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PROGRAMME & ABSTRACT BOOK

1st INTERNATIONAL CONFERENCE ON

POULTRY AND FISH VACCINOLOGY AND DIAGNOSIS

17th -18th JANUARY 2023

Co-organizers:

MALAYSIA
Poultry Health for All

ASIAN FISHERIES SOCIETY
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Programme & Abstract Book

1st International Conference on Poultry and Fish Vaccinology and Diagnosis

"Advancements in animal vaccination and diagnosis: Opportunities and challenges"

17 - 18 January 2023

Co-organizers

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ABOUT THE CONFERENCE

The 1st International Conference on Poultry and Fish Vaccinology and Diagnosis 2023 (1st ICPoFiVD) is the first international conference that aims to gather all the experts in the field of poultry and fish from all around the world. The theme "**Advancements in animal vaccination and diagnosis: Opportunities and challenges**", it covers the latest development in the poultry and fish culture industry, which are strongly supported by advancements in disease diagnostics and vaccination technology. The current advancement would offer more research opportunities, while the challenges should be identified and tackled together for the sustainability of the animal production industry. This conference would be able to provide the best platform to enhance research and innovations through networking, knowledge sharing and collaboration among the participants.

Conference Organizers:

Conference Partners:

Venue:
The Everly Hotel, Putrajaya

Conference Site:
https://www.icpofivd.com/

FOREWORD FROM THE VICE CHANCELLOR UPM

Assalamualaikum WBT and greetings I wish to everyone.

First and foremost, all praise be to Allah the Almighty and the holy Prophet Muhammad (Peace Be Upon Him). I am thankful that by the grace of Allah, we are attending this 1st International Conference on Poultry and Fish Vaccinology and Diagnosis 2023 (1st ICPoFiVD) to share knowledge, information, new ideas, and experiences.

I am pleased that the Institute of Bioscience (IBS) has taken the initiative to organize a conference that gathers two main groups of animals that are important in Malaysia's livestock industries. This conference is in line with Higher Institution Centres of Excellent (HICoE) IBS niche area in animal vaccine and therapeutics. In addition, this conference partners with five important organizations: World's Poultry Science Association - Malaysia branch (WPSA), World Veterinary Poultry Association - Malaysia (WVPA), Malaysian Fisheries Society (MFS), Asian Fisheries Society (AFS), and Informa Markets Malaysia Sdn Bhd. Thus, with all the combined energy and ideas, the theme "Advancements in animal vaccination and diagnosis: Opportunities and challenges" transpired. This theme addresses topics from disease control to secure animal health, food security, and food quality. Reducing the burden of animal diseases and appropriately managing emerging diseases are considered priorities to achieve sustainable livestock systems.

The main objective of this conference is to discuss current innovations and future prospects for poultry and fish therapeutics strategies. It also provides a platform to update latest developments in poultry and fish culture industries, which are strongly supported by advancements in disease diagnostics and vaccination technology. In addition, the conference also aims to gather all experts in these fields to exchange ideas and knowledge, and for future international collaborations.

It is my fervent wish that this conference will help reinforce and enhance even greater unity among us scientists. Together, we forge ahead to face whatever challenges the future may bring to help reach the advancements in animal vaccination and diagnosis.

All the best 1st ICPoFiVD 2023!

Thank you.

DATO' PROF. DR. MOHD ROSLAN SULAIMAN

Vice Chancellor, Universiti Putra Malaysia

FOREWORD FROM THE CHAIR OF THE ORGANISING COMMITTEE

Welcome to the 1st International Conference on Poultry and Fish Vaccinology and Diagnosis 2023 (1st ICPoFiVD)! This is the first conference which combines researchers from two main livestock in Malaysia, poultry and fish. In line with the Institute of Bioscience (IBS) Higher Institution Centers of Excellent (HICoE) niche area, Animal Vaccine and Therapeutics, this conference will enable delegates to share the recent research findings as well as to establish new research relations. We are pleased to partner with the World Veterinary Poultry Association – Malaysia (WVPA), World's Poultry Science Association - Malaysia branch (WPSA), Asian Fisheries Society (AFS), Malaysian Fisheries Society MFS), and Informa Markets Malaysia Sdn. Bhd. in jointly organizing this much awaited event.

Our conference theme for 1st ICPoFiVD is 'Advancements in animal vaccination and diagnosis: Opportunities and challenges'. Disease control, high production, product quality, and reasonable production cost have been the recent main goals of poultry and fish farming. Hence, meeting the consumption requires continuous efficient and goal-oriented animal husbandry to control disease spread and decrease the application of antibiotics. These endeavors will include the development of innovative therapeutics to control infectious diseases, advancement in diagnostics, and ensuring the safety and security of animal products. This conference aims to provide the latest information on vaccine application, immunoprophylactic strategies, diagnostics, and disease management practices in sustainable farming.

In these two days events, there will be many interesting talks from our keynote, plenary, plenary session, invited and technical speakers. We have also received overwhelming participation for oral and poster presentations encompassing various topics related to; poultry disease diagnostics and vaccinology; fish disease diagnostics and vaccinology; and animal health and production aspects of other livestock. I hope everyone will actively engage in all the interesting sessions designed for the conference and actively interact with our sponsors, who will showcase the latest instruments and cutting-edge technology in their exhibition booths. I truly hope these will broaden your horizons and benefit your scientific career in vaccinology and diagnosis for fish and poultry.

I also take this opportunity to thank all our speakers, oral presenters, poster presenters, judges, participants, conference partners, and sponsors for making the 1st ICPoFiVD conference a great success. My deepest appreciation also goes to the organizing committee members for their unfailing commitment and support in making this conference a reality despite all the challenges and obstacles.

Finally, I wish you a fruitful meeting and an enjoyable time at the conference.

PROF. DR. ZUNITA ZAKARIA

Chairperson, 1st ICPoFiVD

ORGANISING COMMITTEE

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Co-Chairperson	:	Assoc. Prof. Dr. Ina Salwany Md Yasin
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		Ms. Noor Nadia Mohd Sohaili
		Ms. Nor Hamizah Mashud

OPENING CEREMONY

1st International Conference on Poultry and Fish Vaccinology and Diagnosis 2023 (1st ICPoFiVD)
Mesmera Hall 4, The Everly Hotel Putrajaya, Malaysia
17 January 2023 (Tuesday)

Time	Programme
08:00	Arrival of Participants/Guest Registration
08:45	Arrival of ❖ YBhg. Dato' Prof. Dr. Mohd Roslan Sulaiman Vice Chancellor UPM ❖ Mr. Modibo Samake, Senior Advisor to the Prime Minister of Cote d'Ivoire ❖ Mr. Eric Ballo, Senior Advisor to the Prime Minister of Cote d'Ivoire ❖ YBhg. Dr. Akma Ngah Hamid Director General, Department of Veterinary Services ❖ YBhg. Prof. Dr. Daud Ahmad Israf Ali Director IBS ❖ YBerusaha Prof. Dr. Zunita Zakaria Chairperson of the 1st International Conference on Poultry and Fish Vaccinology and Diagnosis (1st ICPoFiVD) ❖ YBerusaha Assoc. Prof. Dr. Ina Salwany Md Yasin Co-Chairperson of the 1st International Conference on Poultry and Fish Vaccinology and Diagnosis (1st ICPoFiVD) ❖ YBerusaha Assoc. Prof. Dr. Nurulfiza Mat Isa Co-Chairperson of the 1st International Conference on Poultry and Fish Vaccinology and Diagnosis (1st ICPoFiVD) ❖ YBerusaha Assoc. Prof. Dr. Mohammad Noor Amal Azmai Head of Laboratory Aquatic Animal Health and Therapeutics (AquaHealth)
09:00	National Anthem *'Negaraku'* and *'Putra Gemilang'*
09:05	Prayer Recital
09:10	Welcoming Remarks by ❖ YBerusaha Prof. Dr. Zunita Zakaria Chairperson of the 1st International Conference on Poultry and Fish Vaccinology and Diagnosis (1st ICPoFiVD)
09:25	Officiated by ❖ YBhg. Dato' Prof. Dr. Mohd Roslan Sulaiman Vice Chancellor UPM
09:45	Multimedia Presentation Souvenir Presentation Photography Session
10:00 - 10:30	Refreshments End of Opening Ceremony

SCIENTIFIC PROGRAMME SCHEDULE

1ST INTERNATIONAL CONFERENCE ON POULTRY AND FISH VACCINOLOGY AND DIAGNOSIS 2023 (1ST ICPOFIVD)			
DAY 1 **17 January 2023 – Tuesday**			
08:00 – 08:45	**REGISTRATION**		
08:45 – 10:00	**OPENING CEREMONY** Mesmera Hall 4 YBhg. Dato' Prof. Dr. Mohd Roslan Sulaiman Vice Chancellor UPM		
10:00 – 10:30	**TEA BREAK**		
11:00 – 11:45	**KEYNOTE PROGRAMME 1** Chairman: Prof. Dr. Zunita Zakaria Dr. Akma Ngah Hamid *Department of Veterinary Services Malaysia*		
11:45 – 12:15	**PLENARY PROGRAMME 1** Prof. Dr. Michael Hess *University of Veterinary Medicine, Vienna*		
12:15 – 14:00	**LUNCH BREAK**		
	CONCURRENT SESSIONS		
	SESSION 1 **POULTRY** Mesmera Hall 4 Chairman: Assoc. Prof. Dr. Nurulfiza Mat Isa	**SESSION 1** **FISH** Inspirasi Room 1 Chairman: Dr. Nur Diyana Mohamad Tahir	**SESSION 1** **ANIMAL HEALTH AND PRODUCTION** Inspirasi Room 4 Chairman: Assoc. Prof. Dr. Zetty Norhana Balia Yusof
14:00 – 14:30	**Plenary Session 1 Poultry:** *World Veterinary Poultry Association* *Dr. Teguh Prajitno *PT Japfa Comfeed Indonesia Tbk*	**Plenary Session 1 Fish:** *Malaysian Fisheries Society* Assoc. Prof. Dr. Mohammad Noor Amal Azmai *Malaysia Fisheries Society/ Universiti Putra Malaysia*	**Plenary Session 1 Animal Health and Production:** Dr. Alifah Ismail *Department of Veterinary Services, Malaysia*
14:30 – 14:50		**Oral paper 5** Dr. Azila Abdullah *National Fish Health Research Division*	**Oral paper 10** Assoc. Prof. Dr. Lokman Hakim *Universiti Putra Malaysia*
14:50 – 15:10	**Oral paper 1** Assoc. Prof. Dr. Sharifah Syed Hassan *Monash University Malaysia*	**Oral paper 6** Assoc. Prof. Dr. Ina Salwany Md. Yasin *Universiti Putra Malaysia*	**Oral paper 11** Assoc. Prof. Dr. Samson Soon *Infrastructure University Kuala Lumpur*

15:10 – 15:30	**Oral paper 2** Dr. Joan Molist Badiola *Laboratorios HIPRA*	**Oral paper 7** Assoc. Prof. Dr. Marina Hassan *Universiti Malaysia Terengganu*	**Oral paper 12** Dr. Ruhil Hayati Hamdan *Universiti Malaysia Kelantan*
15:30 – 15:50	**Oral paper 3** Dr. Yu Choo Yee *Universiti Putra Malaysia*	**Oral paper 8** Assoc. Prof. Dr. Natrah Fatin Mohd Ikhsan *Universiti Putra Malaysia*	**Oral paper 13** Dr. Kua Beng Chu *Department of Fisheries, Malaysia*
15:50 – 16:10	**Oral paper 4** Prof. Dr. Zunita Zakaria *Universiti Putra Malaysia*	**Oral paper 9** Assoc. Prof. Dr. Murni Marlina Abd Karim *Universiti Putra Malaysia*	**Oral paper 14** Assoc. Prof. Dr. Annas Salleh *Universiti Putra Malaysia*
16:10 – 17:00	**POSTER PRESENTATION** **&** **HI-TEA NETWORKING SESSION** Mesmera Hall 4		

DAY 2 **18 January 2023 – WEDNESDAY**			
08:00 – 09:00	**REGISTRATION**		
09:00 – 09:45	**KEYNOTE PROGRAMME 2** Mesmera Hall 4 Chairman: Assoc. Prof. Dr. Ina Salwany Md Yasin Prof. Dato' Dr. Mohd Hair Bejo *Universiti Putra Malaysia*		
09:45 – 10:15	**PLENARY PROGRAMME 2** Prof. Dr. Abdul Rahman Omar *Universiti Putra Malaysia*		
10:15 – 10:45	**TEA BREAK**		
	CONCURRENT SESSIONS		
	SESSION 2 **POULTRY** Mesmera Hall 4 Chairman: Dr. Norfitriah Mohamed Sohaimi	**SESSION 2** **FISH** Inspirasi Room 1 Chairman: Assoc. Prof. Dr. Natrah Fatin Mohd Ikhsan	**SESSION 2** **ANIMAL HEALTH AND PRODUCTION** Inspirasi Room 4 Chairman: Assoc. Prof. Dr. Siti Nurbaya Oslan
10:50 – 11:20	**Plenary Session 2 Poultry:** *World Veterinary Poultry Association* *Dr. Marcelo Paniago *Ceva Poultry*	**Plenary Session 2 Fish:** *Asian Fisheries Society* Prof. Dato' Dr. Mohamed Shariff Mohamed Din *Universiti Putra Malaysia*	**Plenary Session 2 Animal Health and Production:** Prof. Dr. Mohd Zamri Saad *Universiti Putra Malaysia/ Bio Angle Vacs Sdn. Bhd.*

11:20 – 11:40	**Oral paper 15** Assoc. Prof. Dr. Nurulfiza Mat Isa *Universiti Putra Malaysia*	**Oral paper 20** Mr. Modibo Samake *Senior Advisor to the Prime Minister of Cote d'Ivoire*	**Oral paper 25** Dr. Faizal Ghazali *Universiti Sultan Zainal Abidin*
11:40 – 12:00	**Oral paper 16** Dr. Ng Kian Yiing *Innovative Diagnostics*	**Oral paper 21** Prof. Dr. Alim Isnansetyo *Universitas Gadjah Mada*	**Oral paper 26** Dr. Norhariani Mohd Nor *Universiti Putra Malaysia*
12:00 – 12:20	**Oral paper 17** Dr. Lau Ka Xin *Ceva Animal Health Malaysia*	**Oral paper 22** *Prof. Dr. Mahanama De Zoysa *Chungnam National University*	**Oral paper 27** Assoc. Prof. Dr. Rozaihan Mansor *Universiti Putra Malaysia*
12:20 – 12:40	**Oral paper 18** Dr. Norfitriah Mohamed Sohaimi *Universiti Putra Malaysia*	**Oral paper 23** *Assoc. Prof Dr. Channarong Rodkun *Chulalongkorn University*	**Oral paper 28** *Dr. Raden Dikky Indrawan *Institut Pertanian Bogor*
12:40 – 13:00	**Oral paper 19** Dr. Tan Sheau Wei *Abadiah Laboratori Sdn. Bhd.*	**Oral paper 24** *Dr. Jérôme Delamare-Debouteville *WorldFish*	**Oral paper 29** *Dr. Ilias Giannenas *Aristotle University of Thessaloniki, Greece*
13:00 – 14:00	**LUNCH**		
14:00 – 14:30	**POSTER PRESENTATION**		
	STUDENT SESSION POULTRY Mesmera Hall 4 Chairman: Dr. Yu Choo Yee	**STUDENT SESSION FISH /ANIMAL HEALTH AND PRODUCTION 1** Inspirasi Room 1 Chairman: Dr. Zarirah Mohamed Zulperi	**STUDENT SESSION FISH /ANIMAL HEALTH AND PRODUCTION 2** Inspirasi Room 4 Chairman: Dr. Nor Yasmin Abd Rahman
14:30 – 14:45	**Student oral paper 1** Ahmad Attahiru Rufai *Universiti Putra Malaysia*	**Student oral paper 1** Hiroaki Saito *Tokyo University of Marine Science and Technology*	**Student oral paper 1** Sridevi Devadas *Department of Fisheries, Malaysia*
14:45 – 15:00	**Student oral paper 2** Siti Nur Bahiyah Azli *Universiti Putra Malaysia*	**Student oral paper 2** Dini Siswani Mulia *University of Muhammadiyah Purwokerto, Indonesia*	**Student oral paper 2** Nur Syafiqah Ishak *Universiti Putra Malaysia*

15:00 – 15:15	**Student oral paper 3** Mohammed Yusuf Zanna *Universiti Putra Malaysia*	**Student oral paper 3** Jumria Sutra *Universiti Putra Malaysia*	**Student oral paper 3** Mohamad Azzam-Sayuti *Universiti Putra Malaysia*
15:15 – 15:30	**NA**	**Student oral paper 4** Nurhikmah Abd. Aziz *Universiti Putra Malaysia*	**Student oral paper 4** Chin Yong Kit *Universiti Putra Malaysia*
15:30 – 15:45	**NA**	**Student oral paper 5** Nur Shidaa Mohd Ali *Universiti Putra Malaysia*	**NA**
15:45 – 16:15	**REFRESHMENTS**		
16:15 – 17:00	**STUDENT PRIZE GIVING AND CLOSING CEREMONY** Mesmera Hall 4		

*** ON-LINE SPEAKERS**

KEYNOTE SPEAKERS

Dr. Akma Ngah Hamid
Director General,
Department of Veterinary Service Malaysia

Dr. Akma Ngah Hamid have 30 years of experience in heading laboratories, divisions, and institute related to veterinary government agencies. Dr. Akma was a graduate with Doctor of Veterinary Medicine, UPM and Master of Food Science, UKM. Her wide experience has served to develop Malaysia livestock industries with high awareness on biosecurity and antimicrobial resistance issues. By focusing on veterinary health program along with her career in government agencies, Dr. Akma has been able to gather information on livestock observation data. She also interacts directly with farmers to discuss the current issues and provide field consultation. Over the years, Dr. Akma's strength at veterinary health has garnered recognition by the farmers communities and livestock industries.

Prof. Dato' Dr. Mohd Hair Bejo
Universiti Putra Malaysia

Prof. Dato' Dr. Mohd Hair Bejo is one of the world's leading experts in the fields of veterinary and avian pathology, as well as animal biotechnology and pathology. He obtained his Doctor of Veterinary Medicine DVM from UPM and PhD in the field of Veterinary Pathology at the University of Liverpool, England. He was the former Dean of Faculty of Veterinary Medicine and was the Director of Putra Science Park UPM. He leads many associations related to academics and poultry. In 2005 and 2015, he has commercialised a safe and effective MyVAC UPM93 Infectious Bursal Disease (IBD) Vaccine and MyHatch UPM93 IBD Vaccine for chickens, respectively for national and international markets. He has 12 Intellectual Property including seven patents, one trademark, one copyright and one trade secret. He has an excellent record of publications and graduated almost 100 postgraduate students.

PLENARY SPEAKERS

Prof. Dr. Michael Hess
University of Veterinary Medicine, Vienna

Prof. Dr. Michael Hess studied veterinary medicine and performed his post graduate education at the Institute of Poultry Diseases, Free University in Berlin. Currently, he is heading the Department for Farm Animals and Veterinary Public Health and the Clinic for Poultry and Fish Medicine. He has a strong interest in poultry health, development of new protection strategies and diagnostic tools. He is a member of various scientific associations, editorial boards and editor-in-chief of the MDPI journal Poultry. He was a founder diplomate and first President of the European College of Poultry Veterinary Science (ECPVS). He has published more than 200 peer-reviewed manuscripts and holds various patents as well as recipient of various prestigious awards.

Prof. Dr. Abdul Rahman Omar
Universiti Putra Malaysia

Prof. Dr. Abdul Rahman Omar obtained his Doctor of Veterinary Medicine from UPM and PhD in Immunology from Cornell University, the USA. He was the Dean of Faculty of Veterinary Medicine and Director of Institute of Bioscience, UPM. His research interest includes the use of biotechnology and immunogenomics approaches in the development of diagnostics, vaccines, and therapeutics against poultry diseases. He has more than 20 years teaching and research experience in avian disease and health. He works closely with various agencies and industries related to poultry health and production. He is also the regional advisor of Global Alliance for Research on Avian Diseases (GARAD) and member of the Advisory Board of Avian Pathology journal.

PLENARY SESSION SPEAKERS

Dr. Teguh Prajitno
World Veterinary Poultry Association (WVPA)

Dr. Marcelo Paniago
Ceva Animal Health Sdn. Bhd.

Assoc. Prof. Dr. Mohammad Noor Amal Azmai
Universiti Putra Malaysia/ Malaysia Fisheries Society

Prof. Dato' Dr. Mohamed Shariff Mohd. Din
Universiti Putra Malaysia

Dr. Alifah Ismail
Department of Veterinary Services, Malaysia

Prof. Dr. Mohd. Zamri Saad
Universiti Putra Malaysia

LIST OF SPEAKERS

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LIST OF POSTERS

ABSTRACTS

KEYNOTE PROGRAMME 1

THE WAY FORWARD FOR VETERINARY VACCINES IN MALAYSIA: REGULATORY PERSPECTIVE

Rohaya Mohd Ali*, Akma Ngah Hamid, Alifah Ismail, Siti Norzubaidah Abdul Rafar, Salleh Sheikh Ibrahim

Department of Veterinary Services, Ministry of Agriculture and Food Security, 62630 Putrajaya, Malaysia

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Abstract

Animal diseases pose a major threat to animal health and can cause economic losses to the livestock industry. Vaccines are an important tool in the prevention and control of animal diseases. To ensure the effectiveness and sustainability of animal disease prevention and control to minimize risks to humans and animals, the government is expected to provide appropriate veterinary vaccine regulations through veterinary legislation. To meet the country's demand for veterinary vaccines, parallel and synergistic with international standards and current global challenges, the Department of Veterinary Services Malaysia (DVS) has provided Veterinary Services and laboratories to support, facilitate and enable industry players to implement them. In this context, DVS has developed the Animal Act 1953 (Amendment, 2013), regulations, guidelines and procedures for veterinary vaccines. Currently, a total of 438 vaccines have been approved and registered in Malaysia according to animal diseases and species. The highest approved veterinary vaccines are the avian vaccines. Malaysia is still far behind in terms of veterinary vaccines developed by local private enterprises. Most of the animal vaccines approved and registered in this country are produced and imported from abroad. The only private veterinary vaccine manufacturer approved and registered in the country is Malaysian Vaccines and Pharmaceuticals (MVP). DVS has always encouraged and supported the private sector to explore and invest in the development and manufacture of veterinary vaccines in the country. Overall, the use of veterinary vaccines in Malaysia has made a great contribution in protecting animals and preventing disease and indirectly reducing the cost of treatment in the event of an outbreak.

Keywords: veterinary vaccine, regulation, legislation, animal health and livestock industry

KEYNOTE PROGRAMME 2

DEVELOPMENT, COMMERCIALISATION AND ADVANCEMENT OF POULTRY VACCINES AND VACCINATION

M. Hair-Bejo

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Abstract

The poultry industry in Malaysia has grown significantly at about 5 % annually and has contributed about 86% of livestock production and 75% of the ex-farm value of the livestock industry in the country. Malaysia is self-sufficient for poultry meat and eggs with the per capita consumption of chicken meat and eggs of 49.7 kg and 370 eggs, respectively; among the highest in the world. However, the industry is not free from many issues and challenges such as high production costs and disease outbreaks. To date, almost all known major poultry diseases have been reported in Malaysia. Prevention and control of poultry diseases involves complex understanding of the interaction between the agent, host and environment, and this can usually be achieved by proper biosecurity, vaccination and flock health management programmes. It is difficult and expensive to always maintain a high level of biosecurity and thus poultry vaccines are powerful tools in disease prevention and control. The development of poultry vaccines involves at least five important phases namely the target product profile, discovery/feasibility phase, early-phase development, late-phase development (field trials) and registration phase. There is no requirement for clinical phase I, II and III trials as in human vaccine. The period of development process for poultry vaccines took as early as 3 to 6 years, whilst for human vaccines for 10 to 20 years. It took 13 and 10 years for the commercialisation of MyVAC UPM93 and MyHatch UPM93 infectious bursal disease (IBD) vaccines, a success story of research, development, and innovation in the country. The application of biotechnology further enhances the advancement of poultry vaccines and vaccination, a new generation of vaccines. These vaccines hold promise for more advantages ahead when compared with the conventional vaccines. It is always a challenge to researchers to strategies and recognize that their research and finding are unique, or novel can be able to obtain the legal protection (intellectual property) and has high market values for commercialisation. The availability of appropriate facilities such as GMP and GLP, legal permissions, master seed production and upscaling, vaccine manufacture and regulatory requirements could delay process of vaccine development and commercialisation. Furthermore, with successive groups of consumers adopting the new technology, its market share eventually reaches the saturation level. In conclusion, the development, commercialisation and advancement of poultry vaccines and vaccination is a journey of an invention to wealth creation.

Keywords: poultry vaccines, development process, commercialisation, technologies, issues and challenges

PLENARY PROGRAMME 1

GLOBAL EMERGENCE OF FOWL ADENOVIRUS (FADV) INFECTIONS IN CHICKENS

Michael Hess

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Abstract

Fowl adenoviruses (FAdVs) are known since decades, being first described in the mid of last century when the viruses were isolated from embryonated eggs highlighting the importance of vertical transmission. So far, a total of 12 serotypes (FAdV-1-8a and 8B-11) are described some of them with a clear link to a certain disease. Inclusion body hepatitis (IBH) was noticed as the first disease due to an FAdV infection already in 1963. Later on, hepatitis-hydropericardium syndrome (HHS) and adenoviral gizzard erosion (AGE) were reported completing the range of FAdV-induced diseases.

Worldwide, the frequency of disease outbreaks due to FAdV infections increased substantially in recent years. The majority of IBH and HHS outbreaks occur in broilers which seem somewhat more susceptible due to fact that both diseases target liver and pancreas, main organs to keep the metabolic balance within an organism. This is somewhat more critical for the fast growing broiler. In comparison, AGE has a completely different pathogenesis with consequences on performance in broilers and layers.

The increasing number of disease outbreaks argues for intensified monitoring which is usually performed by virus isolation or detection by PCR coupled with sequencing to determine the genotype. Serology is mainly done by group-specific ELISA but virus neutralization test is needed to determine serotype-specific antibodies. Serology data are meagerly published but the overall tendency to increase biosecurity in breeders indicates less seroconversion during rearing with consequences on susceptibility and vertical transmission during production. Consequently, in recent years vaccination has gained high importance mainly to be applied in breeders but also in broilers. Vaccine types range from live vaccines, to killed whole virus vaccines up to the use of recombinant proteins as vaccine antigens.

The actual presentation will target the subjects mentioned above and aims to deliver a review and outlook on FAdV-induced diseases.

Keywords: fowl adenovirus (FAdV), inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS), adenoviral gizzard erosion (AGE), PCR, serology, vaccination

PLENARY PROGRAMME 2

POULTRY VACCINE TECHNOLOGIES: CURRENT STATUS AND FUTURE PROSPECTS

Abdul Rahman Omar

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Abstract

Currently, the poultry industry is threatened by either more virulent viruses of endemic diseases or by exotic and emerging diseases that can cause major economic losses to this sector. Catastrophic diseases caused by velogenic Newcastle disease virus (NDV) and highly pathogenic avian influenza (HPAI) H5N1 are not easy to overlook. However, the real challenge is to confront subclinical, immunosuppression and concurrent infections that act in concert with other factors such as management, environment and nutrition, which continuously threaten the entire poultry production system. There is no doubt, poultry vaccine and vaccination equipment have improved over the years. The control of poultry diseases has relied heavily on the use of conventional live and inactivated vaccines. However, genetic engineering technology has been used to generate recombinant vaccines. Some of the recombinant vaccines, especially the viral vector vaccines are available commercially, while many other recombinant vaccines are still in the research and development stages. Do we need recombinant vaccines to control and prevent poultry diseases?. What makes recombinant vaccines attractive and able to provide clear advantages over conventional vaccines when used in commercial birds. This paper reviews the current status and future prospects of poultry vaccine technologies in controlling viral diseases affecting commercial poultry.

PLENARY SESSION 1 POULTRY

NEWCASTLE DISEASE VACCINES: AN UNSOLVED PROBLEM IN THE CONTROL OF NDV– THE INDONESIA EXPERIENCES: LESSONS LEARNED

Teguh Yodiantara Prajitno[1,2,*], Inna Herliana[1], I Wayan Wisaksana Yasa[1], Refiana Lestary[1], Febriana Wulandari[1], Raditya Pradana Putra[1], Irfan Refangga[1], Sri Desintha Dwiharjanti[2], Hugeng Kurniawan[2], Didit Prigastono[2], Yohanes Joko Riyanto[2], Daniel Iki[2] and Maureen Kalona Kandou[1]

[1]Vaksindo Satwa Nusantara, Department of Innovation and Science, Bogor, Indonesia.

[2]Japfa Comfeed Indonesia Tbk, Poultry Breeding Division, Jakarta Indonesia.

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Abstract

Newcastle disease virus (NDV) causes high morbidity and mortality in chickens. Current live and inactivated ND vaccine strains have been in use for about 60 years and are insufficient to prevent severe damages to the reproductive tracts in egg-laying birds when challenged with velogenic NDV. Both, severe lesions and apoptosis in the oviducts of egg-laying hens caused by velogenic NDV strains are associated with the excessive release of inflammatory cytokines, chemokines and lymphocyte infiltration, which contribute to the dysfunction of the oviducts and the decrease of egg production in hens. Investigations into the immune functions of egg laying hens reveal irreversible immunological changes; strong decrease in the number of T-lymphocytes bearing cytolytic capacities and an increase in innate immune cells starting the adolescent phase through the egg-laying phase. We have shown that a low dose intranasal inoculation of velogenic NDV of genotype VIIh at 102 EID50 into broiler breeders above 15-16 weeks of age will result in dysfunction of oviduct and egg production disturbance. These findings suggest that NDV infection may have a greater impact in multi-aged farming system due to the concentration of susceptible poultry in these farms. Despite of intense vaccination, reports of ND outbreaks due to NDV of genotype VIIh and VIIi, sharing only 89% (F protein) and 87% (HN protein) amino acid identity in the protective antigens compared to the ND vaccine strains, have been increased in Asia. Reverse genetics construct Ban/AF, derived from the highly virulent NDV strain Banjarmasin/010, in which the virulent F protein cleavage site motif "RRQKR↓F" was modified to an avirulent motif "GRQGR↓L", was constructed. Our results suggest matching Ban/AF vaccine produces higher neutralizing antibodies and provide better protection than current used ND vaccines.

Keywords: Newcastle Disease, inflammation, immunity, vaccines, matching.

PLENARY SESSION 1 FISH

FEED-BASED VACCINATION IN CONTROLLING BACTERIAL FISH DISEASES: CURRENT ADVANCEMENTS, CHALLENGES, AND OPPORTUNITIES

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Abstract

Vaccination has been used to control disease outbreaks among cultured fish. Demands for commercially available fish vaccines is predicted to increase due to intensification of the aquaculture, increasing disease outbreaks, reducing antibiotic application and industry acceptance. In Malaysia, aquaculture vaccine research and development are progressing, where most studies focused on bacterial fish diseases, including vibriosis, streptococcosis and aeromoniasis. Most of the farmers in this country are from small to medium-sized scales, thus vaccine application should practical and cost effective to the local farmers – introducing the idea of feed-based vaccination. However, efficacy of feed-based vaccine is sometime questionable and "vaccinated fish" need time for acceptance from the publics and even farmers. Several vaccines against bacterial fish diseases have been developed and patented in Malaysia. However, none of it has been commercialized and available to be used at least for local farmers. This presentation describes the current advancement of feed-based vaccination in controlling bacterial fish diseases, while discussing the challenges and opportunities of the feed-based vaccination application Malaysia.

Keywords: bacterial diseases; feed-based vaccination; fish disease; advancement, challenges, opportunities.

PLENARY SESSION 1 ANIMAL HEALTH AND PRODUCTION

THE ROLE OF VACCINES IN PREVENTING ANTIMICROBIAL RESISTANCE (AMR) IN POULTRY IN MALAYSIA

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Abstract

Antimicrobial Resistance (AMR) remains as a major public health crisis and is emerging as a global health threat and also has given a great lost to the industry and economic due to the ineffective medication to treat the diseases. AMR refers to the ability of microorganisms such as bacteria, fungi, parasites and viruses to proliferate despite exposure to drugs designed to kill them or slow down their growth. Generally, antimicrobials in poultry are used for treating infections. However, they have been routinely used for disease prevention and for promoting growth. The indiscriminate use (prolong use and with inappropriate dosage) of antimicrobials in poultry will lead to AMR in human and animals due to the usage. In order to prevent the diseases, vaccines play an important role in developing immunity and protecting animal from viruses, bacteria and other microbials infection. It is one of the alternatives to prevent infection and could help to reduce antimicrobials usage in poultry. Vaccines are highlighted in the Malaysian Action Plan on Antimicrobial Resistance (myAP AMR) as an infection prevention and control (IPC) measures to confront and reduce the threat of AMR. To be widely used, vaccines have to be safe, effective, easy to use, and cost-effective.

Keywords: Antimicrobial resistance, AMR, vaccine, antimicrobial agents, poultry

PLENARY SESSION 2 POULTRY

ADVANCEMENTS IN POULTRY VACCINOLOGY AND ITS IMPORTANCE IN FIGHTING NEW AND OLD ENEMIES

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Abstract

Since Edward Jenner inoculated James Phipps with cow pox in 1796 and this procedure was called "vaccination" (*vacca* = cow, in Latin), the evolution of this method of disease prevention has developed remarkably. In 1870, Pasteur, working with a chicken disease - Fowl Cholera, developed the concept of artificial attenuation of a microorganism. Later, in 1920's, Gaston Ramon developed a method to inactivate antitoxins with formalin, creating toxoids that were used for immunization. The first generation of vaccines, thus, includes mostly killed or attenuated live microorganisms.

In 1953, Watson & Crick revealed to the world *"the secret of life"* and therefore new horizons to the biological research were opened. More recently, with the outstanding development of the knowledge on molecular technology, the possibility of developing better vaccines became a reality.

Today, vaccines are developed based mostly on two main drivers: the evolution of the poultry production and the progress of molecular biology. There are several vaccines commercially available that were developed using cutting-edge technologies such as immune complex, vector, virus-like particles, sub-unit, gene-deletion, reverse genetic and others. Molecular biology has dramatically enlarged the range of possibilities offered to creative researchers. Several tracks can be followed, and many concepts are waiting to be proven, developed, and turned into products.

Finally, there have been much more changes in vaccinology during the past 10 years than during the previous 30 years. The limits for the creativity of research today lies on outdated regulations that will have to be, sooner or later, modernized to take into consideration such innovations that will help producers to meet the colossal challenge of feeding more than 9.5 billion mouths in few decades.

Keywords: vaccinology, vaccine technology, disease protection

PLENARY SESSION 2 FISH

EVOLUTION OF MALAYSIAN SHRIMP CULTURE TOWARDS A MORE SUSTAINABLE FUTURE

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Abstract

The Malaysian shrimp industry is currently experiencing a transition in its practice from being traditional and high-risk to reliable and sustainable in its production methods. Traditional practices of the late 20th century used post larvae from wild-caught Penaeus monodon, which considerably reduced its gene pool due to high susceptibility to diseases. Disease control and treatment also saw the indiscriminate use of antibiotics which resulted in low inconsistent yields of *P. mondon* and a shift to the production of *Penaeus vannamei*, a specific pathogen-free stock (SPF) imported from Hawaii. However, after two decades, the *P. vannamei* culture began seeing a similar crisis of diseases as faced earlier by P. monodon which saw a shift in preference for *P. monodon* using SPF stocks.

After six decades of challenges, the industry resorted to strict adoption of biosecurity practices, which include fencing, roofing and scientific-based information. Practices include reducing water exchange and treating it well to provide a healthier environment for the shrimps; introduction of a nursery system to reduce risks in grow out; and antibiotics replaced with probiotics towards consistent harvest yields. Partial harvests are done three to five times in a cycle to maintain stable biomass, and MyGAP was introduced for good aquaculture practices.

The common diseases now seen in *P. monodon* are WSSV and acute hepatopancreatic necrosis disease (AHPND). In *P. vannamei* there are two other diseases, infectious myonecrosis virus (IMNV) and enterocytozoon hepatopenaei (EHP).

Disease control measures include manipulating water pH, reducing temperature fluctuations, using HDPE pond liners, introducing shrimp toilets, disinfecting all facilities during the 'break cycle' phase, using footbath and doubling paddle wheels to give >6 ppm dissolved oxygen. In addition, PCR is also used to monitor shrimp viruses throughout the production cycle.

Keywords: shrimp diseases; Malaysian shrimp industry; sustainable shrimp culture

PLENARY SESSION 2 ANIMAL HEALTH AND PRODUCTION

Research and Development in Livestock Vaccine: a Consideration

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Abstract

Diseases are an important factor that influence the success of a livestock farm. Therefore, disease prevention through herd-health program is important, and herd health program includes vaccination. In developing an animal vaccine, the costs of vaccine production and the acceptance by end-users are often neglected. Generally, advanced, state-of-the-art vaccines such as subunit, recombinant and DNA vaccines are expensive but more effective, and suitable for large/commercial farms. Traditional vaccines such as autogenous and whole-cell killed vaccines are cheap but less effective, and suitable for smallholder farms. Considering that between 40% and 60% of ruminant and aquaculture farms, particularly in the Southeast and South Asia are smallholdings, with farm size of <2ha and family-run with limited capital, developing cheap, less labor intensive and fairly effective vaccines that fulfil their requirements should be considered.

Keywords: Animal vaccine, research, development

ORAL PAPER 1

LACTOCOCCUS DISPLAYING SURFACE-MULTIPLE INFLUENZA PROTEIN ANTIGENS AS PROBIOTIC-ORAL VACCINE AGAINST AVIAN INFLUENZA

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Abstract

Worldwide, improvements to `strengthen' influenza vaccines, that can induce both strong humoral, cell-mediated immunity (CMI), as well as mucosal immune responses for protection against all subtypes and strains of influenza viruses, are still ongoing. Using *Lactococcus lactis* strain M4 as a delivery vehicle- constructs carrying the HA1, M2 and NS1 ORFs of genes of the highly pathogenic avian influenza virus H5N1 with glycine-serine linkers were ligated into *L. lactis* vector that results in the transformed *L. lactis* to express the multiple antigens on the surface of the Lactococcus bacteria. Constructs of Lactococcus displaying (i) recombinant (r) *L.lactis*-HAI alone (ii) rL.lactis-NS1+M2 were combined in oral feeding trials resulting in a composite *rL.lactis*-HA1+NS1+M2 vaccine. The immunizations were conducted on two-week-old chickens orally on three consecutive days with 1 x 10^9 cfu/mL of r*L. Lactis* in 100 µL of 5 % sucrose solution. The immunization was repeated two weeks later. The immune sera were collected after one week of final dose of each immunization**.** Haemagglutination humoral antibodies were successfully elicited by the vaccine. The r*L. Lactis*–M2 and NS1 vaccine resulted in highly elevated IFN-α in the serum of vaccinated chickens at 39 days post-vaccination. For the HA1+NS1+M2 vaccine, cytokines, IL-8, IL-12, and IL-4 were expressed at higher levels compared to the non-vaccinated controls, however, they were shown to be statistically not significant. The GMT HI responses (n=5) elicited by the r*L lactis*-HA1 were 12.0-18.4. The GMT HI responses (n=5) elicited by the r*L.lactis*-HA1+NS1+M2 was 27-37. If the oral route of vaccination can result in humoral immunity development, we, therefore, believe that strong protective mucosal immunity will be developed using this vaccine. Unfortunately, to date, we do not have the opportunity to conduct efficacy or challenge studies due to the unavailability of a BLS-3 facility. More constructs of the vaccines and vaccination regimes are needed to develop the best vaccine that can provide high and protective humoral, CMI, as well as mucosal immunity, and more importantly too, a vaccine that completely neutralizes field avian influenza viruses without shedding the infecting-field viruses.

Keywords: Avian Influenza; recombinant Lactococcus; surface-displayed antigens; vaccine

ORAL PAPER 2

COCCIDIOSIS VACCINATION IN BROILERS FOR AN EFFICIENT GUT HEALTH & BETTER ECONOMIC PERFORMANCE

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Abstract

Coccidiosis is one of the most widespread, costly and important diseases in commercial poultry. Also, this disease favours a worsened gut health and is able to predispose to enteric dysbiosis such as necrotic enteritis (NE). Anticoccidial drugs have been the most widely used prevention tool against coccidiosis in broilers and they still are in several countries. However, resistances of *Eimeria* field strains due to prolonged use of these drugs have been widely demonstrated, favouring the appearance of coccidiosis outbreaks or subclinical infections and, consequently, other enteric issues. Moreover, the ever-increasing need to reduce antibiotic use, such as ionophore antibiotics, and search of alternatives have drawn the attention of poultry producers towards the use of coccidiosis vaccines.

Coccidiosis vaccines attenuated by precociousness are indicated to decrease the intestinal lesions caused by specific *Eimeria* species and have been demonstrated to reduce the resistances of *Eimeria* field strains and restore the sensitivity to the anticoccidial drugs, being an alternative solution to continuous usage of coccidiostats through a rotational programme or a continuous vaccination programme. Additionally, the usage of these kind of vaccines has been demonstrated to be able to reduce the intestinal lesions by a NE disease triggered by a previous coccidiosis infection and improve the productive losses associated with the disease.

ORAL PAPER 3

NEXT-GENERATION SEQUENCING FOR AVIAN RESEARCH: OPPORTUNITIES AND CHALLENGES

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Abstract

The advances in high-throughput or massive parallel sequencing using next-generation sequencing (NGS) technologies have revolutionised and propelled the field of genomic research in recent years. Basic and clinical research have been tapping into the endless potentials and opportunities arising from the transition of traditional Sanger sequencing to NGS but the adoption of NGS in avian research is still lagging. In this talk, a brief overview of the current trends in avian research involving NGS will be given. Different applications of NGS in avian research will be covered such as whole genome sequencing of either pathogens or hosts, transcriptomic studies that detect changes in the gene expression levels and metagenomic studies that analyze genetic material from a community of microorganisms. Future perspectives of NGS in avian research will also be discussed, including the potential use of NGS as a powerful tool to establish definite diagnosis or to uncovering underlying genetic defects. The talk will also explore the challenges faced in implementing NGS into routine diagnostics and surveillance at different stages starting from sample preparation, library preparation, sequencing to downstream bioinformatics analyses. By addressing these hurdles, we could bridge the gap between NGS implementation and avian research as well as encourage multidisciplinary NGS-based research to improve the diagnosis, prevention and control of avian diseases.

Keywords: genomic, whole genome sequencing, diagnostics, metagenomics, transcriptomics, chicken.

ORAL PAPER 4

CHARACTERISTICS OF *Salmonella enterica* SEROVAR ENTERITIDIS ISOLATES FROM CHICKENS AND CHICKEN MEAT PRODUCTS IN MALAYSIA

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Abstract

Salmonella enterica subspecies enterica serovar Enteritidis is a serotype of global public health significance. It is one of the most common strain responsible for human infection worldwide. This study includes Salmonella enterica serovar Enteritidis isolated from fresh chicken meat, ready-to-eat chicken meat as well as from cloacal swabs of live chickens in some selected locations within the central region of peninsular Malaysia. We investigated the phenotypic antimicrobial resistance profile as well as the molecular characteristics of the Salmonella isolates including the virulence gene profiles and multilocus sequence types. All of the tested isolates were found to possess resistance to tetracycline, gentamycin, streptomycin, and sulfadimidine and were predominantly subtyped into ST11 and ST1925 using the MLST method. The isolates were also found to harbour several virulence genes, with multidrug-resistance characteristics. The study provides an assessment of the AMR, virulence determinants and the MLST subtypes of S. Enteritidis isolates circulating in Malaysia.

Keywords: Salmonella, Enteritidis, Malaysia

ORAL PAPER 5

VACCINATION AS PREVENTIVE STRATEGY IN COMMON FISH DISEASES IN MALAYSIA

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Abstract

World aquaculture production has been growing steadily at 5.2% per year since 2000-2019 with major production is finfish. Asia is the main producer represent almost 70% of the total world production. In Malaysia, aquaculture activities contributed around 24% of the total fisheries production and almost 1.8 million MT was for human consumption. The high aquaculture activities may lead to the introduction of diseases in the system, thus a good management practices and biosecurity in aquaculture health will be the main control and prevention strategies. Started with the early detection, control and prevention will be made easier to contain the fish disease outbreak. Vaccination in fish nowadays has gained an important interest to the Asian countries in comparison to the salmonid culture countries, however delivery system might be an issue for aquaculture which depending on several factors. These 3 components or scopes has been applied in the R&D activities by NaFisH since 9th Malaysia Plan and has obtained several success stories, that will be presented in this seminar.

Keywords: Malaysia, fish diseases, preventive strategy, vaccination

ORAL PAPER 6

FISH VACCINES DEVELOPMENT AGAINST VIBRIOSIS IN MALAYSIA: STATUS AND CHALLENGES

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Abstract

Aquaculture currently accounts for 46% of total fish production, with Asia accounting for more than 91% of worldwide aquaculture production. However, intensive farming systems usually increase the stress level in fish due to high stocking density and high feeding levels, resulting in decreased water quality. Disease such as vibriosis is common and significant in tropical countries as it was commonly found in farmed marine fish and shellfish in the region. Treatment of antibiotic-resistant infections with existing antibiotics has become more challenging with the emergence of multidrug resistance in aquatic microorganisms. Several vaccines against warm water vibriosis have been experimentally tested in marine fish with promising results in Indonesia, Thailand, Vietnam, and Malaysia. However, these regions' commercial and licensed fish vaccines against warm-warm vibriosis are still limited. In Malaysian aquaculture, the use of vaccines is still in an early developing phase, with most efforts focused on creating vaccines against bacterial infections, such as vibriosis. The present study briefly describes the prevalence of vibriosis in Malaysia and how present vaccines are developed and applied. Limitations and gaps in research and development on fish vaccines are also discussed.

Keywords: vaccination, vibriosis, marine fishes, limitations

ORAL PAPER 7

HISTOPATHOLOGY FOR DIAGNOSIS OF SHRIMP DISEASES

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Abstract

In Malaysia, shrimp production is very important in terms of food supply and contribution to economic growth. The industry is now dominated by the culture of *Litopenaeus vannamei*. However, shrimp production by aquaculture has been seriously impacted by diseases. Disease outbreaks cause serious economic losses in several countries and affect survival of the industry. The most important diseases of cultured penaeid shrimp are viral or bacterial aetiologies, but less important diseases are fungal and protozoan. Actually, the shrimp disease treatment is not easy, often, it is more complex than disease prevention. Diagnostic is an important method in shrimp health management and disease control. Some diagnostic methods are used for surveillance and confirmatory diagnosis. Recently, the diagnostic methods for shrimp disease investigation either used traditional methods or application of modern biotechnology. The traditional methods are pathology, traditional microbiology and the application of serological methods. However, modern biotechnology needs the rapid and sensitive diagnostic methods for shrimp disease detection such as PCR (Polymerase chain reaction) / RT-PCR (reverse transcriptase PCR) and real time PCR. Histopathological examination of the tissue biopsy for the identification of infectious disease is an important diagnostic tool. Histopathology is a study of disease at the tissue and cellular levels. It is important for diagnosis and also to investigate the severity of the infections. The common stain used for histopathology slides is hematoxylin and eosin (H&E) and various special stains also established to identify the specific affected tissue.

Keywords: Shrimp; infectious disease; histopathology; diagnostic method

ORAL PAPER 8

QUORUM QUENCHERS AS DISEASE CONTROL IN AQUACULTURE

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Abstract

Quorum sensing (QS) is a bacterial cell-to-cell communication whereby bacteria regulate gene expression through the presences or absence of small signal molecules. Bacteria use this mechanism to monitor its population density, the surrounding environment and regulate various activities and behaviors. This includes pathogenesis towards aquaculture hosts such as shrimp and fish. Interference of the signal molecules through quorum quenching (QQ) activities was shown to improve the survival and growth of the cultured organism. This includes inhibitions of the signal from various aquatic organisms such as algae and probiotic bacteria either through enzymatic degradation or chemical inactivation to antagonistic as well as agonistic activities. This paper focuses on summarizing the recent findings on quorum quenching activities particularly involving algae and bacteria interactions.

Keywords: quorum, sensing, quenching, diseases, vibriosis

ORAL PAPER 9

BENEFICIAL ROLE OF *Bacillus amyloliquefaciens* AS POTENTIAL PROBIOTIC IN AQUACULTURE

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Abstract

Beneficial microorganisms such as probiotics, are one of the promising solutions that could help reduce antibiotic use in aquaculture. This study evaluated the properties of *Bacillus amyloliquefaciens* isolated from the blue swimming crab (*Portunus pelagicus*) as a potential probiotic. One of the ongoing research projects that uses this strain is the development of multi-strain probiotics (MSP) incorporated feed for red hybrid tilapia as a disease control and growth-promoting tool. *Bacillus amyloliquefaciens* is combined with two other probiotic strains from the genera of *Lysinibacillus* and *Enterococcus* to create MSP1, a multi-strain probiotic. MSP 1 inhibited the growth of *Aeromonas hydrophila* (13.0± 0.6 mm) and *Streptococcus agalactiae* (15.3± 0.8mm) observed in *in vitro* well diffusion assay. MSP 1 at a concentration of 10^8 CFU mL^{-1} completely inhibited the growth of *A. hydrophila* in co-culture assay. Furthermore, *B. amyloliquefaciens* also demonstrated antimicrobial activity against *Vibrio parahaemolyticus* in spot and well diffusion assays with significant inhibition zones of 5.3 ± 0.01mm and 4.4 ± 0.06 mm respectively. In a co-culture assay, this strain at a concentration of 10^6 CFU mL^{-1} also showed a significant reduction of *V. parahaemolyticus*. In *in vivo* study, this strain significantly improved the survival rate (88%) of white shrimp, *Litopeneaus vannamei*. Supplementation of microfeed with L9 (10^8 CFU mL^{-1}) enhances the survival of shrimp challenged with *V. parahaemolyticus*. The ability of *B. amyloliquefaciens* to form biofilm was tested and the highest biofilm formation was observed at 24 hr, with optical densities of 3.67nm. Thus, *B. amyloliquefaciens* has a high potential for use in aquaculture industries. This strain has been shown to have excellent properties in terms of pathogen antagonism, biofilm formation, and host survival rate.

Keywords: Probiotic, *Bacillus amyloliquefaciens*, aquaculture, antagonism, survival, biofilm

ORAL PAPER 10

ALTERNATIVE LEAVES FOR PROTEIN SOURCE FOR POULTRY FEED: THE SUGGESTIVE LEVEL AND THE ISSUES OF USING IT.

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Abstract

It is always worth considering an alternative protein source, especially when prices for conventional ingredients increased. The rise in the price of animal feed components in the international market is a major challenge to poultry industries, and small farmers are the most effected once. Recently, the use of *Trichanthera gigantea* (*Tg*) leaves or known locally as *ketum ayam* and *Azolla pinnata* (*Ap*) as alternative protein sources in poultry diet is becoming more popular in Malaysia, especially in small scale village chicken farmers. However, in this country, there is lack of scientific evidence on the effectiveness on both leaves to replace the protein source. With its relatively high protein content, approximately 23% in *Tg*, and 24.82% in *Ap*, the leaves may have a potential to be used as a partial replacement for alternative protein source in broiler feed. Experiment was conducted to determine the effect of varying levels: 0, 5, 10 and 15% of dried *Tg* and *Ap* leaves meal inclusion in broiler diet on growth performance and carcass yield at grower-finisher stage using a completely randomized design. A decreasing trend in growth performance as *Tg* level increased was seen which it reduced final body weight and cumulative weight gain and increased cumulative feed conversion ratio (FCR) at 15% inclusion. It is increased the gizzard weight at 10% and 15% levels, reduced dressing percentage at 15% level, shrink the liver at 5% level, and produced the highest abdominal fat content at 10% level. The inclusion of *Ap* up to 15% in broiler chicken feed ration showed no adverse effect on the growth performance, nutrient digestibility, meat production and meat quality of the birds. In conclusion, both leaves have a potential to be a protein source in broiler diet as a up to 10%. However, the adverse effect observed on the higher inclusion.

Keywords: *Trichanthera gigantea*, *Azzola pinnata,* broiler chicken, growth performance

ORAL PAPER 11

APTAMER-BASED ANTIBACTERIAL AND ANTIVIRAL STRATEGIES IN VETERINARY SCIENCE

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Abstract

Aptamers are single-stranded RNA or DNA oligonucleotides that forms unique three-dimensional (3D) structures that can bind a wide range of molecules that include proteins, toxins, and even metal ions. Due to their high specificity, affinity and ease of production, these nucleic acid ligands are now replacing conventional antibodies in diagnostics and therapeutics. Aptamers are also advantageous as it can be designed to specifically target any molecule, does not have a batch-to-batch variation, is stable at room temperature, and are fast and inexpensive to produce. Aptamers' unique affinity towards bacteria and viruses also makes it an ideal antibacterial and antiviral agent. Aptamers have demonstrated remarkable *in vitro* inhibitory effects against pathogen-specific virulence factor(s). We developed a new approach to establish high binding aptamers via a process called *In silico* Conformational Aptamer Development by Design (ICADD) which is efficient, low cost and rapid. Good binding aptamers can now be designed from natural tRNA sequences with specific folding properties that can specifically bind a pathogen similar to an antibody. The interactions between an aptamer and its target molecule can be further analysed through three-dimensional (3D) structural modelling to predict and optimise binding affinities on the target molecule via computational molecular docking. We share our research experiences on aptamer development for diagnostic and antimicrobial applications against veterinary pathogens. We will review and discuss aptamer-based antibacterial and antiviral strategies in veterinary medicine.

Keywords: Aptamer; antibacterial; antiviral; pathogen-specific virulence factor; ICADD

ORAL PAPER 12

IDENTIFICATION AND ANTIBIOTIC RESISTANCE PATTERN OF *Vibrio* SPECIES ISOLATED FROM DISEASED ASIAN SEABASS, *Lates calcarifer* IN EAST COAST, MALAYSIA

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Abstract

Vibrio bacteria particularly members of the Harveyi clade, are the causative agents of vibriosis. Due to mass mortality events, this disease impact on economic losses in aquaculture farms. This study was conducted to check the presence of *Vibrio* species diversity in farmed Asian seabass in East Coast, Malaysia. The study found several species of *Vibrio* that are potentially pathogenic to Asian seabass as well as to human. Varying degrees of antimicrobial resistance were also observed among the isolated vibrios. Further investigations are needed to identify the risk factors that could trigger disease outbreaks in Asian seabass farms with Vibrio strains. Fish health surveillance programme should be implemented effectively to prevent disease outbreaks and will help to improve Asian seabass production in Malaysia.

Keywords: Molecular characterization, Antimicrobial resistance, seabass, vibriosis

ORAL PAPER 13

ON-SITE SOLUTION FOR SHRIMP HEALTH DETECTION

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Abstract

The Shrimp Health On-Site Spotter (SHOS-Spotter) was created in response to shrimp farmers concern~~s~~ due to acute and high mortalities of shrimp culture. Drastic environmental changes, toxins or infection caused disturbance in the normal physiological function and digestion process of cultured shrimp. Unhealthy ~~s~~hrimp often shed hepatopancreatic (HP) cells into the tubules, and subsequently ended in shrimp's gut where health condition can be determined. SHOS-spotter model was developed based on diseases caused by Early Mortality Syndrome (EMS)/Acute Hepatopancreas Necrosis Disease (AHPND) and the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) in farmed shrimp. In 2011, EMS and AHPND caused an estimated loss of USD1.3 billion to farmers, while in 2015, another emerging disease caused by EHP was detected, whereby the affected shrimp tend to be smaller with high variation in size due to slow growth syndrome. Hence, farmers experienced an increased in the operation costs. Both diseases caused significant impact to shrimp farming and the current diagnostic method for disease detection required skilled labour, time-consuming and costly. Therefore, a rapid and practical method is needed at the farm level. SHOS-Spotter is an on-site method that can detect shrimp health within 1-3 hours. This innovative early detection method was based on the determination of shrimp's health status via HP's cell observation in the shrimp's gut. The severity of damage or shedding of HP's cells was determined by scoring values of 0, 1, and 2. It requires only 3 steps to operate the SHOS-Spotter. The first step is to remove the entire gut from the shrimp, followed by HP cell observation in the gut under a portable microscope and finally the interpretation of the gut scorecard, with score 0 referring to healthy shrimp, score 0 and 1 for unhealthy shrimp with some symptoms, while scores 0, 1 and 2 refer to unhealthy shrimp having acute or late mortality or variation in size. Laboratory and field trials of SHOS-spotter showed shrimps' gut score readings correlate with AHPND positive cases and they were further confirmed using PCR (Polymerase Chain Reaction) and histology results with an accuracy of 95 to 98%. Thus, the SHOS-Spotter invention can be used as an indicator of shrimp's health.

Keywords: AHPND, early detection, hepatopancreas, rapid, shrimp health

ORAL PAPER 14

INCORPORATING VETERINARY PATHOLOGY IN VACCINE DEVELOPMENT AND ITS CHALLENGES

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Abstract

In veterinary medicine, veterinary pathology is one of the core fields of study. It largely contributes in disease diagnosis, as well as understanding how diseases progress, termed as pathogenesis. In general, the application of veterinary pathology basically revolves around the classic epidemiologic triad involving the host, agent, and environmental factors. One of the initial step to design a vaccine involves understanding the pathogenesis of a particular disease. This includes information on the natural routes of infection, dosage of infection, and roles of environment as potential stressors. Prior to vaccine testing, researchers must be able to re-create the disease in the laboratory. Thus, pathologists play an important role in developing assessment of potential animal models and re-creation of a disease. This stage can be challenging especially for diseases with various manifestations such as acute, chronic, and carrier. During the vaccine testing stage, pathologist play role in diagnosing cause of death, assessment of tissues for lesions and structures related to immunity. While the application of pathology and the role of pathologist are important in the process of vaccine development, challenges are inevitable. Pathology is a highly-specialized field, with very limited number of pathologists worldwide, and pathologists may not be interested in research, as well as are not familiar with all animal species.

Keywords: vaccinology, vaccine, veterinary pathology, veterinary medicine

ORAL PAPER 15

*cf*UPMT27 A CRISPR BASED FOWL ADENOVIRUS VACCINE CANDIDATE

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Abstract

Fowl adenovirus (FAdV) is an important causative agent of devastating disease known as inclusion body hepatitis (IBH) with significant economic impact due to high mortality and poor chickens' production. FAdV is claimed to be an endemic among poultry species globally as infection cases were reported in studies from various geographical regions. In Malaysia, the first IBH outbreak was reported in the year 2005 due to FAdV serotype 8b (FAdV-8b) infection. Since then, the number of IBH nation cases has increased, widely involving major poultry producing areas. The high infection rate in the poultry farms had pressure experts to develop commercial vaccines as a control and prevention method against FAdVs, worldwide. This study aimed to develop live attenuated CRISPR-based vaccine of FAdV by modifying its' fiber gene using Cas9-guide RNA Ribonucleoproteins. A guide RNA (gRNA) was designed specifically to the targeted gene, synthesized and delivered to the viruses residing inside the chicken embryo liver (CEL) cells. The modified strain was subjected for sequence validation, rescued and subjected to serial passage to determine its pathogenicity and genetic stability in specific-pathogen-free (SPF) chicken embryonated eggs. Further pathogenicity and immunogenicity study were carried out in SPF chickens. Interestingly, the results show the fiber gene was successfully mutated with amino acid substitution at position 179 (Tyrosine-aspartate) identified. The live modified virus was rescued in SPF chicken embryonated eggs with predominant delayed mortality. Localization study shows the modified strain localized the CEL cells at 48 hours and cause low CPE after 72 hpi compared to the wild-type strain as early as 24 hours with high CPE within 48 hours hpi. This modified strain namely *cf*UPMT27 replicates efficiently in the cell and induced high antibody titer after single dose (10^6) vaccination either via subcutaneous or oral route inoculation. No mortality was observed, and chicken bodyweight increased consistently. Hence, suggesting the virus may physically mutated and expressed the non-proper functioning of the fiber protein; a potential vaccine candidate for IBH disease.

Keywords: Fowl adenovirus (FAdV); Inclusion body hepatitis (IBH); Cas9-guide RNA Ribonucleoproteins; CRISPR; FAdV vaccine

ORAL PAPER 16

POULTRY ELISA RESULTS INTERPRETATIONS

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Abstract

Enzyme-Linked Immunosorbent Assay (ELISA) is an immunological plate-based assay designed to detect and quantity soluble substances like antibodies and antigens in biological matrices like blood, serum, plasma, tissues etc. Indirect ELISA is commonly used for disease monitoring, vaccination monitoring as well as vaccination date prediction. Optical densities of both positive and negative controls must fall in between the ranges suggested by the kit manufacturer for an iELISA test to be deemed valid and reference serum must achieve expected titer. After that, by comparing the mean titer and coefficient of variations (CV) of an iELISA test to a given baseline, we can differentiate between a case of good vaccination, a case of suspected challenge and that of a vaccination failure.

Keyword: ELISA, Vaccination Monitoring, Disease Monitoring, Baseline

ORAL PAPER 17

NEWCASTLE DISEASE: TAKING CHARGE OF NDV TRANSMISSION

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Abstract

Newcastle Disease Virus (NDV) has been classified as Avian Orthoavulavirus 1, with only one serotype. It is a poultry disease which is highly contagious and causes severe mortality in poultry. Newcastle Disease (ND) is endemic in Malaysia, with waves of outbreaks happening periodically, thus being a risk to national food security. Disease control has become even more challenging with larger and more intensive poultry productions, as well as co-infection with other pathogens. Through advancements in diagnosis, and field experiences, several key strategies are suggested to control and stop the transmission of Newcastle Disease. This presentation outlines the practical approaches of biosecurity, farm cleaning and disinfection, monitoring, data traceability, agile farm management, as well as application of vaccines. Moreover, awareness of producers towards ND vaccines is continuously growing and thus the technology of ND vaccines is evolving to meet modern expectations. Overall, a measurable disease control strategy as well as the use of new technology vaccine program that reduces the re-excretion of the NDV are key towards effective control of Newcastle Disease

Keywords: Newcastle Disease, disease control strategies, poultry, transmission, vaccine.

ORAL PAPER 18

DETERMINATION OF OPTIMUM INACTIVATION PERIOD FOR FOWL ADENOVIRUS SEROTYPE 8B ISOLATE OF MALAYSIA FOR VACCINE DEVELOPMENT

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Abstract

Fowl adenoviruses (FAdVs) is a non-enveloped DNA virus, ubiquitous and highly resistant in environment. High pathogenic FAdV has worldwide endemic and caused serious economic losses in cases of inclusion body hepatitis (IBH), gizzard erosion and hepatitis-hydropericardium syndrome (HHS). In Malaysia, serotype 8b was identified as a major strain circulated in commercial poultry farms and necessitate effective control measure to overcome the disease outbreaks. Knowledge on FAdV inactivation at optimum period remain scanty and crucial for development of safe inactivated vaccine in chicken. It was objective of the study to determine the optimum period of FAdV serotype 8b inactivation for vaccine development. FAdV isolate, UPM1137, at fifth passage in chicken embryo liver (CEL) cells was treated with 0.002M binary ethyleneimine (BEI) at 5 different period intervals at 20 hours(h), 24h, 28h, 32h and 36h. Safety tests were conducted in specific pathogen free (SPF) chicken embryonated eggs (CEE), primary CEL cells and commercial broiler chickens. It was demonstrated that CPE was absent throughout the trial in CEL cells after inoculated with five treated inoculums. However, in SPF CEE, 100% embryonic mortality were recorded following inoculation with FAdV inoculums treated at 20h, 24h and 28h. No mortality was recorded in embryos after inoculated with FAdV inoculums treated at 32h and 36h. Neither mortality, nor gross or histological lesions were observed in the broiler chickens using inoculums treated at period 32h and 36h via subcutaneous route. Subsequently, FAdV inoculum treated at 32h induces high antibody titre in chickens at day 14pi compared to that FAdV inoculum treated at 36h. These results suggest that the optimum period for FAdV inactivation at titre $10^{11.5}TCID_{50}$/ml was 32h by using 0.002M BEI concentration. Thus, the inactivated FAdV inoculums at 32h is safe and suitable to be used for future vaccine formulation.

Keywords: Fowl adenoviruses (FAdVs), inclusion body hepatitis (IBH), inactivation, safety, vaccine

ORAL PAPER 19

PERFORMANCE OF KYLT® PMV-I PATHOTYPING KIT, A MULTIPLEX REAL-TIME PCR METHOD IN DETECTION AND DIFFERENTIATION OF NDV PATHOTYPES

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Abstract

Newcastle disease (ND) remains as a major threat in poultry industry in Malaysia despite the intensive vaccination program have been implemented. Sporadic ND outbreaks have been reported throughout the years in commercial poultry farms in Malaysia. ND has associated with high mortality in chickens and drop in egg production which led to the huge economic losses. A diagnostic method which allows rapid and accurate detection and differentiation of NDV pathotypes is important in controlling the spread of ND. In this study, the performance of a multiplex real-time PCR method (Kylt® PMV-I Pathotyping Kit, AniCon Labor GmbH) was evaluated in terms of its accuracy, specificity and sensitivity in detection and differentiation of NDV pathotypes. A total of 20 NDV isolates included 17 virulent NDV and 3 avirulent NDV were used in the evaluation. The F cleavage site amino acid sequence of all NDV isolates were sequenced and determined. In addition, the performance of the multiplex real-time PCR method also evaluated with the various types of sample format such as swabs, organs and FTA cards. The result showed that the multiplex real-time PCR precisely detected and differentiated the NDV pathotypes. The real-time PCR result was in good agreement with the F cleavage site amino acid sequence analysis. In conclusion, the multiplex real-time PCR method evaluated in this study is proven as an accurate and useful tool in detection of NDV.

Keywords: Diagnostics; ND outbreaks; NDV pathotypes; multiplex real-time PCR

ORAL PAPER 20

PSTACI - OPPORTUNITIES FOR INVESTMENTS AND INTERNATIONAL COLLABORATIONS TO TRANSFORM AQUACULTURE INTO A DYNAMIC GROWTH ECONOMY OF COTE D'IVOIRE

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Abstract

The PSTACI (Programme Strategique de Transformation de l'Aquaculture en Cote d'Ivoire) is formulated with the vision to develop the aquaculture sector domestically to increase the current production from 4,500MT to 120,000MT within 10 years. PSTACI aims to develop. PSTACI aims to develop the aquaculture industry through a set of transformation strategies that will address the key challenges with a strategic approach. With a combination of the creation of the new opportunities from technical assistance, key government incentive and providing the infrastructure for long term development of the industry, and strategic supply chain PSTCI is targeting and accelerated industry growth that will propel the industry to achieve a target of135,000 tons of local production in 10 years, covering all spectrum of the industry requirement. The above efforts would be able to provide quality inputs that the aquaculture industry need, technology know-how, financial options, marketing and distribution. The hope is for the local future farmer can benefit from the program and further enhance the main objective for the government of Cote d'Ivoire to embark in such an initiative.

ORAL PAPER 21

FISH VACCINE DEVELOPMENT IN INDONESIA

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Abstract

Aquaculture is the fastest-growing sector among agribusiness, contributing to a significant increase in global fish production. Intensive aquaculture faces serious problems of diseases outbreak, climate change, and environmental deterioration. Vaccination is thought to be a costly and technically effective method of controlling fish diseases, but there are numerous constraints in developing the vaccine. This paper describes the development of vaccines and commercially available fish vaccines in Indonesia. This paper discusses vaccine development and commercially available fish vaccines in Indonesia. Commercial fish vaccines developed by Indonesian researchers are scarce, despite the fact that fish vaccine research has been ongoing since the 1980s. Almost available commercial fish vaccines developed by Indonesian researchers are bacterial fish vaccines such as Aeromonas, Streptococcus, Vibrio, and Edwarsiella vaccines. Very limited imported fish vaccines are available in Indonesia. All commercial fish vaccines are registered by the Ministry of Marine Affairs and Fisheries (MMAF) Republic and freely accessed through the Information System of Fish Medicine portal (http://www.sibatik.kkp.go.id/web/daftar-izin/). Registered local fish vaccines in Indonesia are Caprivac-Vibrio L, Caprivac-Aero L, and Caprivac-Icta® for diseases caused by Vibrio, Aeromonas, and *Edwarsiella ictalurii*, respectively. Recently, MMAF released Caprivac-Hidrogalaksi, a vaccine for Aeromoniasis and streptoccociosis. Other registered vaccines are Aquavac Strep Si, Aquavac Strep Sa, Aquavac Strep Sa-Si, Alpha Ject Micro 1 TiLa, and Aquavac® Irido V. In 2012 MMAF launched Fish Vaccination Movement to improve the understanding and applying fish vaccine at the farmer level. However, the use of the vaccine in Indonesian aquaculture is hampered by factors such as farmers' limited understanding of vaccines, small-scale aquaculture business, inconsistency policy, vaccine distribution, and a lack of available automatic vaccination machines.

Keywords: aquaculture, pathogen, bacteria, virus, vibriosis, aeromoniasis, streptocociosis

ORAL PAPER 22

CHITOSAN BASED NANOPARTICLES IN FISH HEALTH MANAGEMENT

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Abstract

Prevention and control of infectious diseases are the most difficult constraints in aquaculture worldwide. Advanced technologies must be applied to develop efficient and safe therapeutic agents for fish disease control, and among them nanotechnology is considered as a “new era” for sustainable development of aquaculture industry. Chitosan is a cationic polysaccharide derived from chitin, which can be mainly found in the exoskeleton of crustaceans. Structurally, chitosan composed of co-polymers of D-glucosamine (β-1–4-linked 2-amino-2-deoxy-d-glucose) and N-acetyl-D-glucosamine (2-acetamido-2-deoxy-d-glucose) which has a linear backbone linked through glycosidic bonds.

Chitosan has been intensively studied in various industries such as food, pharmaceutical, cosmetic, and bioengineering. Properties of chitosan can be upturned by changing the chemical structure as well as incorporating external nanoparticles with its polymer matrix. Our results related to antimicrobial, immunomodulating, wound healing, vaccine adjuvant, and drug delivery properties of chitosan nanoparticles and its composite forms such as chitosan silver nanocomposites suggesting that chitosan based nanomaterials could be immerging new therapeutic agents for fish disease management.

Keywords: antimicrobial; aquaculture; chitosan; nanoparticles; wound healing, drug delivery; nanotechnology; pharmaceutical

ORAL PAPER 23

NEEDLE-FREE FISH NANO VACCINES FOR PREVENTION OF BACTERIAL FISH DISEASES

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Abstract

Bacterial diseases are the major diseases that threaten cultured fish worldwide. The diseases have a devastating effect on the wide range of economical cultured fish species e.g. *Lates calcarifer* (Asian sea bass), Tilapia (*Oreochromis* spp.), etc. Fish can be protected from those diseases by effective vaccines. Most of the licensed effective fish bacterial vaccines are vaccines for injection. Even though injection vaccination can provide many advantages for the prevention of diseases in cultured fish, however, this method also has many disadvantages such as it is not appropriate for vaccination in the early stage and the small size of cultured fish, fish needs to anesthetize before vaccination, fish may get infected by environmental pathogens at the injection site, fish may get very stressful from the vaccination method, time-consuming, etc. Therefore, needle-free vaccination is a novel trend in fish vaccination that can bring fruitful benefits for the prevention of bacterial diseases in the mass production of cultured fish. Our current research was mainly based on the development of novel mucoadhesive nano-encapsulated vaccines for immersion against fish bacterial infections such as *Flavobacterium oreochromis*, *Franciscella orientalis*, and *Aeromonas veronii*. The cationic polymer-based nanoparticles were combined with an antigen obtained from bacterial cells. Various parameters including transmission electron microscopy (TEM), physiochemical properties; zeta potential, and polydispersity index (PDI) were determined for the formulated nano vaccines. *In vivo* study, the immunologic and protective effects of the needle-free vaccination (immersion vaccination with nano delivery system vaccines) by the prepared nano vaccines were determined by challenging the experimental fish species on a laboratory scale. The results revealed promising immunologic and protective effects of the formulated nano vaccine for the experimental fish species. Moreover, the formulated nano vaccine candidates have more potential when compared to killed-whole cell immersion vaccination. We also see promising efficacy of our nano vaccine after immersion vaccination to mass fish production at the industrial farming scale. It can be summarized that needle-free vaccination with nano vaccines has significant immunologic and protective efficacies against major fish bacterial diseases. Additionally, it can be used in the mass production of the cultured fish farming industry to boost fish immunity against harmful fish bacterial diseases.

Keywords: needle-free, nano vaccines, prevention, bacterial fish diseases

ORAL PAPER 24

DIAGNOSIS IN A FISH FARMER'S BACKPACK

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Abstract

Fish underpin future nutritional security, supplying high quality protein, iron, iodine and vitamin A that are critical to childhood development and deficient in many staple foods. In 2018, 54.1 million tonnes of fish were produced by farming, generating US$138.5 billion and directly employing 19.3 million people, mostly in developing nations. With expansion and intensification, disease losses are increasing and are a priority for the FAO sub-committee on aquaculture. In most developing countries, disease mitigation comprises over-stocking to compensate, and use of readily available antibiotics. Indeed 67 different antimicrobials are used in the 11 major producing countries, contributing to the global pool of antimicrobial resistance (AMR). Accurate identification of the causes and sources of infectious disease is essential for implementation of evidence-based treatment, biosecurity and prevention. Pathogen genomics can provide sufficiently detailed information but has, to date, been too expensive and time consuming. Lab-in-a-backpack uses nanopore sequencing technology and low-cost, low-waste sample preparation to generate whole pathogen genome sequence data from diagnostic samples on the farm without laboratory support. Our simplified safe workflow includes a cloud-based identification tool that returns near real-time information about the pathogen using any laptop or smartphone. This enables evidence-based treatment, epidemiological tracing, AMR surveillance and the production of simple low-cost locally produced 'autogenous' vaccines to protect the next crop. These big-data-informed but locally implemented solutions align well with FAO's recently proposed Progressive Management Pathway for Improving Aquaculture Biosecurity, and can deliver real advances in local economy, nutritional security, antimicrobial stewardship and animal welfare.

Keywords: Oxford Nanopore Technologies, bacterial genomic, cloud-based identification tools, serotyping, multi-locus sequence typing

ORAL PAPER 25

EXPRESSION OF PROTEASE-ACTIVATED RECEPTOR 2 IN MAMMARY TISSUE DURING EXPERIMENTALLY INDUCED CLINICAL MASTITIS IN DOES

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Abstract

Protease-activated receptor 2 (PAR2), a cell surface G protein-coupled receptor, plays an important role in the induction and amplification of the inflammatory response through proteolytic cleavage by trypsin-like serine proteases. In ruminants, increased protease activity has been observed in milk from mastitis animals. This study aimed to determine the presence of PAR2 protein and quantify PAR2 mRNA expression in the goats' healthy and inflamed mammary gland tissue due to infection caused by *S. aureus*. Thirty mammary gland tissues (15 mastitis and 15 control) were used. The presence of PAR2 in the mammary gland tissue was detected by immunohistochemical (IHC) staining, while real-time PCR was used for mRNA expression analyses. In addition, a routine histopathological examination was performed to determine the degree of inflammation and changes in the microstructure of mammary glands. In the mastitis group, PAR2 staining is found in the interstitial tissue of the mammary gland, with staining being observed in both mononuclear and polymorphonuclear cells. PAR2 mRNA expression was detected in all mammary gland samples. PAR2 mRNA expression levels in the mastitis group ($P<0.05$) were ten times higher than in the control group. Furthermore, the gene expressed significantly high ($P<0.05$) in mammary gland tissue with severe clinical signs. Histopathological examination revealed atrophy of the alveoli, loss of mammary gland epithelium and inflammatory cell infiltration in the interstitial connective tissue. These results reflect on the pathogenicity and the virulence factors produced by bacterial strains, which are modulated by a complex transcriptional regulator network. Expression of PAR2 in mammary gland tissue is a novel and significant finding which supports the hypothesis that serine proteases are involved in the development of mastitis.

Keywords: goat; mammary gland; mastitis; PAR2; *S. aureus*

ORAL PAPER 26

DECISION SUPPORT ON RUMINANT AND AQUACULTURE FARM USING ANIMAL HEALTH AND PRODUCTION ECONOMIC MODELS

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Abstract

The animal agriculture needs to produce animal products fit for human consumption sustainably and the production system must be resilient to climate change. Animal production and health economics model could be used to get insights and support decisions of climate-smart agriculture. We present certain challenges in building the bioeconomic models in Malaysia and the findings of the models. First, an enterprise budget is a useful tool to compare the profit margin of different farm systems as it does not require a lot of inputs. Using inputs from survey at different dairy buffalo farms, the model found the costs of milk is the lowest in commercial farm of more than 50 cows as compared to smaller size farms. Second, partial budget model is a simple and useful tool when studying change in production management. Our previous study using partial budget model showed cross-bred Murrah buffalo gave a higher economic benefit as compared to Sawah when nutrition was improved before breeding the cows. Third, additional information when studying impacts of animal health intervention for instance disease prevalence, case fatality and growth performance could enable a realistic model to support decision of change. We used such inputs in stochastic bioeconomic model to estimate costs of grow-out Asian Seabass, costs of mortality and benefit of vaccination against *Vibrio* sp. Research using stochastic bioeconomic model has also been done for Dutch farms to support dairy young stock rearing showed the total costs of rearing is 14% of the milk cost price. While in Malaysia, the total costs of rearing dairy heifer were similar across different farm size. What differed were the growth performance of different farm system due to different management showed by first calving age of 30 months for small-scale as compared to 24 months in commercial dairy farms. Despite similar costs of rear per animal, inefficiencies at herd level occurred as small-scale farms rear all youngstock and have less replacement available. The Dutch dairy farm model included costs due to calf diseases, whereas the model for Malaysia dairy farm unable to include such input due to lack of information. Finally, as the total costs of rearing a young stock were expensive, decision to rear optimal number of young stocks (70%) on Dutch farm was supported using the stochastic bioeconomic model. We believe farms record keeping in Malaysia should be improved. Most small-scale farms lack record keeping that can be used to evaluate to quickly support animal health intervention and reduce poverty. Training is needed for stakeholders to increase adoption of digital technology and investment for digitalization is needed to ensure sustainability along the value chain.

Keywords: animal health, production, bioeconomic model, sustainable farming.

ORAL PAPER 27

SUBCLINICAL MASTITIS IN BUFFALOES IN PENINSULAR MALAYSIA: ITS PATHOGENS AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES

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Abstract

The diversity of bacterial composition in buffalo milk in the presence of intramammary infection is not vastly explored in Malaysia as compared with other dairy industry animals such as cattle, goats, and sheep. Moreover, identification of bacterial pathogens associated with bubaline mastitis is essential to understand the aetiology of this infection which in turn helps create new strategies to control it. This study aims to and to identify the pathogens causing the subclinical mastitis and their antimicrobial susceptibility profiles in buffaloes in several states in Peninsular Malaysia. To achieve this, 10 dairy buffaloes' farms were selected from several states in Peninsular Malaysia with a total of 184 lactating buffaloes identified. Subclinical mastitis (SCM) infection was diagnosed with California Mastitis Test and milk was collected aseptically and subjected to bacterial culture. Gram-staining was done on the isolated bacterial colonies and were then subjected to Analytical Profile Index (API) tests according to their cell morphologies to determine the causative agents of SCM buffalo milk in this investigation. Antibiotic Susceptibility Test (AST) using Kirby-Bauer disk diffusion method was done on the bacterial isolates discovered using 10 types of antibiotics commonly used to treat mastitis. The most prevalent causative agent was *Coagulase Negative Staphylococcus (CNS)* (37.3%) while AST results revealed that, Gram-positive bacteria (GPB) were most susceptible to amoxycillin/clavulanate (93.9%), tetracycline (92%), gentamicin (92%) whilst Gram-negative bacteria (GNB) to enrofloxacin (100%), trimethoprim/sulfamethoxazole (93.5%), and tetracycline and gentamicin (90.3%) in order. In contrast, GPB and GNB were most resistant against cloxacillin (52% and 84% respectively). Conclusively, high prevalence of subclinical mastitis caused by *Coagulase Negative Staphylococcus* in buffaloes of several states of Peninsular Malaysia, and amoxycillin/clavulanate and enrofloxacin might be appropriate antimicrobial agents in the treatment of bovine mastitis.

Keywords: antibiotics; antimicrobial susceptibility; buffaloes; mastitis; pathogens

ORAL PAPER 28

POULTRY PRODUCTION AND CURRENT STATUS OF VACCINE DEVELOPMENT AND BUSINESS IN INDONESIA

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Abstract

The poultry industries provide protein to Indonesians and contribute to the economy. Poultry populations have been steadily increasing, and this trend is likely to continue as interest in this field has grown over the last decade. The future expansion of the poultry business is hampered by a number of reasons, including poultry immunity, health, and productivity. Foodborne illnesses and poultry are indissolubly linked. The management of foodborne and zoonotic pathogens is a substantial challenge to the poultry industry. Infectious diseases such as Avian Iinfluenza (AI), and Newcastle Disease (ND), Infectious Bronchitis (IB) and Gumboro are major health concerns for the Indonesian poultry industry. Furthermore, the global trend of removing antibiotic growth promoters has already impacted poultry production, making bird health management more difficult. Vaccination is an effective method of preventing the occurrence and spread of a variety of diseases amongst chickens. Indonesia is self-sufficient in poultry vaccine production and exports the vaccine to other countries. Indonesia is the world's largest exporter of poultry vaccines, sending the majority to Pakistan, Vietnam, and India. This paper aims to review the current progress of vaccine in the poultry chain, as well as the industry's strategic future, and to discuss current and potential poultry strategies. This review summarizes recent developments in Indonesian poultry industry growth, poultry diseases, pathogenesis, risk factors, and vaccine development.

Keywords: poultry diseases, poultry production, vaccine, vaccine business

ORAL PAPER 29

HERBAL EXTRACTS AS AN ALTERNATIVE FOR CHICKEN DISEASE CURE: *IN VITRO EVALUATION FOR ALTERNATIVE STRATEGIES TO REDUCE THE USE OF ANTIBIOTICS IN SYSTEMATIC POULTRY FARMING*

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Abstract

Origanum vulgare subsp. hirtum, *Thymus vulgaris* and *Salvia fructicosa* are aromatic plants commonly found in Mediterranean countries and traditionally used in Greece as a remedy for humans, since they are well known as potent antibacterial, antioxidant and anti-inflammatory agents. The aim of this study was to investigate essential oils (EOs) derived from plants cultivated in the mountainous region of Epirus, Greece, for their inhibitory activity against key microorganisms with relevance to avian health, while also assessing their antioxidant and anti-inflammatory activity. The total phenolic content (TPC) of EOs was estimated according to the Folin-Ciocalteu method, while antioxidant capacity was tested through the EOs' ability to scavenge free radicals by means of the DPPH, ABTS and the FRAP assays. Antibacterial and anti-inflammatory effects were examined by the agar disc diffusion method and the LOX inhibition test, respectively. Furthermore, the EOs' ability to inhibit the invasion of sporozoites of Eimeria tenella (Wisconsin strain) along with any toxic effects were assayed in Madin-Darby bovine kidney (MDBK) cells. The antioxidant activity of the EOs was found in descending order oregano > thyme > sage. The antimicrobial effects of thyme and oregano were equivalent and higher than that of sage, while the anti-inflammatory effect of thyme was higher compared to both sage and oregano. Intracellular invasion of sporozoites was evaluated by detection of E. tenella DNA by qPCR from cell monolayers harvested at 2- and 24-hours post-infection. Parasite invasion was inhibited by the addition of oregano essential oil at the concentration of 100 μg/ml by 83% or 93% after 2 or 24 hours, respectively, and was higher compared to thyme and sage addition which had similar effects but at a less intensive level. The cytotoxic assessment of all three essential oils revealed that they had no effect on MDBK cells compared to dimethyl sulfoxide (DMSO), used as the control substance. The supplementation of oregano, thyme and sage essential oils had a potent antioxidant, anti-inflammatory, antimicrobial and anticoccidial in vitro effect that is comparable to synthetic substances or approved drugs, justifying the need for further evaluation by in vivo studies in broilers reared in the absence of antimicrobial and anticoccidial drugs or synthetic antioxidant and/or anti-inflammatory compounds

STUDENT ORAL PAPER 1

AN OVERVIEW OF AVIAN MYCOPLASMOSIS IN MALAYSIA

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Abstract

Avian mycoplasmosis is a type of bacterial infection that affects birds. It is caused by species of mycoplasma, which are small bacteria that lack a cell wall, a feature that makes it resistant to antibiotics that targets the cell wall. Among the many species of mycoplasma affecting poultry, the world organization for animals health (OIE) has listed *Mycoplasama gallicepticum* and *Mycoplasma synoviae* as the two species that are most pathogenic. Mycoplasma infections can cause a range of symptoms in birds, including acute and chronic respiratory disease, synovitis, air sacculitis, severe weight loss, egg shell abnormalities and decreased egg production. In Malaysia, avian mycoplasmosis has been reported in various poultry species and has been identified as a major cause of respiratory problems in commercial and backyard poultry farms in the country. A number of studies have been conducted on the disease in Malaysia, with a focus on the prevalence, distribution and molecular characterization the disease, as well as the various risk factors that contribute to its occurrence. These studies have shown that avian mycoplasmosis is a common problem in Malaysian poultry, and that it can have significant economic consequences for farmers. Risk factors for the disease in Malaysia include poor biosecurity measures, high bird density, and the presence of other respiratory diseases. Poor management practices can also increase the risk of the disease in poultry. Prevention and control of the infection in Malaysia involves implementing good biosecurity measures, such as proper hygiene and disinfection, as well as vaccination and the use of antibiotics. The use of vaccines has been shown to be effective in reducing the incidence and severity of avian mycoplasmosis in poultry, and is recommended as a control measure for the disease. However, despite all of these efforts, there is a lack of information regarding the optimization of detection techniques (culture and PCR), data on the strain types that are currently available, data on the antibiotic susceptibility profile of the field isolates. Additionally, diagnosis of the disease is still a challenge due to the organism's exacting nature and the laborious isolation process. In conclusion, avian mycoplasmosis is a significant problem in Malaysia, affecting various poultry species and causing economic losses for farmers. This overview highlights the need for further research towards a broarder understanding of the epidemiology and impact of the disease in the country and to develop effective diagnostic and control measures.

Keywords: Mycoplasmosis, Avian, Diagnosis

STUDENT ORAL PAPER 2

FUNCTIONAL PREDICTION OF *DE NOVO* UNI-GENES FROM CHICKEN TRANSCRIPTOMIC DATA FOLLOWING INFECTIOUS BURSAL DISEASE VIRUS-CHALLENGED AT 3-DAYS POST-INFECTION

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Abstract

Infectious bursal disease virus (IBDV) is one of the major threat to the poultry industry, especially towards the nation's nutrient and food security. IBDV is a highly pathogenic strain that would cause high mortality and immunosuppression symptoms observed in chickens. Understanding the underlying genes that play roles in the disease manifestation is now a global interest in order to control and curb future outbreaks. Hence, we had identified novel genes that elucidated the pathogenicity of IBD in chickens following IBDV-infection by employing the advance RNA-Sequencing bioinformatics protocol. After sequencing and aligning, a set of sequences retrieved from IBDV-infected chickens unmapped to chicken reference genome were de novo assembled, clustered and analysed. 10,282 uni-transcripts were assembled and 618 uni-transcripts were screened as the most significant sequences to known genes, as determined via BLASTX searches. Based on the differentially expressed genes (DEG) analysis, 18 commonly downregulated and 12 commonly upregulated uni-genes were present in all six inbred lines with false discovery rate of q-value <0.05. However, only 13 downregulated and 9 upregulated uni-genes were reported with BLAST hits against Non-redundant and Swiss-Prot databases. Meanwhile, the genome ontology enrichment keywords of these DEGs were associated with apoptosis, cell signaling and immune response. Consequently, the Weighted Gene Correlation Network Analysis R package was used to predict the functional annotation of the remaining non-significant BLAST hits unknown uni-genes. Interestingly, the functions of the five unknown downregulated uni-genes were predicted to be related to the cell surface functions, while the three unknown upregulated uni-genes may plays role in the innate immune response. This *de novo* transcriptomic profiling provides valuable information for investigating molecular mechanisms underlying host-pathogen interaction in short time. These thorough results had elucidated and supported the current molecular knowledge of IBDV infection and chicken's defense mechanisms against viral infections.

Keywords: avian, bursal, infectious bursal disease, RNA-Sequencing, transcriptomic

STUDENT ORAL PAPER 3

MORPHOLOGY OF IMMORTALIZED CHICKEN BONE MARROW DERIVED DENDRITIC CELL-LINE FROM SPECIFIC PATHOGEN FREE CHICKENS

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Abstract

Dendritic cells are professional antigen-presenting cells. In chickens, the primary chicken bone-marrow derived dendritic (ch-BMDCs) cell-line has limited life span that can only be cultured within the few first passage and yet there is no any available immortalized ch-BMDCs cell-line, thus, this study aimed to immortalized and characterized primary ch-BMDCs cell line, using an oncogenic virus; Simian virus40 Large-T antigen (SV40LT-Antigen), that was cloned into a Lentiviral vector (pLVX-EF1alpha-Puro-vector) and it's presence was detected using restriction digestion method & sequencing. Thus, immortalization of the Primary ch-BMDCs cell-line derived from bone marrow cells of specific pathogen free chickens was achieved by transfecting SV40LT-antigen into Lentivirus competent cell-line and the supernatant was harvested, which was used for transduction of the Primary ch-BMDCs, and eventually the immortalized cells were selected using 5ug/ml Puromycin selective antibiotic, confirmation of the immortalized cells was done using PCR method. Morphologically, the primary ch-BMDCs and the immortalized ch-BMDCs were indistinguishable, however the immature untreated cells appear as rounded cells while the matured cells treated with LPS showed a stellate shapes appearance. Therefore, this finding shows that the immortalized ch-BMDCs cell-line can be used to study the course of poultry viral diseases.

Keywords: Avian Viruses; Bone marrow; Chicken Dendritic cell; Immortalized cell line.

STUDENT ORAL PAPER 4

AN EFFICIENT LIVE ATTENUATED VACCINE ADMINISTRATION METHOD PROVIDES PROTECTION FOR GOLDFISH AGAINST HERPESVIRAL HEMATOPOIETIC NECROSIS CAUSED BY CYPRINID HERPESVIRUS 2

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Abstract

Herpesviral hematopoietic necrosis causes huge economic losses in goldfish *Carassius auratus* industry. A live attenuated vaccine was developed by propagating the causative virus, CyHV-2, in ginbuna CFS cell line 7 times and further in carp KF-1 cell line 8 times (P7-P8). The vaccine showed no virulence reversion even after 5 times passaged in fish and the standard bath vaccination for 2 hours provided high protection against virulent virus challenge. However, the vaccine administration must be improved to reduce vaccine cost. Therefore, we studied the efficient vaccine administration methods and vaccination temperature range. The efficiency of improved administration methods for P7-P8: dipping for different durations and showering, a variation of spray vaccination, for 10 s were compared in goldfish at 25ºC by subsequent challenge with virulent CyHV-2 at 21 days post-vaccination (dpv). To examine the vaccination temperature range, goldfish vaccinated by the showering method were reared at 15ºC, 20ºC, 25ºC and 30ºC, which are virus permissive temperatures *in vitro*, for 3 weeks and then challenged at 28 dpv after shifting each rearing temperature to 25ºC. The dynamics of P7-P8 in the organs of fish vaccinated at each temperature was measured by qPCR. The dipping (even for 10 s) and showering vaccination methods showed high protective efficacy with relative percentage survival (RPS) more than 80%. Showering vaccinated fish reared at 15ºC, 20ºC, 25ºC and 30ºC showed 73%, 78%, 100% and 78% RPS, and peak virus load (spleen or kidney) were around $10^{3.7}$, $10^{6.8}$, $10^{6.5}$ and $10^{4.6}$ virus DNA copies/mg, respectively. Vaccine virus was not detected at 21 dpv in all groups. The showering vaccination method, which required small quantity of P7-P8 culture solution and short handling time, can reduce vaccine cost and labor. This vaccine may be applicable in goldfish aquaculture since the vaccination can be conducted from 15ºC to 30ºC.

Keywords: Goldfish; Herpesviral hematopoietic necrosis; Live attenuated vaccine; Showering vaccination; Vaccine administration

STUDENT ORAL PAPER 5

ISOLATES POTENCY OF *Aeromonas* sp. AS A VACCINE CANDIDATE

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Abstract

Freshwater fish farming often has to cope with the problem of bacterial disease such as aeromoniasis or motile *Aeromonas* septicemia (MAS) which is caused by *Aeromonas* sp. Vaccine is one of effective ways to control the bacterial disease. However, the quality of bacterial isolates used as vaccine candidates sometimes decreases. Therefore, it is necessary to look for new isolates that have the potential as vaccine candidates. This research aims to examine the potential of *Aeromonas* sp. isolates as the vaccine candidate. Bacterial isolation was carried out on infected catfish obtained from aquaculture ponds. Survey methods and purposive sampling techniques were used to determine the area of catfish farming ponds, in addition to random purposive sampling techniques that were used to take samples of infected catfish. Samples were obtained from aquaculture ponds in three provinces on Java Island, i.e., DIY Yogyakarta, Central Java and West Java. The Koch's postulate test for ten isolates was applied on healthy catfish. The isolates were put into pathogenicity test using lethal $dose_{50}$ (LD_{50}). Data were then analyzed descriptively. The results of Koch's postulate test showed that pathogenic bacteria caused the diseases in catfish, including *Aeromonas* sp. Appearing clinical signs in catfish included skin erosion, skin depigmentation, hyperemia, and hemorrhagic; inflammation of the abdomen, ascites, abscesses, and abdominal dropsy; paleness of the kidneys, lesions, and hemorrhagic; paleness and lesions of the liver and gills. *Aeromonas* sp. indicated high virulence with LD_{50} values of, 7.61×10^3 CFU/mL to 1.49×10^5 CFU/mL. This study concluded that ten isolates, comprising of *A. hydrophila* (Ah1), *A. caviae* (Ac1), *A. veronii* bv *veronii* (Av1, Av2, Av3, and Av4), and *A. dhakensis* (Ad1, Ad2, Ad3, and Ad4), indicated high virulence and large potential as the vaccine candidate.

Keywords: *Aeromonas* sp., vaccine candidate, lethal dose, Koch's postulates.

STUDENT ORAL PAPER 6

GUT MICROBIOME CHANGES OF ASIAN SEABASS (*LATES CALCARIFER*) FOLLOWING FEED-BASED VACCINATION AGAINST VIBRIOSIS

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Abstract

This study describes the effects of oral feed-based vaccination against vibriosis on gut microbiome of Asian seabass. Asian seabass that cultured in floating cages were divided into two groups, which is non-vaccinated group that fed with commercial pellet and vaccinated group that was fed with commercial pellet which incorporated with the vaccine. The feed-based vaccine was administrated at weeks 0, 2, and 6. The gastro-intestinal wall and contents were collected on weeks 0, 2, 6 and 10, and were processed for high throughput 16S amplicon sequencing. Fish of both treatments were then challenged on week 10 post-vaccination with 10^8 CFU/mL of live, virulent *V. harveyi* Vh1. For field vaccination, the growth performance, survival rate and IgM antibody levels were higher in vaccinated fish. Moreover, the survival of the challenged fish was higher in the vaccinated fish. The alpha diversity of both non-vaccinated and vaccinated group showed significant ($p < 0.05$) differences, particularly in diversity and richness of microbiota that were further supported by PCoA with PERMANOVA. Phylum Proteobacteria was the most dominant in non-vaccinated group and both pre- and post-challenge followed by Firmicutes and Fusobacteria, while in vaccinated group, Firmicutes was the most dominant and followed by Proteobacteria and Fusobacteria. At genus level, non-vaccinated group dominated by *Vibrio*, while vaccinated group were dominated by *Clostridium*. Further identification of *Vibrio* to species level showed that *Vibrio harveyi* was dominantly present in non-vaccinated group compared to the vaccinated group. LEfSe analysis identified 10 potential taxa markers that differentiated the vaccinated and non-vaccinated fish, where five taxa were associated with each vaccinated and non-vaccinated group, respectively. This study revealed that feed-based vaccination aids microbiome changes, which facilitates the gut mucosal immune response of the fish against bacterial infection.

Keywords: 16S amplicon, Asian seabass, disease, feed-based vaccine, vibriosis.

STUDENT ORAL PAPER 7

PROTEOMIC ANALYSIS ON SKIN MUCUS OF HYBRID GROUPER (*Epinephelus fuscoguttatus* ♀ ×*Epinephelus lanceolatus* ♂) REVEALS DISEASE -RESISTANT BIOMARKER AGAINST *Vibrio alginolyticus*

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Abstract:

Fish skin mucus is an important component that provides first line defence of physical and chemical barrier against pathogens and toxins. The continuous production and slough off regularly of the mucus able to prevent pathogen invasion through the skin. Despite its crucial roles in fish health, the molecular properties of the mucus content that involve in the prevention of pathogen invasion are yet to be fully understood. Herein, groupers were being exposed to *Vibrio alginolyticus* by immersion challenge to simulate a natural infection scenario in order to identify *Vibrio*-resistant and -susceptible strains. Comparative proteomic analysis was then approached using liquid chromatography–mass spectrometry (Hybrid Quadrupole Orbitrap technology) to explore the changes in the mucus proteome of *Vibrio*-resistant and -susceptible groupers. *Vibrio*-resistant groupers showed no observable clinical sign of infection after immersion challenge, whereas the *Vibrio*-susceptible groupers presented hemorrhagic- and/or non-hemorrhagic ulceration of the skin. Proteome profilling of the mucus samples identified 1488 identified proteins included the immune-related proteins, namely Cystatin B, Complement Component C6, Complement factor 1, Allograft inflammatory factor 1, Deleted in malignant brain tumors protein, MHC class 1 and Annexin A1. These proteins were found to be significantly abundant in the mucus of the Vibrio-resistant grouper. Further analysis revealed two-potential biomarkers that correspond to the disease-resistant phenotype, which include 3-hydroxybutyrate dehydrogenase type 2 and L-rhamnose-binding lectin SML.

Keywords: grouper; skin mucus proteome; *Vibrio alginolyticus*; *Vibrio* resistant

STUDENT ORAL PAPER 8

FEED-BASED BIVALENT VACCINE: IMMUNOGENICITY AND PROTECTION AGAINST STREPTOCOCCOSIS AND MOTILE AEROMONAD SEPTICEMIA FOR RED HYBRID TILAPIA (*Oreochromis* spp) CULTURE

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Abstract

Streptococcosis and Motile Aeromonad Septicemia (MAS) are the main bacteria-based diseases in tilapia culture. *Streptococcus agalactiae* and *Aeromonas hydrophila* are the most virulent bacteria infecting the tilapia culture. These cause mass mortalities and economic losses. Although antibiotics may be implemented for disease treatment, there are some clear drawbacks such as drug resistance issues. Vaccination is an effective method of preventing diseases and contributes to economic sustainability. This study aims to investigate the immuno-protective efficacy of the feed-based bivalent vaccine against the causal agent of Streptococcosis and MAS. The feed-based bivalent vaccine containing formalin-killed *S. agalactiae* and *A. hydrophila* with 10 % palm oil was developed and delivered orally at 5 % of the fish's body weight. The proximate composition of the bivalent vaccine was found within the standard range of the tilapia feed composition. The palatability of the bivalent vaccine was found to be up to 96.39 ± 1.27 %, and it was more stable (79.00 %) in static water up to 7 hours of leaching time than the control ($p \geq 0.05$). Results also found that the bivalent vaccine does not affect the fish's growth performances. Lysozyme activity in vaccinated fish samples (serum, gut lavage and skin mucus) was significantly ($p \leq 0.05$) higher than the unvaccinated fish after vaccination weeks. The IgM antibody levels in the serum, gut lavage and skin mucus of vaccinated fish were increased significantly ($p \leq 0.05$) after vaccination when tested on *S. agalactiae* and *A. hydrophila* antigens than the unvaccinated fish. The bivalent vaccine offered high protective efficacy against *S. agalactiae* (80.00 %) and *A. hydrophila* (90.00 %), and partial cross-protective efficacy against *S. iniae* (63.33 %) and *A. veronii* (60.00 %). The findings proved that the feed-based bivalent vaccine could be a remarkable approach for large-scale Red hybrid tilapia culture immunization.

Keywords: Red hybrid tilapia; Streptococcosis; Motile Aeromonad Septicemia; Feed-based bivalent vaccine; Immuno-protective

STUDENT ORAL PAPER 9

AN OVERVIEW OF THE ANTIMICROBIAL RESISTANCE STUDY IN SHRIMP AQUACULTURE IN MALAYSIA

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Abstract

Antimicrobial resistance (AMR) is now extensively regarded as one of the greatest risks to human and animal health. Aquaculture is one of the sectors, which is believed to have high levels of antibiotic use although exact figures of antibiotic usage are difficult to be established due to the lack of surveillance. Amidst these circumstances, shrimp aquaculture has more challenges due to high disease burden caused by various viruses, bacteria, fungi and parasites faces greater need for antimicrobial treatments. As a result, multiple classes of antimicrobials such as tetracyclines, fluoroquinolone, quinolones, sulfonamides, chloramphenicol and nitrofuran have been frequently used to treat and prevent diseases in shrimp farming. The industry which is directly connected to the aquatic environment is likely to pose a main risk to the widespread dissemination of AMR. Therefore, assessment of AMR in shrimp aquaculture is essential to update on emergence, spread and sources of antimicrobial resistance. Hence, this paper aimed to provide an overview of the AMR studies that have been conducted in shrimp aquaculture in Malaysia. The findings showed that a number of AMR studies have been performed and antimicrobial resistant pathogens such as *Vibrio* species and *Salmonella* have been frequently isolated from shrimp aquaculture environment in Malaysia. However, the studies lack of data on antimicrobial usage patterns and linkages between the usage and resistance frequencies observed. Besides, there are very limited recent studies and data on AMR in shrimp aquaculture in Malaysia. Therefore, more research on AMR and shrimp aquaculture's antibiotic usage patterns is necessary to stop its spread and to support the National Action Plans (NAPs) on AMR.

Keywords: Antibiotics, antimicrobial resistance (AMR); aquaculture; Malaysia; shrimp

STUDENT ORAL PAPER 10

SEROLOGICAL AND MOLECULAR SURVEILLANCE OF WEST NILE VIRUS IN RUMINANTS IN PENINSULAR MALAYSIA

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Abstract

West Nile Virus (WNV) is an emerging arbovirus that has spanned many parts of the world and remains a threat to humans and animals. Previous work in Malaysia has documented evidence of antibodies developed against WNV in wild birds, macaques and swine and the detection of WNV antigens in wild birds and bats. While many studies were carried out to investigate the prevalence level of WNV transmission in Malaysia, the status of WNV circulating among ruminants in Malaysia remains unknown. In this study, a total of 112 serum samples were collected from cattle and goats in an attempt to screen anti-WNV IgG using a competitive enzyme-linked immunosorbent assay (c-ELISA). The WNV antibodies were detected in both cattle's and goats' serum samples. In addition, 112 nasopharyngeal swab samples were undergone for RNA extraction prior to WNV RNA screening by RT-PCR. However, none of the samples tested positive for WNV RNA detection. With regards to the antibodies that persisted in the ruminants tested, the findings gathered could assist in understanding WNV transmission risk in a wide range of hosts, including ruminants and poultry.

Keywords: West Nile Virus, ruminants, c-ELISA; antibodies; RT-PCR

STUDENT ORAL PAPER 11

THE PREVALENCE AND PATHOGENICITY OF TWO MOST NOTORIOUS *Aeromonas* spp. AFFECTING CULTURED FRESHWATER FISHES IN PENINSULAR MALAYSIA: *A. dhakensis* and *A. hydrophila.*

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Abstract

Aeromonas spp. are ubiquitous in water bodies ranging from environmental water to aquaculture water. This genus poses a threat to a myriad of hosts, including freshwater fishes. High stocking density and improper farm practices can increase the susceptibility of cultured freshwater fishes to diseases. *Aeromonas hydrophila* and *A. dhakensis* are among commonly identified species causing Motile Aeromonas septicemia (MAS) in cultured freshwater fishes, thereby causing huge economic losses to some countries. Therefore, this study characterized a large collection of *Aeromonas* spp. that were collected from nine important fish farms in Peninsular Malaysia, and to compare these two species based on the presence of virulence genes and pathogenicity in red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) from selected isolate of each species. A total of 78 isolates were obtained from fishes with clinical symptoms of MAS that grew on *Aeromonas* selective agar, were Gram-negative and were oxidase-positive, indicating these as presumptive *Aeromonas* spp. Further identification was made using phylogenetic analysis of *gyrB* genes of the isolates. Results show that 68% of those isolates were *A. dhakensis,* whereas 32% were *A. hydrophila.* Isolates of *A. hydrophila* (80%) and *A. dhakensis* (4%) were observed to harbor all virulence genes studied (8 virulence genes). Selected isolate of each species, *A. hydrophila* 8TK3 and *A. dhakensis* 4PS2, were found to produce LD_{50} of 10^5 CFU/ml and mortalities can be observed as early as 24 h post-challenge with current disease model. These findings suggested that these species pose an alarming threat to freshwater aquaculture with the presence of multiple important virulence genes. However, further studies at genomic level are warranted so as to determine the responsible genes that play a significant factor in the virulence of *Aeromonas* spp. to further elucidate the pathogenesis of *Aeromonas* spp. in cultured freshwater fishes.

Keywords: *A. hydrophila*, *A. dhakensis.* freshwater fish, tilapia, virulence

STUDENT ORAL PAPER 12

BACTERIAL GENE EXPRESSION AND HISTOPATHOLOGY OF BLACK TIGER SHRIMP, *Penaeus monodon* POSTLARVAE UPON FED WITH DIFFERENT *Lactobacillus* sp. STRAINS AS FEED SUPPLEMENT PROBIOTICS AND *Vibrio parahaemolyticus*, CAUSATIVE AGENT OF HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IMMERSION CHALLENGE

Yong Kit, Chin.[1], Karim, Murni.[2], Mohammad Noor Azmai, Amal.[1], and Md Yasin, Ina-Salwany.[1,2]*

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Abstract

Acute hepatopancreatic necrosis disease (AHPND) is a newly emerging marine shrimp disease that severely damaged to global shrimp aquaculture industries. In this study, *Lactobacillus* sp. was chosen as probiotic candidate against *Vibrio parahaemolyticus* positive-AHPND. Six hundreds of Black tiger shrimp *Penaeus monodon* postlarvae (PL15) were distributed into 4 treatments groups which 3 replicate tanks which 50 shrimps each. The 4 treatment groups were fed different with (i) basal feed, 10^8 CFU/g of *L. plantarum* (ii) strain L20, (iii) strain KD2 and (iv) strain T31 supplemented feeds respectively, for 35 days with 3 times daily with 4% body weight. Then, 80 shrimps from each treatment's shrimps transferred to 4 aquariums with 20 shrimps per aquarium as replicated accordingly. Pre-challenge sampling was conducted. All aquariums proceeded for 6×10^5 CFU/ml *V. parahaemolyticus* S2-4 immersion challenge test. Post-challenge sampling was conducted. Cephalothorax and abdomen of shrimps were separated after anaesthetized, RNA was extracted and cDNA was synthesized, qPCR was conducted for gene expression analysis. Shrimp were fixed in formalin after anaesthetized, dehydration and wax embedment was conducted, staining was procced for histology observation. In gene expression analysis, high viability of L20 was indicated by up regulated GHKL domain containing protein in pre-challenge abdomen and post-challenge cephalothorax of L20 treatment group. L20 reduced PirA and PirB toxin quantity of S2-4 were indicated by down regulated the toxin in post-challenge shrimps' cephalothorax for L20 treatment group. The survival of L20 treatment group' shrimps showed normal structures of shrimp hepatopancreas and intestine. Overall, *L. plantarum* L20 was chosen because it effectively inhibited *V. parahaemolyticus* S2-4 positive-AHPND in vitro and effectively improved survival and gastrointestinal microbiota of *P. monodon* postlarvae. Hence, feed-based containing *L. plantarum* L20 potentially prevents penaeid shrimp disease and potentially increases global income in aquaculture production.

Keywords: *Lactobacillus plantarum*; *Penaeus monodon*; *Vibrio parahaemolyticus*; Acute hepatopancreatic disease

POSTER

Efficiency of binary ethylenimine against low pathogenic H9N2 and H5N2 Avian Influenza viruses

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Abstract

Vaccination using inactivated virus can be as one of the methods to control the virus's spread. Avian influenza virus H9N2 and H5N2 are known to be a low pathogenic avian influenza virus (LPAI). The objective of this study is to inactivate the LPAIs using binary ethylenimine (BEI). First, BEI at a concentration of 0.002 M was prepared and mixed with the known egg infectious dose (EID) of the H9N2 and H5N2 AIV subtypes. Then, the mixture was inoculated into 9-day-old specific-pathogen-free (SPF) chicken embryos and left for 120 hours. The process was repeated for three times and heamagglutination assay (HA) was performed using the harvested allantoic fluid from the infected eggs at every passage. Morphology of the embryos was also observed. At BEI concentration of 0.002 M, H9N2 was completely inactivated within 24 hours, proven by the normal growth (complete survival) of the embryos after 120 hours of inoculation, and undetectable hemagglutination activity after three passages. However, at the same BEI concentration treatment to the H5N2, the inoculated chicken embryos showed stunted growth after 120 hours and HA titer of 2^8 was detected during the first passage. Therefore, this preliminary result suggests that 0.002 M BEI may have different inactivation activity against different AIV subtypes. A further experiment using different BEI concentrations shall be performed on H5N2 subtype to determine its optimum inactivation efficiency.

Keywords: BEI; Low Pathogenic Avian Influenza Virus; Virus Inactivation, H9N2, H5N2

POSTER

ISOLATION AND CHARACTERISATION OF GENOTYPE VII NEWCASTLE DISEASE VIRUS IN MALAYSIA

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Abstract

Newcastle Disease (ND) is caused by Newcastle disease virus (NDV), an economically important disease that caused significant financial losses to the poultry sector. Despite the widespread use of vaccines, the current vaccine and vaccination program cannot control ND in commercial chickens where frequent outbreaks of velogenic NDV have been reported in this region, including Malaysia. This study's goal is to characterise NDV isolates from diagnostic cases obtained from different states in Malaysia between 2020 and 2022 based on the sequence and phylogenetic analysis of the partial fusion (F) gene of NDV. Thirteen NDV isolates were chosen and phylogenetically characterised. Reverse transcriptase-PCR (RT-PCR) was used to amplify the partial F protein region of the NDV. The sequence of the amplified regions was analysed and compared with published sequences retrieved from GenBank. Pathogenicity study of one of the NDV isolates (UPM008) was characterised based on Intracerebral Pathogenicity Index (ICPI) and Mean Death Time (MDT). Analysis of the F protein cleavage site's amino acid sequence showed the presence of three different multiple basic amino acid motifs, 8 isolates with 112RRRKRF117, 3 isolates with 112RRQKRF117, and 1 isolate with 112KRQKRF117 which are typical of velogenic strains. Out of thirteen isolates, only one isolate (UPM129) belongs to Genotype VII.I.I and the rest of the isolates belong to genotype VII.2. The pathogenicity study showed the NDV strain UPM008 has an ICPI value of 1.75 and an MDT value of 59.2, indicating the virus is of a velogenic pathotype. The ongoing outbreak of genotype VII NDV needs continuous surveillance for a better understanding of ND outbreaks and the development of better control and prevention strategy.

Keywords: genotype VII; Malaysia; NDV; virulent

POSTER

GENE EXPRESSION ANALYSIS OF THE INNATE IMMUNE SYSTEM DURING EARLY VIBRIOSIS INFECTION OF BROWN-MARBLED GROUPER (*Epinephelus fuscoguttatus*)

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Abstract

The present study reports on the representative of innate immune genes in the brown-marbled grouper (*Epinephelus fuscoguttatus*) fingerlings with the focus on juvenile fish. This fish stage is a critical stage in mariculture fish since reliance on innate immune mechanisms is required for survival particularly during transfer from nursery ponds to grow-out cages. Here, we report the molecular cloning of partial open reading frames and expression patterns of some innate immune genes (*hep*-1, TRIM39, *lec*E, STEAP4, PGPR-SC2, *tlr*5, *il*6, ATF-3, *lec*M, CDA, *il*8, and CA6). The gene expression profiles of innate genes were investigated for mRNA extracted from the spleen of brown-marbled grouper fingerlings at 4 h post challenges against *Vibrio alginolyticus*. The present result demonstrated that *hep*-1, TRIM39, *tlr*5, *il*6, and CDA were expressed between 10 to 20-fold change. Higher expression profiles were observed on *lec*E, STEAP4, PGPR-SC2, and *lec*M that were expressed more than 20-fold change. While the highest expression was shown by PGPR-SC2 gene which expressed more than 70-fold change. All the genes of interest are related to the pathogen recognition system with a crucial role in the activation of innate immune response stimulated by PAMPs from *V. alginolyticus*. Significant changes in mRNA levels of target genes in the spleen, indicated that these genes are good molecular marker candidates for infection detection.

Keywords: *Epinephelus fuscoguttatus,* innate immunity, gene expression, grouper, vibriosis

POSTER

ANTIGEN-SPECIFIC ANTIBODY PRODUCTION IN HYBRID GROUPER (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) INDUCED BY IMMUNOGENIC PEPTIDES DERIVED FROM BETANODAVIRUS

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Abstract

Betanodavirus is a significant pathogen that causes viral nervous necrosis (VNN) in a wide range of fish species. It could cause up to 100% mass mortalities on the infected farmed fish that is in larval stages. Red-Spotted Grouper Nervous Necrosis Virus (RGNNV) has an extensive range of host-compatibility across warm-water fish species as it can infect most grouper species including hybrid grouper. To date, there is no viable strategy to combat this virus infection. Vaccine is known to be an effective prophylactic and therapeutic agent against infectious diseases. This study aims to identify immunogenic peptides against grouper betanodavirus using reverse vaccinology approach. Nucleotide sequence of the grouper nervous necrosis virus strain was retrieved from the National Center for Biotechnology Information (NCBI). B- and T-cell epitopes were predicted using the Immune Epitope Database analysis resource and the NetCTL 1.2 server, respectively. The predicted B- and T-cell epitopes were analysed for its respective antigenicity, immunogenicity, physico-chemical properties, and surface accessibility. Selected epitopes were then synthesized for immunization assay to examine antigen-specific antibody production in grouper. Five epitopes (1 B-cell and 4 T-cell epitopes) were selected based on the immunogenicity score. The B-cell epitope has the highest immunogenicity score of 0.9333. Physico-chemical properties analysis of the B-cell epitope shown highest GRAVY score of 0.516, and it was predicted to possess the longest estimated half-life of 30 hours. Structural analysis shown that the B-cell epitope possess the largest accessible surface on the virus particle. Immunization of grouper with the selected B-cell epitope induced the highest antigen-specific antibody production as compared to grouper that was treated with the selected T-cell epitope, and the negative control group. This study demonstrated the feasibility of reverse vaccinology approach in the selection and design of immunogenic peptides as the potential vaccine candidate against VNN in grouper.

Keywords: antibody; betanodavirus; grouper; peptides; viral nervous necrosis (VNN).

POSTER

DEVELOPMENT OF A COLORIMETRIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY FOR THE DETECTION OF PATHOGENIC *Streptococcus agalactiae* SEROTYPE III IN MALAYSIAN AQUACULTURE

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Abstract

Infections by *Streptococcus agalactiae* particularly serotype III have been reported to cause major outbreaks affecting local tilapia producers and farmers. Concurrent to the advancement of serotype-specific conjugate vaccine for GBS, a rapid and accurate detection with a point-of-care (POC) application is compulsory to reduce the losses due to the disease. The study aims to establish a colorimetric detection tool using gold nanoparticles (AuNPs) probe conjugated with serotype-specific capsular polysaccharide subunit (*cpsI*) gene of *S. agalactiae* TP 486 E, a serotype III strain isolated from infected red hybrid tilapia, via loop-mediated isothermal amplification (LAMP) assay. The LAMP reaction was optimised prior to the AuNPs probe preparation. The hybridisation of the LAMP amplicon and AuNP probe was then optimised for the best visualisation for assay detection. The specificity and sensitivity of the assay were tested to compare the efficiency of the assay with conventional PCR. LAMP amplicons were successfully amplified using the designated LAMP primers. The optimal LAMP reaction condition of the *cpsI* gene in TP 486 E comprises 6 mM $MgSO_4$ with the incubation temperature and time of 65ºC for 60 min, respectively. The optimal ratio and most distinct colour for visualisation of the assay after salt induction for the strain was at 4:6 of LAMP amplicon: AuNP probe. On the other hand, 0.05 mM of $MgSO_4$ was selected as the best salt concentration for aggregation for the strain. No cross-reactivity was detected in the LAMP assay when tested along the other non-specific aquatic pathogens indicating a high-specificity reaction. The detection limit recorded was 4×10^3 CFU/ml, 100 times more sensitive than the conventional PCR (4×10^5 CFU/ml). The test is completed in less than an hour from DNA extraction to the assay. The developed LAMP-AuNP assay has great potential for use in the detection of serotype-specific pathogenic aquatic GBS with a POC application.

Keywords: gold nanoparticle labeling; loop-mediated isothermal amplification; red hybrid tilapia; streptococcosis; *Streptococcus agalactiae*

POSTER

EFFICACY OF WHOLE-CELL TRIVALEN VACCINE AGAINST VIBRIOSIS IN CULTURED HYBRID GROUPERS IN INDONESIA

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Abstract

Multiple infection of bacterial species are often occured under natural farm conditions. The infections would cause a much more significant loss compared to a single infectious agent. Vaccination using multiple antigens is an essential strategy to prevent diseases in aquaculture. This study aimed to evaluate the immune response of grouper induced by whole-cell trivalen vaccine, composed of novel V. harveyi, V. parahaemolyticus and Photobacterium damsela subspecies damsela formalin killed cells (FKC). The protective potential of trivalen vaccine was examined in a hybrid grouper model. Fish were intraperitoneally vaccinated with FKC and challenged with a lethal-dose of *V. harveyi*, *V. alginolyticus*, *P. damsella* subspecies *damsela*, *V. parahaemolyticus*, *V. fluvialis*, and *V anguillarum*. The results showed that vaccination at 107 cells/fish induced protection in hybrid grouper with RPS value of 65% or more against pathogenic Vibrio spp.. the protections were dependent on the vaccine dose and lasted at least up to three months. The immunological analysis revealed the vaccine ability to elevated respiratory burst ability and bactericidal activity of macrophages at 7 d post-vaccination, enhance serum antibody titers and serum bactericidal activity, as well as higher expression of IgM, IgT, CD8a, TNFa, IL-1b genes, which were involved in both innate and adaptive immunity. This vaccine induced no apparent abnormalities in fish conditions even at 10 time higher dose. The present vaccine can be a promising product for effective immunization in grouper farming.

Keywords: Vibrio, Photobacterium, RPS, innate immunity, adaptive immunity

POSTER

DEVELOPMENT OF A MICROBIAL IMMUNOSTIMULANT AGAINST *Vibrio parahaemolyticus* CAUSING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN SHRIMP

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Abstract

Acute hepatopancreatic necrosis disease (AHPND) imposes a serious threat to shrimp production through mass mortality of post-larval shrimp within the first 30 days of stocking in ponds. Hence, this disease has resulted in USD 1 billion loss to the global shrimp industry. In this study, we developed an inactivated bacterial immunostimulant as a potential protection against the AHPND pathogen. The bacterial immunostimulant was prepared using *V. parahaemolyticus* (AHPND isolate strain C4B) cells as per the method described in the previous study using a heat-killed technique. The administration strategy for the immunostimulants delivery is through orally mixed with commercial diet. The PLs (approximately PL 15) were first acclimatized in the main holding tank for seven days upon delivery. The standard operation culture condition was practiced and the water quality parameters were maintained at the optimal level. The treatment group for this study were as follows, G1: Commercial feed, G2:$1x10^3$ CFU kg/feed, G3:$1x10^5$ CFU kg/feed, G4:$1x10^7$ CFU kg/feed, G5: $1x10^3$ CFU kg/feed + 2% *Sargassum*, G6:$1x10^5$ CFU kg/feed + 2% *Sargassum*, G7:$1x10^7$ CFU kg/feed + 2% *Sargassum*, G8: Commercial feed with 2% *Sargassum*. After four weeks, the shrimp in each treatment group were challenged through immersion technique with *V. parahaemolyticus* strain C4B, with a challenge dose, $1x10^7$ CFU/ml, using tank inoculation method. After four weeks of the treatment period, G1 showed the highest percentage (>70%) of survival compared to other treatment groups. Meanwhile, G8 showed the highest growth performance. After the challenge study, G6 showed a higher survival percentage (>60%) compared to other groups. The addition of Sargassum to the immunostimulant worked synergistically increased the survival rate of the infected shrimp. These findings on the development of the immunostimulant will potentially contribute to new knowledge and are important in controlling APHND in shrimp culture systems in an effort towards sustainable aquaculture food security.

Keywords: shrimp, AHPND, vibriosis, immunostimulant, aquaculture

POSTER

THE POTENTIAL OF BROWN SEAWEED, *Sargassum polycystum*, AS NATURAL DIETARY SUPPLEMENT ON GROWTH PERFORMANCE, PROTEIN AND FAT CONTENT OF RED TILAPIA, *Oreochromis spp*. FINGERLINGS

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Abstract

Sargassum polycystum, a marine brown seaweed, contains nondigestible selective fermented polysaccharides and their derivates, which are potential prebiotic compounds that benefit the host by stimulating the growth of beneficial bacteria while suppressing the growth of pathogenic bacteria. The current study was designed to determine the feed efficiency, growth performance as well as the composition of crude fat and protein in red tilapia fingerlings fed with supplemented with three graded levels of powdered seaweed (0.0, 1.0, and 2.0 %) into diet formulation for 56 days. The results showed that survival, feed consumption and efficiency, crude fat, protein and fatty acid and growth performance were better in fish fed the 1.0 and 2.0 % seaweed supplemented feed compared to the control. Since *Sargassum polycystum* powder is natural, its inclusion as a supplement did not harm human health or the environment, meeting food safety and biosecurity requirements.

Keywords: brown seaweed, feed efficiency, prebiotic, red tilapia fingerlings *Sargassum polycystum*.

POSTER

IN VITRO ANTAGONISTIC ACTIVITY AND BENEFICIAL CHARACTERISTICS OF MULTI-STRAIN PROBIOTICS TOWARDS AQUATIC PATHOGENS

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Abstract

Probiotics application in aquaculture is one of the preventive measures to potentially reduce the use of antibiotics which leads to the emergence of antimicrobial resistance genes. The usage of multi-strain probiotic (MSP) has more benefits and increased potential on a host as opposed to the usage of a single-strain probiotic (SSP). This study concentrated on the *in vitro* evaluation of single and multi-strain probiotics in terms of strain compatibility, antagonistic potential, biofilm formation capability and stress tolerance. MSP 1 which consists of potential probiotics *Bacillus amyloliquefaciens* (L11), *Enterococcus hirae* (LAB3), and *Lysinibacillus fusiformis* (SPS11) significantly inhibited the growth of pathogens (*Aeromonas hydrophila* and *Streptococcus agalactiae*) on both spot and well diffusion assays. On the other hand, MSP2 consisting of *Lysinibacillus fusiformis* strain SPS11 and A1, and *Lysinibacillus sphaericus* strain NAS32 also showed significantly high inhibitory zone against *Vibrio parahaemolyticus* and *Vibrio harveyi*. Both MSPs also significantly reduced the pathogen count in co-culture assay. MSP1 in the co-culture assay reduced *A. hydrophila* count from 9.89 ± 0.1 CFU mL^{-1} to 2.14 ± 0.2 CFU mL^{-1} ($p < 0.05$). MSP2 at concentrations 10^4, 10^6, and 10^8 CFU mL^{-1} showed a significant reduction ($p < 0.05$) in *Vibrio* count for both *V. harveyi* and *V. parahaemolyticus*. The biofilm formation of both MSPs were significantly higher ($p < 0.05$) than its constituent SSPs and the pathogens. MSP1 and 2 produced the highest biofilm at a 48-h interval with optical density reading of 10.09 ± 0.4 nm and 3.46 ± 0.01 nm ($p < 0.05$) respectively. The SSPs in both MSPs showed resistance to high temperatures (80, 90, and 100 °C) and a wide range of pH (2 to 9) which further highlights the strains beneficial characteristics. This *in vitro* assessment demonstrates that MSP1 and 2 could be further tested as multi-strain probiotics on selected aquatic host.

Keywords: multi-strain probiotics; single-strain probiotics ; antagonism; strain compatibility; properties; biofilm; stress tolerance

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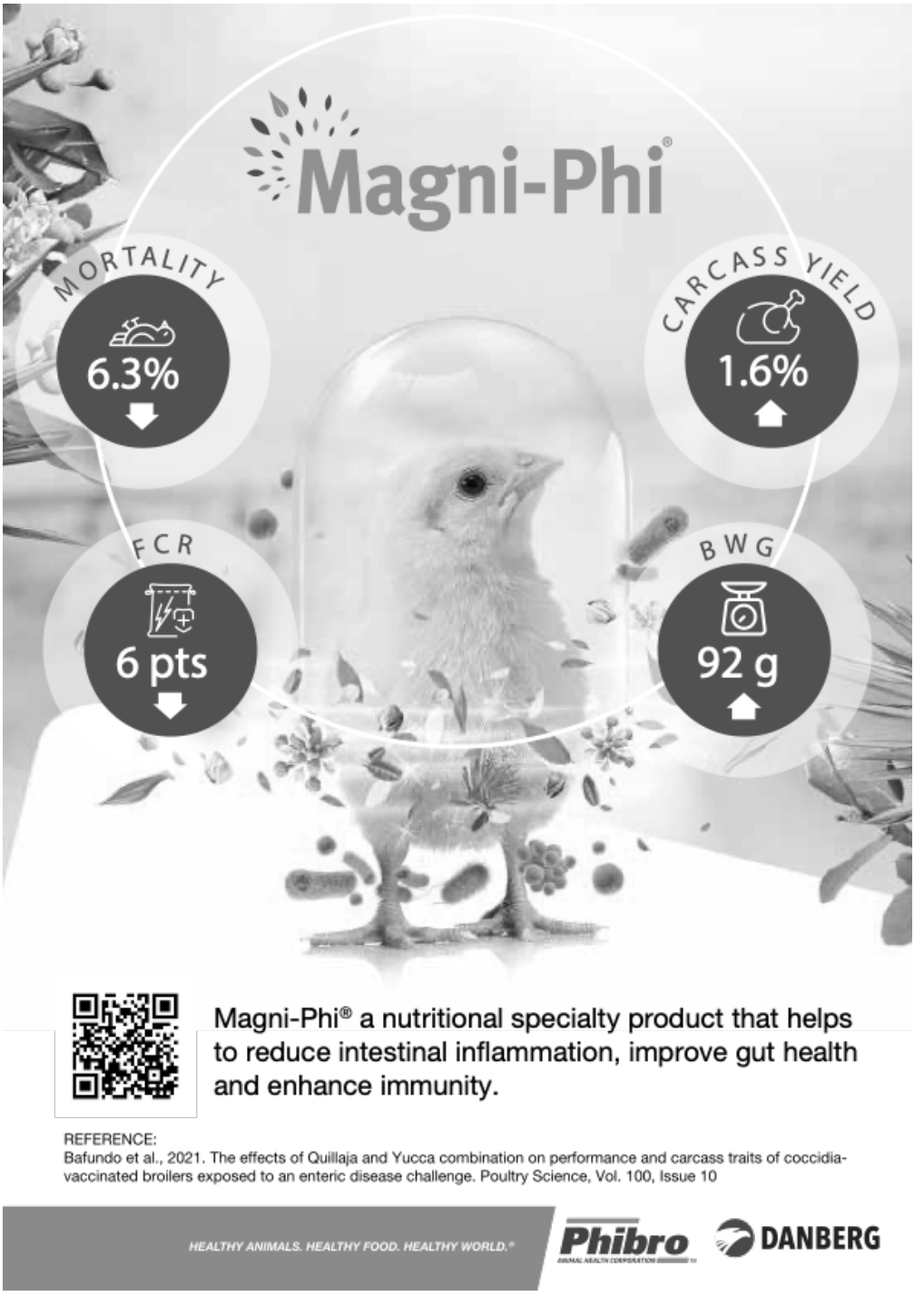
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