth status every week. According to grow density to judge the urine impact on *Leptospira*. Results showed that the minimal impact on all leptospiral strains was the urine of swine and goats after just one week they can grow very well after urine treatment. All leptospiral strains were poorly cultured after both cow and dog urine treatment. The intermediate pathogenic *L. inadai* could grow after both rat and human urine treatment. In addition to the effect of urine pH, there are other influencing factors that impact the *Leptospira*, and the sooner *Leptospira* are excreted via animal urine in a suitable environment the better their survivability.

Keywords: *Leptospira*, urine, EMJH medium

PB6

Detection of cat-scratch disease (*B. henselae*) of blood and saliva in cat by using iiPCR and Nested PCR methods

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Bartonella henselae (*B. henselae*) is the main pathogen of cat-scratch disease (CSD or felinosis), which is classified as *Rickettsia*. The main purpose of this study was to investigate the methods of sampling and detection of specimens when cats were suspected of being infected with *B. henselae*. We compared insulated isothermal PCR (iiPCR) and Nested PCR by testing *B. henselae* specimens which come from the blood and saliva of cats. These samples source included Pingtung Animal Shelter, Tainan Vet Hospital, and Stray Cats Protection Association. In 40 specimens, the 6 positive samples and 34 negative samples of *B. henselae* had been detected by Nested PCR and iiPCR. The similarity rate of both for positive *B. henselae* was as high as 100%. In the chi-square test, there are no significant differences between Nested PCR and iiPCR. Comparing detection time, iiPCR is completed faster than Nested PCR. These results then, provide clinical veterinarians options with more effective detection methods when they encounter suspected cases of *B. henselae* infection.

Keywords : *cat-scratch disease*; *Nested PCR*; *insulated isothermal PCR*; *Veterinary Clinical Diagnostics*.

PB7

Establishment of Deep Corneal Stromal Ulcer Animal Model in Rats and Investigation of Features in Optical Coherence Tomography and Histopathology.

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Corneal ulcer is a common ocular disease in veterinary ophthalmology, which not only causes the patient's discomfort, but also leads to serious complications. The most common reason causing deep corneal stromal ulcer in small animals is mechanical trauma. There was no surgery induced deep corneal stromal ulcer animal model in rats. The aims of the study were to establish a stable deep stromal ulcer animal model in rats and understand the stromal healing process and features by optical coherence tomography (OCT) and histopathology. Lamellar keratectomy was used to induced deep stromal ulcer. Evaluations were performed by ophthalmic examinations, OCT, histology, and immunohistochemistry of markers of corneal stromal healing. Post-op OCT cross-sectional images confirmed deep stromal ulcer and showed increased stromal signal intensity in the surgical treated stroma. At post-op days 3, the OCT images revealed hyper-reflectivity in both corneal epithelial and stromal areas compared to the healthy corneal tissues, and the epithelial thickness was noticeable thicker than the healthy epithelium. At post-op day 7, the signal intensity and the thickness of the epithelium were comparable to the undamaged epithelium. The hyper-reflectivity stromal layers were still present. The histological analysis showed moderate infiltration of neutrophils and mild number of macrophages at post-op day 3. There were multifocal of myofibroblasts located in the wounded stromal tissue at post-op day 7. Based on the results of this study, a deep stromal ulcer animal model was stably established in rats, and the stromal healing response was studied in detail. This surgery induced deep stromal ulcer animal model in rats could be used in future studies on efficacy of different treatment modalities for deep corneal stromal ulcers in animals.

Keywords: Corneal stromal ulcer, OCT, myofibroblast

PB8

Estimation of Origins and Insertions of Canine Stifle Ligaments

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Accurate estimation of the origins and insertions of stifle ligaments is crucial for surgical treatment planning and guidance, implant designing and the reconstruction of subject-specific computational models used for predicting muscle and ligament forces. Especially, knowledge regarding the footprint locations of cranial cruciate ligament (CrCL) on the bones is required for performing the intra-articular CrCL repair. However, it is difficult to observe the femoral footprint of the CrCL in small and medium-sized dogs in surgery. Preoperative prediction of CrCL endpoint location may be a viable alternative. Previous research has established radiography-based methods to quantify the endpoint locations in 2D planes. The objective of the study was to propose model-based description and prediction methods for the exploration of 3D endpoint locations of the CrCL, caudal cruciate ligament (CaCL), and lateral and medial collateral ligaments (LCL and MCL) in the canine stifle. The source of data required for model building is twofold. First, statistical shape models (SSMs) of the femur and tibia were built from 54 CT-derived bone surface models of healthy Taiwan dogs. Second, the ligament information together with femoral and tibial shapes were recruited from CT scans of 19 hindlimb specimens (9 left hindlimbs and 10 right hindlimb) with radio-opaque marking on the ligament footprints. The SSMs were subsequently transformed to best fit the bone shapes of 19 specimens. Following the shape transformation, all specimens' ligament footprints were mapped onto the corresponding deformed SSMs, from which the centroid coordinates were obtained and expressed in barycentric coordinates (i.e., the endpoint location of the ligament). The average and dispersion of 19 ligament endpoints were analyzed. On the other hand, the SSMs of the femur and tibia imbedding with averaged endpoint locations were referred to as the deformable shape template (DST) used for estimating ligament endpoint locations. In the present study, the individual ligament endpoints were estimated following a leave-one-out cross-validation framework. The DST built from 18 out of 19 samples was transformed to best fit the bone shapes of the left-out sample, and the ligament endpoints were transformed accordingly to provide the estimated endpoint location of each ligament. The results showed that the dispersions of all the ligament endpoints were below 2.1 mm. The means \pm standard deviations of the predictive endpoint errors (i.e., Euclidean distance between estimated endpoint and true endpoint) in the femur and tibia were found to be less than 1.81 ± 1.02 mm and 2.09 ± 1.29 mm, respectively. The shape transforms on the DST provides an alternative way to estimate the 3D endpoint locations of the stifle ligaments, which can be applied for pre-operative planning in the anatomical repair of deficient ligaments and as well in the computational modeling of the stifle joint.

Keywords: ligaments, stifle, statistical shape model, bone templates, subject-specific

PB9

Protective efficacy against *Vibrio harveyi* in shrimp induced by feed containing anti-*V. harveyi* IgY encapsulated in PLG microparticles

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Vibrio harveyi is an important pathogen of marine fish and invertebrates (particularly prawns). Antibiotics are so far used to prevent most bacterial diseases in shrimp. However, overuse of antibiotics may lead to drug residues in the environment and aquatic organisms or emergence of drug-resistant bacteria. These chemical substances, therefore, have been prohibited to conduct in aquaculture in numerous countries. Thus, there is an urgent need to develop other possible alternatives to combat aquatic diseases. Since invertebrates lack an effective specific immune system, it is ineffective to induce the active immunity through vaccination. So, induction of the passive immunity will be a feasible method to protect invertebrates from infection. Immunoglobulin IgY (IgY) is avian immunoglobulin that can be stored in egg yolk. Specific IgY can be isolated and purified from eggs laid by laying hens immunized with antigens. Through oral passive immunization, IgY can be used in target animals for immune prevention and treatment. In this study, quantified *V. harveyi*(BCRC13812) was inactivated in 0.3% formalin. Laying hens were inoculated intramuscularly and subcutaneously four times at 21-day intervals with inactivated *V. harveyi* emulsified with Freund's incomplete adjuvant. Eggs were collected daily and the average total IgY content per egg was 61.32 mg following analysis. The purified IgY from eggs could recognize *V. harveyi* proteins after Western blot analysis. Moreover, anti-*V. harveyi* IgY showed an effective minimum inhibitory concentration to *V. harveyi*, indicating that IgY prepared in the study displayed a bacteriostatic effect. Thus, the purified IgY possessed the potential to prevent *V. harveyi* infection. In the challenge test, 1×10^7 CFU of *V. harveyi*, which could cause 100% mortality rate in shrimp (*Litopenaeus vannamei*) by 7 days, will be used as the challenge dose in protective assays in the future. The purified IgY will be encapsulated with poly (lactic-co-glycolic acid) to form controlled-release microparticles and mixed with the shrimp feed. The protective efficacy induced by the shrimp feed containing anti-*V. harveyi* IgY microparticles will be evaluated in shrimp.

Keywords: *Litopenaeus vannamei*, *Vibrio harveyi*, passive immunization, IgY (immunoglobulin Y), poly(lactic-co-glycolic acid) (PLG)

PB10

Effects of Oral Supplementation on Dry Eye Disease in a Rat Model
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Dry eye disease is a common ocular surface disorder which is characterized by tear film instability and ocular surface damage. Although the pathogenesis of dry eye is not thoroughly understood, previous studies have showed that inflammation and oxidative stress are both involved in disease progression. Eyedrops such as artificial tears are used as initial treatment to help maintain moisture of ocular surface, however, the residence time of artificial tears are relative short which may need frequently applying. Under this premise, we are interested in oral supplementations in aid of soothing dry eye symptoms. In this study, we investigated the effects of fish oil and lutein complex supplements on a dry eye disease induced by excising exorbital lacrimal gland in rats. Sixteen female Sprague-Dawley rats aged 7 to 8 weeks old were randomized to four groups (saline, fish oil, high dose lutein complex and normal dose lutein complex). Schirmer tear test, non-invasive tear film break-up time and fluorescein scoring were investigated at baseline and weekly after dry eye induction for 4 weeks. A significant improvement in tear volume, tear break-up time and fluorescein score were observed in the rats fed with fish oil and lutein complex (500mg/kg/day and 100mg/kg/day) compared with the control group which was fed with saline. The result suggests that dietary supplements, such as fish oil and lutein, are beneficial in alleviating dry eye symptoms.

Keywords: dry eye disease, oral supplement, fish oil, lutein

PB11

Development of Skin Damage Model Induced by Ultraviolet B Irradiation in Mice

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The skin long-term exposed to sunlight will cause skin aging and sunburn, which can lead to many wrinkles. Ultraviolet B (UVB) that contained in sunlight, can cause sunburn, inflammation, redness, peeling and skin cancer. In this study, Institute of Cancer Research (ICR) mice irradiated by UVB for continuous five days to cause skin damage. According to our results, UVB irradiation caused a significant decrease in the skin moisturizing properties, the expressions of catalase, superoxide dismutase, hyaluronic acid, and collagen type I. Furthermore, UVB irradiation caused a significant increase in the IL-1 β , IL-6, and malondialdehyde of mice. Under the histopathologic examination, UVB exposure would increase the thickness of the skin and cause sunburn to skin cells. Based on the above results, we successfully established the UVB-irradiated skin damage model. In the future, we can use this mouse model in skin care products development.

Keywords: skin damage model, mice, ultraviolet B

PB12

Establishment of a Mouse Model with Colitis via Dextran Sulfate Sodium Administration

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Inflammatory bowel disease is a multifactorial chronic disease involved in ulcerative colitis and Crohn's disease, which are the chronic intestinal disorders that result from dysfunctional or abnormal epithelial and immune responses to intestinal microorganisms. Many studies have demonstrated that colitis can be induced in animals to investigate inflammatory colitis. Thus, we wish to establish a mouse model with colitis via dextran sulfate sodium (DSS) administration and apply the mouse model to investigate the potential treatments. In this study, mice were grouped and respectively treated with 2% DSS under two periods. Data showed the clinical and macroscopic changes of DSS-treatment mice in body weight, stools, colon weight, colon length, colon weight/length ratio, and myeloperoxidase activity. Inflammatory colitis was induced in mice successfully by DSS via drinking water administration in this study. Taken these results together, this mouse model of DSS-induced colitis will enable us to investigate the potential treatments included anti-inflammatory drug selections and therapeutic strategies for inflammatory colitis in the future.

Keywords: colitis, mouse model, dextran sulfate sodium

PB13

Establishment of a Mouse Model with Retinal Lesion via Light-induction

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Retinal lesion is a common disease of the eye, which was caused by aging or trauma. Some may mildly affect the vision, while others severely may lead to blindness. This study let mice exposed under 600-1,000 lux light-emitting diode (LED) light 12h/12h light-dark cycle to induce retinal damage. In this model, LED-exposed mice' outer nuclear layer (ONL) and out segments / inner segment thickness were thinner than the normal mice. The glial fibrillary acidic protein positive cells displayed in the ONL when mice were exposed to LED light. The data also showed the gene expressions of the inflammatory factors increased by the LED light. Up to the results, we established a light-induced mouse retinal lesion model, which mimics the retinal damage from LED light. In the future, we can use this mouse model in health supplements development or medicines screening.

Keywords: retinal lesion, mouse model, light-induced

PB14

Epidemiology of Porcine Circovirus Type 3 in Central Taiwan from 2020 to 2021

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Porcine circovirus type 3 (PCV3), an emerging disease, was first described in 2015 in the USA. Subsequently, PCV3 has been reported in several countries worldwide. To investigate the PCV3 prevalence in Taiwanese pig farms, we collected 138 samples from 50 farms between 2020 and 2021 and examined them by polymerase chain reaction (PCR). The sample and herd positive rates for PCV3 were 27.5% and 42.0%, respectively. PCV3 has been detected in most counties in central Taiwan and possibly all over the whole island. Seventeen positive samples were subjected to PCR with another three pairs of primers to assemble the complete PCV3 genome sequence and performed phylogenetic analysis. The 17 PCV3 whole genomic sequences in this study showed a 98.95-100% similarity. The phylogenetic analysis revealed that PCV3 isolates were clustered with the Taiwanese strain, Chilean strain, and Japanese strain, indicating that PCV3 might come from multiple sources.

Keywords: porcine circovirus type 3, prevalence, phylogenetic analysis

PB15

Comparison of Leptospiral Serovars in Dogs and Wild Small Mammals in Taiwan

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Pathogenic *Leptospira* can infect almost all mammals throughout the world. Wild small mammals are the major carrier who have no clinical symptoms after leptospiral infection. In addition, the carrier can shed *Leptospira* in urine throughout life. Dogs, as companion animals in close contact with people, when dogs are infected with pathogenic *Leptospira* could make people exposure its infection. Sera samples of dogs and wild small mammals are collected and the antibody titers are detected by the microscopic agglutination test (MAT) during 2021-2022 and 2016-2021, respectively. The goal of the study was to compare the correlation of leptospiral serovars between dogs and wild small mammals by the MAT. From 2021 to April 2022, a total of 78 sera samples of dogs were collected from different areas (40 from the northern area, 26 from the central area, 10 from the southern area, and 2 from the eastern area) and a total of 472 sera samples of wild small mammals (59 from the central area and 413 from the southern area) for MAT measurement. Results showed the dog's leptospiral serovars following *L. santarosai* serovar shermani was 15% (6/40) as the major serovar, *L. interrogans* serovar autumnalis was 7.5% (3/40) in the northern area; *L. interrogans* serovar canicola (11.5%, 3/26) and *L. interrogans* serovar icterohaemorrhagiae (11.5%, 3/26) were the dominant in central Taiwan; *L. santarosai* serovar shermani (20%, 2/10) and *L. interrogans* serovar autumnalis (20%, 2/10) were dominant in southern Taiwan. The comparison of leptospiral serovars between the dogs and the wild small mammals showed the wild small mammals have more leptospiral serovars than dogs. In southern Taiwan, *L. interrogans* serovar icterohaemorrhagiae is the major serovar (3.9%, 16/413), followed by *L. interrogans* serovar pomona (3.4%, 14/413) in the wild small mammals. We also found *L. interrogans* serovar kennewicki, *L. borgpetersenii* serovar Javanica, and *L. borgpetersenii* serovar poi in the wild small mammals. In conclusion, there are many serovars carried by wild small mammals, and whether there correlated with the dog's leptospirosis still needs study in the further.

Keywords: *Leptospira*, wild small mammals, dogs, serovars, MAT

PB16

Monitoring of bacterial pathogens in poultry farms with Hazard Analysis and Critical Control Points (HACCP) system

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In this study, the HACCP system was used to monitor bacterial pathogens in poultry farms. The study design had three parts. 1. Conducted hazard analysis in poultry farm. 2. Conducted water quality control, and decided CCP of water supply system in poultry farm. 3. Adding β -Glucan into feed to investigate the effect of reducing infection of pathogenic bacteria in chickens. A farm with 5000 chickens that 18-week-old chickens were selected. Anal sampling was performed before and after adding β -Glucan into the feed for three weeks. A total of 10 anal swabs were sampled from the front, middle and rear sections of the same poultry building, and were transported to the laboratory under refrigerated storage on the day of sampling. Samples were cultured and tested by using 3M™ Petrifilm™ Quick Screen Plates. The result of the rapid screening test is that the positive rate of *Salmonella* spp. reduced from 70% to 0% after adding β -Glucan into feed, and the results of polymerase chain reaction and electrophoresis analysis confirmed that the sample was *Salmonella* spp. The results of this study showed that the β -Glucan added can reduce the infection of *Salmonella* spp. and also other pathogenic bacteria in chickens. The CCP set by applying the HACCP management system for hazard analysis in poultry farms maybe improve management methods, reduce the antimicrobial preparations used, and expect effectively to monitor pathogenic bacterial infections in poultry farms.

Keywords: β -Glucan , diet , poultry , HACCP , *Salmonella* spp.

PB17

A Dog with Infected Paraprostatic Cyst and Severe Electrolytes Imbalance

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An 11-year-old, intact male Dachshund was presented to NTUVH for hematuria. At presentation, ultrasound showed prostatomegaly with heterogeneous echogenicity and a paraprostatic cyst. Drainage of the cyst was performed under ultrasound guidance at the clinic and the fluid was sent for examination and bacterial culture. Urinary tract infection and prostatitis were suspected, and an antibiotic was prescribed. The patient deteriorated dramatically after going home, with blood dripping from the penis and decreased activity, so was brought back to the hospital the next day. Severe electrolytes imbalance and dehydration were found, and the patient was hospitalized. During hospitalization, the patient underwent fluid therapy for electrolytes correction and blood transfusion. Meanwhile, a canal communicating the urinary bladder and the paraprostatic cyst was discovered under ultrasound. After being stabilized, the patient underwent an exploratory laparotomy for paraprostatic cyst biopsy and resection. Histopathology result of the biopsied paraprostatic cyst revealed chronic-active inflammation, and the result of cystic fluid bacterial culture was positive for *Enterococcus faecium*. Even though the canal connecting the cyst and the bladder was repaired during surgery, it was formed again several weeks after. Since no clinical signs are shown afterward, no further treatment was given and the patient carried a new bladder with a connected cyst without complications. Paraprostatic cyst is rarely reported in veterinary medicine. It is considered as an embryological remnant of Mullerian duct. Most patients discovered this structure incidentally until it reaches sufficient size to suppress other organs and causes clinical signs of the urinary tract. Hematuria or pyuria may be noted if an infection happened. Spontaneous urethral fistulation may result. Surgical intervention for resecting the paraprostatic cyst is needed for long-term control. In the present case, the patient had a huge paraprostatic cyst discovered and accompany by infection and hematuria. Fluid accumulated in the cyst which became a third space led to severe electrolytes imbalance. A fistula was formed between the bladder and the paraprostatic cyst. Even though surgery failed to repair the fistula, the patient showed no clinical signs afterward.

Keywords: paraprostatic cyst, prostatitis, electrolytes imbalance, dog

PB18

The Impact of Preservation Methods of Dog and Cat Urine Samples on the Result of Quantitative Bacterial Culture

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Urinary tract infection (UTI) is a common disease in veterinary practice. The diagnosis of bacterial UTI is based on the presence of related clinical signs and detection of pathogens through quantitative bacterial culture (QBC). Current guidelines for QBC suggested that samples should be inoculated immediately or stored under refrigeration and inoculate within 24 hours. Despite this, some studies have shown that storage conditions can affect the result of QBCs and even lead to false results. The major goal of our study was to compare the results of dog and cat urine QBC between (1) immediate streak plate, (2) streak plate after urine sample stored at room temperature for 24 hours, and (3) streak plate after urine sample stored at 4°C for 24 hours. The other goal of the study was to find out correlations between positive bacterial culture results and the results of urinalysis. We included 49 cat urine samples and 30 dog urine samples in our study. QBC results of cat urine samples storing at room temperature and under refrigeration for 24 hours were all consistent with immediately cultured results. As for dog urine samples, those storing under refrigeration for 24 hours had consistent results with immediately cultured results, but 2 of the urine samples storing at room temperature yielded different results. However, the overall agreement was still high (kappa 0.84). Presence of pyuria and bacteriuria were highly correlated with positive QBC results for cats, and absence of pyuria and bacteriuria could rule-out possibility of positive QBC results. As for dogs, absence of pyuria and bacteriuria could rule-out possibility of positive QBC results. The results of this study suggested that it was unnecessary to refrigerate urine samples for QBC during shipping to other laboratories within 24 hours. In addition, presence or absence of pyuria and bacteriuria can help to predict QBC results.

Keywords: quantitative bacterial culture, cat, dog, urinalysis

PB19

Investigation and Comparison of the Efficacy of Gelatin-Epigallocatechin gallate Nanoparticles with Hyaluronic Acid and Commercial Artificial Tears on Keratoconjunctivitis Sicca Associated Keratopathy in Rats

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Keratoconjunctivitis sicca (KCS), also known as dry eye syndrome, is a disorder commonly presented in dogs. It is categorized into two main types: qualitative and quantitative KCS. Regardless of the type of KCS, artificial tears are used to relieve KCS-related symptoms. Among different types of artificial tears, those containing hyaluronic acid (HA) has been proved to be of better performance in different objective tests. Other than traditional medical treatment options, the use of nanoparticles has been increasingly more popular as it can overcome certain barriers of drug delivery of eyedrop application. HA is one of the applicable ingredients that can improve performances of nanoparticles. In this experiment, a KCS rat model was established through excision of exorbital lacrimal gland (ELG) and infraorbital lacrimal gland (ILG). Commercial artificial tears containing different main active ingredients were applied to KCS-induced rats. In addition, an antioxidant epigallocatechin gallate, in combination with gelatin and hyaluronic acid, were used to produce nanoparticle eye drops. The efficacy of the artificial tears and gelatin-epigallocatechin gallate with hyaluronic acid nanoparticle (GEH) on the cornea in KCS rat models were evaluated. Other than common ophthalmic assessments including palpebral opening, Schirmer's tear test (STT), fluorescein staining and slit-lamp biomicroscopic examination, advanced tools such as Optical Coherence Tomography (OCT) and Ocular Surface Analyzer-VET (OSA-VET) were also adopted for evaluation of treatment outcomes, which resulted in successful imaging in this experiment. Results showed that KCS rat model was successfully established through excision of ELG+ILG, with significantly lower post-surgery STT values, smaller palpebral opening, shorter non-invasive tear break-up time, higher degrees of corneal irregularity and more severe corneal lesions by fluorescein staining compared to pre-surgery. Treatment groups showed more favorable results compared to KCS group in different examinations. Specifically, GEH group had significantly larger palpebral opening, longer non-invasive break-up time, less corneal irregularity compared to KCS group. While all groups had no significant changes on corneal epithelial thickness observed under OCT, only GEH group, carbomer-containing tears group and hyaluronate-carbomer containing tears group showed no significant changes on corneal stromal thickness along time. It is concluded that other than the use of commercial artificial tears, nanoparticles may be utilized for future treatment options on KCS patients.

Keywords: Keratoconjunctivitis sicca, dry eye, artificial tears, nanoparticles

PB20

Development of Recombinant Protein Adjuvants Based on *Vibrio harveyi* Flagellin Gene in *Epinephelus coioides*

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Vibrio harveyi is a gram-negative bacterium that has flagella which are useful for locomotion. This bacterium is an important pathogen causing vibriosis in shrimp and some marine fish, including the orange-spot grouper (*Epinephelus coioides*) can become infected with these pathogens. In *V. harveyi* virulent genes such as chitinase, exotoxin, metalloprotease, serine protease, hemolysin and flagellin genes have been identified. The toxin produced by *Vibrio harveyi* flagella can be a source of infections in fish. The purpose of this research is to look at how gene responses in orange-spot grouper (*Epinephelus coioides*). Binding of *VhFliA* to TLR5 could activate the innate and adaptative immune system to stimulate production of cytokines. This study used *VhFliA* and mutants gene and analyzed cytokine (IFN γ , IL-1 β , IL-6, IL-8, and IL-10) and also IFN γ downstream gene (MHC class I, MHC class II, CD4, CD8) immune response gene. Determine the responsiveness of *Vibrio harveyi* flagellin (*VhFliA*) gene expression in the spleen and head kidney of grouper. Total RNA was extracted from the spleen and head kidney, and qRT-PCR was used to analyze it. The post-injection observation period is repeated several times. There are four different treatments: EGFP as a control, wild-type *VhFliA*, and two mutagenesis of wild-type *VhFliA* (Δ MH-*VhFliA* and Δ DOMH-*VhFliA*). We've found an upregulation in cytokines and downstream genes. In this study, we identified and characterized *V. harveyi* flagellin A (*VhFliA*) gene. We investigated the in vivo activities of recombinant *VhFliA* protein and its mutant forms (Δ MH-*VhFliA* and Δ DOMH-*VhFliA*) in grouper. This study will provide the development of epidemic prevention strategies in aquaculture species using recombinant *VhFliA* and its mutants protein

Keywords: *Vibrio harveyi*, flagellin, toll-like receptor 5, Immune response, *Epinephelus coioides*

PB21

Development of a Novel Scoring System for the Assessment of Neurological Function in Dogs

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Applying a scoring system to evaluate the disease progression or functional changes has been commonly applied in human medicine. It is also gradually utilized in veterinary medicine over the recent decades. Scoring systems allow evaluation and documentation of specific topics and reduce subjective bias or recall bias between evaluations. Previous published semi-quantitative scoring systems for the functional evaluation of the neurological system in dogs mainly focused on traumatic brain injury or acute spinal cord injury. Currently, no validated scoring system is available for evaluating the functional changes of the complete nervous system in dogs with chronic diseases. This study aims to develop a scoring model encompassing both the brain and spinal cord function. Neurological tests regularly performed clinically were incorporated with the Texas Spinal Cord injury Scale to form a semi-quantitative neurological evaluation system. The ability of the scoring system to present different severity of neurological signs in dogs with various neurolocalization is evaluated and validated. A 10% change from the baseline of the score defines a functional improvement or deterioration. A total of 40 dogs, in which 26 dogs were localized to a forebrain, brainstem, or peripheral vestibular system problem and 14 dogs were localized to a spinal cord problem, were recruited for score distribution evaluation. Twenty-one out of 40 dogs were used to evaluate the intra- and interobserver reliability. Results showed good to high intraobserver reliability (ICC ranges for two observers: 0.801-1.00) in the majority of the sub-tests, except spinal palpation and vestibular eye movement. Except for moderate agreement in spinal palpation, good to excellent interobserver reliability (ICC range: 0.792-1.00) between two observers was also demonstrated, supporting overall the ease of use and good agreement between observers. Cronbach's alpha value indicated a good internal consistency of the scoring system between groups of sub-tests evaluating forebrain, brainstem and spinal function (Cronbach's alpha ranges: 0.701-0.758). Limitations include small sample size and limited variations in neurological deficits collected in the study. Current results concluded that the developed scoring system is feasible to be used as a semi-quantitative method to evaluate functional neurological changes, but further evaluation of the sensitivity to detect changes may be required.

Keywords: scoring system, neurological function, dogs

PB22

The Effects of Equilibrative Nucleoside Transporter 1 (ENT1) Inhibitor on Insomnia

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Insomnia is one of the most common sleep disorders with a high morbidity and affects approximately 30%-40% of the general population. Hypnotic is a class of psychotropic drugs which the primary function is to induce sleep or treat insomnia, but also having different adverse effects. Hypnotics either can enhance the activity of inhibitory neurons or inhibit excitatory neurons. Gamma-aminobutyric acid (GABA) is one of the most important inhibitory neurotransmitters and is regarded as a sleep regulator. Many sedatives and hypnotics are GABA analogs which increase the efficiency of GABA action through GABA receptors (mainly GABAA receptors). GABA and its upstream regulators play an essential role in sleep initiation. Adenosine is one of the most focused upstream regulators, which promotes sleep and shows hypnotic effects as GABA. However, there is no adenosine analogs as hypnotic medication due to severe cardiovascular outcomes. Our previous studies have demonstrated that adenosine 2A receptors (A2ARs) are involved in the activity of GABAergic neuron regulation in the ventral lateral preoptic area (VLPO), and adenosine analog T1-11 exhibits a hypnotic effect. Another study found that A2AR positive allosteric modulator (PAM) enhances adenosine signaling at the A2AR and induces NREM sleep without affecting blood pressure or heart rate in wild type mice after intraperitoneal administration, which also reveals the potential direction of hypnotic drug development. In this study, we want to investigate the role of adenosine transporter ENT1 inhibitor in insomniac rodents. Caffeine administration and change of the cage bedding are two different stressors using to induce acute insomnia in rodents, and electroencephalograms (EEGs) are used to record and analyze the sleep-wake activity. The results showed that the ENT1 inhibitor dose-dependently increased NREM sleep within the first 6 hours of the dark (active) period. We also revealed that the ENT1 inhibitor can dose-dependently block the insomnia effects caused by caffeine or stress. The series of experiments can demonstrate the feasibility of a new drug development.

Keywords: insomnia, adenosine analogs, sleep disrupt

PB23

Treatment of Acute Pancreatitis in One Dog with Traditional Chinese Veterinary Medicine

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Male Mixed dog intact 12 years old. Anorexia and vomiting for 3 days admitted to Veterinary Medical Teaching Hospital National Chung Hsing University, Taichung, Taiwan. Clinical presentation includes weakness, abdominal pain and lower body temperature (BT:37.8°C). Immunologic assays with SNAP cPL test (IDEXX Laboratories, Inc) result positive. Abnormal results of routine clinic pathological features in CBC and serum biochemical profile are BUN 150mg/dl (7-27mg/d), Creatinine 15g/dl (0.5-1.8 g/dl), Alkp 300mU/ml (23-212 mU/ml), TP 8.7g/dl (5.2-8.2 g/dl), RBC 5.24 10⁶/ul (5.5-8.5 *10⁶/ul), HGB 11.1 g / dl (12-18 g / dl), PCV 29.3% (37-55 %), PHOS 15.4 mg/dl (2.5-6.8mg/dl). Initial diagnosis is severe Acute Pancreatitis. Treat using Traditional Chinese Veterinary medicine. Including enema with liquid composed of Da Huang (*Rheum*)、Chuan Xiong (*Ligusticum*)、Huang Qi (*Astragalus*)、Jin YinHua (*Lonicera*)、Pu Gong Ying (*Taraxacum*)、Bai Mao Gen (*Imperata*)、Yi Mu Cao (*Leonurus*), aquapuncture (Acupoints: BL18, BL19, BL20, BL21, BL22, ST36, ST37, ST44) and oral Chinese herbal (Da Chai Hu Tang 1g* Tid). Significant improvement after treatment. Conclusion : Traditional Chinese Veterinary Medicine treatment may provide a new option for acute pancreatitis in dog.

Keywords : Acute Pancreatitis , Chinese Herbal, Aquapuncture, Enema

PB24

Chemo-Drug Loaded Hydroxyapatite-Gelatin Composite Microspheres for Local Treatment in a Subcutaneous Xenograft Model

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Chemotherapy is a widely used technique for cancer treatment, which is mainly administered by intravenous route in clinic. However, achieving sufficient drug concentration in the target tissue without causing side effects has been a challenge. Thus, new cancer therapies that are more targeted to tumor tissue, such as local chemotherapy, have gained increasing attention in recent decades. Hydroxyapatite - gelatin composite microspheres (HAM) are crystals that can be simply prepared by wet-chemical methods. The porous surface properties have also shown the advantages of carrying a greater amount of pharmaceutical molecules. Chemo-drugs, including paclitaxel (PTX) and doxorubicin (DOX), have been successfully loaded into HAM particles with drug loading concentrations of 0.036 mg PTX/mg and 5 mg DOX/mg, respectively. To confirm the in-vivo anti-tumor ability and side effects of locally administered drug-loaded HAM, human osteosarcoma G-292 cell line was used for the subcutaneous xenograft model. The tumor-bearing mice were randomly divided into four groups of 5 mice each: (1) Tumor control without treatment, (2) low dose intratumoral drug-loaded HAM (3) high dose intratumoral drug-loaded HAM, and (4) systemic administration. Treatments were given when the tumor volumes reached 0.1 cm², followed by a 14-day observation. Clinical observations showed significant tumor volume reduction in both local treatment groups, the histological examination also showed a greater tumor tissue necrosis ratio in both drug-loaded HAM groups. Immuno-histological analysis revealed that local treatments induce more significant tumor cell apoptosis and inhibition of tumor cell proliferation as well. Systemic administration of DOX caused cell injuries in the crypt of the intestine, ovary, spleen, and bladder, but those injuries were minor or absent in the local treatment groups. However, a drug leakage was noticed when DOX-HAM samples suspended within normal saline were given intratumorally, which then caused skin ulceration and spleen white pulp cell apoptosis in a dose-dependent manner. In summary, intratumoral drug-loaded HAM can reduce tumor volume, and cause more necro-apoptotic tissue within the tumor, which shows its potential in new cancer treatment systems, yet further study is required to improve the drug-loaded-HAM retention within the tumor.

Keywords: Hydroxyapatite -gelatin composite microspheres, osteosarcoma, nude mice

PB25

Multiple Prolonged Stress Disrupts Behavioral and Neuronal Activities in Rats

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Post-traumatic stress disorder (PTSD) is a severe psychological disorder developed in traumatized humans and animals. In rodent, chronic PTSD syndrome exhibits psychological and physiological abnormalities during fear memory processing, including intrusive, avoidance, negative alteration, and hyperarousal symptoms. These symptoms can be developed in many animal models, such as using the electric footshock stimulation, social defeat stress, and predator stress. However, the most existing model used a single arrangement as the stimulator, and the comprehensive multi-stressor stimuli have less been discussed. Therefore, a seven-day multiple prolonged stress (MPS) was conducted in rats by randomizing the given stressors and modifying the stressors' manipulating period and intensity from the single prolonged stress (SPS) model to cause the prolonged impairment of fear extinction on PTSD. MPS consists of four different stressors, including the 2-h restraint, 20 mins of force swim, 12 times of inescapable footshock (each shock paired with a cue tone) within 10 mins, and a short term of anesthesia with isoflurane per day for seven consecutive days. The extended fear memory is evaluated by long-term fear retrieval-induced startle responses and the increased corticosterone concentrations after the MPS manipulation. The theta oscillation coherence in the circuitries between the prefrontal cortex, basal lateral amygdala and ventral hippocampus is correlated with the behavior alteration in memory retrieval and behavioral anxiety tasks. Moreover, the MPS and fear retrieval also triggered the irregular sleep-wake activity to disrupt fear memory consolidation. Together, our results from the fear extinction deficit, neuronal connectivity, anxiety behavior, and sleep alteration indicated the integrated chronic effects of MPS could successfully conduct a PTSD-like model in rodents.

Keywords: PTSD, MPS, fear, neuronal activity, anxiety, sleep

PB26

**Synovial Fluid Analysis of Rescued Sea Turtles in Taiwan (2019-2021)
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Septic arthritis is one of several illness that frustrates clinicians working with sea turtle rescuing. Difficulties encountered during rehabilitation include unclear pathogenesis, delay-onset, long-term treatment requirement and poor prognosis. To diagnose arthritis, arthrocentesis had been used as one of minimal-invasive tools prior to advanced surgical biopsy. Previous case reports mentioned the cytology of synovial fluid from affected sea turtles. To the best of our knowledge, the characteristics of normal synovial fluid in sea turtles, however, had not been well-described. During 2019-2021, 16 rescued sea turtles were recruited in this study. Inclusion criteria were as follow: normal limb motor functions, no radiographic evidence of joint lesion and no hematologic or plasma biochemistry evidence of systemic infection. Arthrocentesis was performed at bilateral shoulder joints. Qualitative analysis of the synovial fluid including color, texture, and cytology were obtained. Normal synovial fluid presented transparent to light straw color. The string could be pulled to over 2 centimeters. Cytology was dominated by mononuclear cells, occasionally with blood contamination. The present study reported characteristics of normal synovial fluid of rescued sea turtles in Taiwan. The results of the study could provide basic diagnosis data for comparison to sea turtles with septic arthritis.

Keywords: sea turtle, septic arthritis, arthrocentesis, cytology

PB27

Application of HACCP to Monitor the Hazard Factor in Horse Farm — the Impact of Mycotoxins in Concentrations of Fecal Bacteria

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In horses feeding management, in order to maintain horses health state, hindgut microbiota is one of the most important factors and the contamination of mycotoxins may influence the balance of hindgut's microbiota. The application of hazard analysis critical control point (HACCP) can be an effective tool to prevent the potential risk in horses feeds from being contaminated by mycotoxins. The research aimed to discuss if mycotoxins have an impact on the *Escherichia coli* and the anaerobic bacteria. This positive rate of commercial horse feeds (n = 16) and forage (n = 3) are Fumonisin (0%, minimal detection amount (MDA) > 5,000 ppb) , Zearalenone (0%, MDA> 200 ppb) , Deoxynivalenol (23.52%, MDA> 1 ppm) and Aflatoxin B1 (0%, MDA> 10 ppb) , and all positive results of deoxynivalenol came from the same farm (DON-positive farm) . The feces of clinical healthy adult horses (n = 28) were collected from two different farm, farm A (n = 18) and farm B (n = 10) , and the sex and the body condition score of the horses at the time of sampling were recorded. Mann-Whitney-Wilcoxon Rank Sum was performed in statistical analysis. Both of the mean concentration of aerobic count and *E. coli* did not differ between DON-positive farm and DON-negative farm, and so as the body condition scores. The relationship between mycotoxins and concentration of aerobic count and *E.coli* shows little connection in this study while the confirmed quantity technique for the positive result of deoxynivalenol was not performed. Noted that the mean body condition score of DON-positive farm is 4.5 while DON-negative farm is 5.8. The HACCP system may be used to monitor the hazard factor to influence the health condition in horse farm.

Key words: HACCP, Horse farm, mycotoxin, fecal bacteria

PB28

Development of a Colloidal Gold-Based Immunochromatographic Strip for Rapid Detection of Classical Swine Fever virus

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Classical Swine Fever (CSF) is caused by the genus *Pestivirus* of the family *Flaviviridae* virus. The genome of CFSV is very closed to *Bovine Viral Diarrhea* in cattle and *Border's Disease* in sheep. CSF is a highly contagious porcine viral disease. The clinical signs of CSF are very different, the mortality rate from high mortality (95% to 100%) to mild, even no clinical signs. It is very important to have the laboratory tests to detect antibody and the virus antigen for disease prevention and treatment. There are several conventional test methods for CSFV, such as virus isolation, RT-PCR or ELISA. All the methods are time-consuming and with high cost. Moreover, there is no commercialize immunochromatographic strip (ICS) for CSFV antigen detection. In the present study, a competition colloidal gold-based ICS was developed with synthetic E2 peptide and anti-E2 peptide polyclonal antibodies was developed to specifically detect CSFV antigen. First, the linear peptide located in the extracellular surface domain of the E2 protein was predicted by bioinformatics analysis. Two special peptides sequence (12-14 aa) with high degree surface antigenicity and high conserved between different CSFV isolates were chosen and synthesized. Two SPF (Specific Pathogen Free) rabbits were immunized with 2 synthetic polypeptides to produce 2 polyclonal antibodies. After dot blot analysis, one of the polyclonal antibodies has the better recognition effect for LPC and wild type CFSV, TD96, but not BAS. The competition colloidal gold-based ICS was established with this peptide and polyclonal antibody. Moreover, the sensitivity of ICS for CSFV was evaluated with commercial LCP vaccine. The detection limitation of homemade ICS is $10^{0.85}$ RID50. The homemade competition ICS with rapidity, convenience and on-site screening property provides a useful tool for the surveillance and diagnosis of CSFV infection in clinical test and may improve the eradication efficiency of CSF in Taiwan.

Keyword: Classical swine fever, immunochromatographic strip, antibody

PB29

**Case Report: Nasal Adenocarcinoma in A Canine
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A 12-year-old spayed female Sheltie dog with history of chronic kidney disease and choleliths presented with clinical signs of unilateral epistaxis, hemorrhagic nasal discharge, sneezing and stertorous breathing for approximately 11 months. The dog was diagnosed with a mass located in the right nasal cavity via rhinoscopy at a local animal hospital and which the case was subsequently transferred to National Chiayi University Veterinary Teaching Hospital. Radiography and computed tomography findings suggested the presence of an intranasal tumor. The computed tomography three-dimensional reconstruction demonstrated focal lysis of right frontal bone, maxillary bone, zygomatic bone, palatine bone and turbinate without invading cribriform plate, and thus, the tumor was classified as modified Adams' stage 3 nasal tumor. Adenocarcinoma in the nasal cavities was diagnosed based on Tru-cut biopsy and histopathological examinations. Thoracic radiography and cytological examination of bilateral mandibular lymph nodes via fine needle aspiration displayed no evidence of tumor metastasis. Low-dose metronomic chemotherapy was performed which cyclophosphamide and piroxicam were orally administered once daily at a dosage of 15.0mg/m² and 0.3mg/kg respectively. The dog presented clinical signs of hematemesis, hematochezia, and neurological signs after 14 days from the treatment and subsequently died. Survival time was approximately 9 months from diagnosis.

Keywords: canine, intranasal tumor, adenocarcinoma, metronomic chemotherapy

PB30

The Feasibility of Diffusion-weighted Magnetic Resonance Imaging for the Diagnosis and Prognosis Prediction in Dogs with Meningoencephalitis of Unknown Etiology

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Meningoencephalitis of unknown etiology (MUE) is a group of non-infectious diseases of the central nervous system in dogs. The histopathological examination serves as the only gold standard for the definitive diagnosis. Due to the difficulty and complications of the brain biopsy, clinicians usually achieve the clinical diagnosis of MUE based on the signalment, neurological examination, MRI characteristics, and cerebrospinal fluid (CSF) analysis. Diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) map is an MRI technique that detects the microscopic mobility of water molecules. It has a significant role in diagnosing various brain diseases, such as cerebral ischemia and tumor characterization. Its role in diagnosing viral encephalitis in humans has also been documented. This retrospective study aims to describe the DWI and ADC values in canine MUE and investigate their value for the diagnosis or outcome prediction. Dogs with neurological signs indicating a brain problem, MRI and CSF findings consistent with inflammatory disease, and negative results in pathogen screening tests were included. Dogs with necrotic lesions on MRI were excluded as they present as another sub-type of MUE. ADC map was created by calculating the signal intensities on DWI at two b-values (0 and 1000 s/mm²). For each dog, lesions were first identified in the conventional MRI sequences. Corresponding areas in the ADC map then were placed with multiple circular regions of interest (ROIs). ADC values were generated from the ROIs by imaging software. In addition, five circular ROIs were placed within the normal-appearing white matter area of the other cerebral hemisphere, representing the ADC value of normal brain tissue. The Mann-Whitney U test was applied to compare ADC values between the lesions and normal white matter. If the results are significantly different, further analysis is conducted to identify the association between ADC value and various factors, including age, body weight, CSF results, MRI findings, and short-term survival time. Sixteen dogs were included. The ADC values of the lesion were significantly different from the normal white matter area. However, after categorizing the lesion region into cerebrum, cerebellum, and brainstem, only lesions in the cerebrum and brainstem showed significantly different ADC values from the normal white matter. No association was identified between the ADC value and all the factors. In conclusion, for dogs with MUE causing cerebral and brainstem lesions, DWI may have potentials to aid the diagnosis. Further study is warranted to compare the ADC values between MUE and other brain diseases. Based on the results, the ADC value could not aid the outcome prediction. However, the small sample size is a major limitation of this study.

Keywords: meningoencephalitis of unknown etiology, diffusion-weighted imaging, apparent diffusion coefficient

PB31

Diagnosis of Thymoma and Thymic Lymphoma in Rabbits (*Oryctolagus cuniculus*)

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Thymoma and thymic lymphoma are the top two differential diagnosis of mediastinal neoplasms in rabbits. However, biopsy may be too invasive for rabbits and sometimes cytology alone is inconclusive for definitive diagnosis. In dogs, flow cytometry and PCR for antigen receptor rearrangement (PARR) had been used to differentiate mediastinal mass routinely. To achieve the goal of diagnosing thymoma and thymic lymphoma with low invasive approach in rabbits, we try to investigate the potential of flow cytometry to distinguish these two neoplasms in clinical cases. Fine needle aspirated samples collected from mediastinal mass of visiting patient and cadaver were analyzed by flow cytometry and cytology. The results of above mentioned tests were correlated with final diagnosis of histopathology postmortem. We expect a higher ratio of CD4+CD8+ lymphocytes in thymoma samples. By matching histopathology diagnosis with flow cytometry and cytology, we expect our study could demonstrate that flow cytometry is effective tools for discriminating the two major mediastinal neoplasms in rabbits.

Keywords: flow cytometry, thymoma, thymic lymphoma, mediastinal neoplasm, rabbit, *Oryctolagus cuniculus*

PB32

**Expression of Androgen Receptor in Canine Hepatoid Gland Tumors
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Hepatoid glands are modified sebaceous glands only found in Canidae and Marsupial. Neoplasms of the hepatoid glands, including hepatoid gland adenoma, epithelioma and carcinoma, are common in dogs. The aim of this study was to investigate the expression of androgen receptor (AR) in various types of canine hepatoid gland tumors (HGTs). In total, 47 dogs with 68 HGTs were enrolled. Among the HGTs, 40 (59%) were diagnosed as an adenoma, 20 (29%) were epithelioma and 8 (12%) were classified as a carcinoma. The mean age of dogs was 11.8 years old (ranged from 7 to 15). In the breeds with HGTs, mixed-breed dogs (12; 25.5%) predominated in the population, followed by Toy poodle (5; 10.6%), Beagle (4; 8.5%) and Husky (3; 6.4%). Regarding the gender incidence rate of tumors, most of the HGTs occurred in male dogs, especially in intact male dogs (87%), fewer in castrated male dogs (12%) and only one case occurred in an intact female dog (1%). Expression of AR in HGTs was determined by immunohistochemical staining and western blotting with anti-AR antibody (anti-androgen receptor clone AR441, diluted 1:50 for immunohistochemistry and diluted 1:200 for western blot). Results showed that all types of HGTs presented positive reaction of AR in nuclei. According to the immunoreactive score system and statistical analysis, total expression scores of adenoma and epithelioma were significantly higher than those of carcinoma (both $p < 0.05$). By western blotting for anti-AR antibody, showed a distinct band of 110 kDa which is consistent with the predicted molecular weight of the AR protein. In conclusion, the incidence of HGTs is obviously higher in intact male dogs but lower in castrated male dogs and female dogs. Also, adenoma and epithelioma showed significantly stronger expression of AR when compared with carcinoma.

Keywords: Hepatoid gland tumors, androgen receptor, immunohistochemical staining, western blot method, male dogs

Index (presenting author)

A

Andre Giovanni · 54

B

Benji Brayan Ilagan Silva·7

C

Chen Yi-Fei · 2

Chen Pin-Yu· 69

Chan Man-Ha·53

Chiang Yi-Shan ·48

Chang Shang-Lun ·70

Chung Hsyang-Hsun · 9.10

H

Hsu Hai-Wei ·28

Hsu Shin-Ji·53

Hsu Ching-Ching ·54

Hung Yu-Fan ·52

Hung Shao-Wen ·49.50.51

Huang Yu-Ling·31

Huang Hua-Chen·30

Huang Yu-Cheng·60

Hsieh Feng-Cheng·33

Hsieh Wan-Ru·62

K

Ko I-Ting·13

Kuo Chuan-Te 9

Kao Chen-Yung21

Yang Shun-Nung ·22

L

Lu Chih-Chun ·65

Li I-Chen · 47

Li Chia-Ling ·39

Lee Yu-Chi ·42 ·43

Lee Fan ·15

Lee Ann Nee ·6

Lee Hao ·45

Lin Yi-Chen · 47

Lin Yu-Ying ·46

Lin Bing-Ing ·27

Lin Chiu-Hui ·14

Liao Chen-Yin ·61

M

Man Hoi-Yan ·32

S

Su Hsiang-Pei· 64

Shih Huei-Ming ·41

T

Tsai Shen-Pang ·66

W

Wu Jia-Shuan · 25

Wang Po-Hsiang 40

Wong Wen-yi ·59

Y

S
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