NETWORK OF AQUACULTURE CENTRES IN ASIA-PACIFIC

Twenty First Meeting of the Asia Regional Advisory Group on Aquatic Animal Health

REPORT OF THE MEETING

Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand

17-18 November 2022

Prepared by the NACA Secretariat

Preparation of this document:

This report was prepared by the 21st Asia Regional Advisory Group on Aquatic Animal Health (AG) who met virtually in Bangkok, Thailand on 17-18 November 2022.

The Advisory Group was established by the Governing Council of the Network of Aquaculture Centres in Asia-Pacific (NACA) in 2001 to provide advice to NACA members in the Asia-Pacific region on aquatic animal health management, through the following activities: (a) evaluate disease trends and emerging threats in the region; (b) identify developments with global aquatic animal disease issues and standards of importance to the region; (c) review and evaluate the Quarterly Aquatic Animal Disease reporting programme and assess the list of diseases of regional concern; (d) provide guidance and leadership on regional strategies to improving management of aquatic animal health including those under the framework of the Asia Regional Technical Guidelines; (e) monitor and evaluate progress on Technical Guidelines implementation; (f) facilitate coordination and communication of progress on regional aquatic animal health programmes; (g) advise in identification and designation of regional aquatic animal health resources, as Regional Resource Experts (RRE), Regional Resource Centres (RRC) and Regional Reference Laboratories (RRL); and (h) identify issues of relevance to the region that require depth review and propose appropriate actions needed. Members of the Advisory Group include invited aquatic animal disease experts in the region, representatives of the World Organisation for Animal Health (WOAH) and the Food and Agricultural Organization of the United Nations (FAO), collaborating regional organisations such as SEAFDEC Aquaculture Department (SEAFDEC AQD) and WOAH-Regional Representation in Asia and the Pacific (WOAH-RRAP), and the private sector.

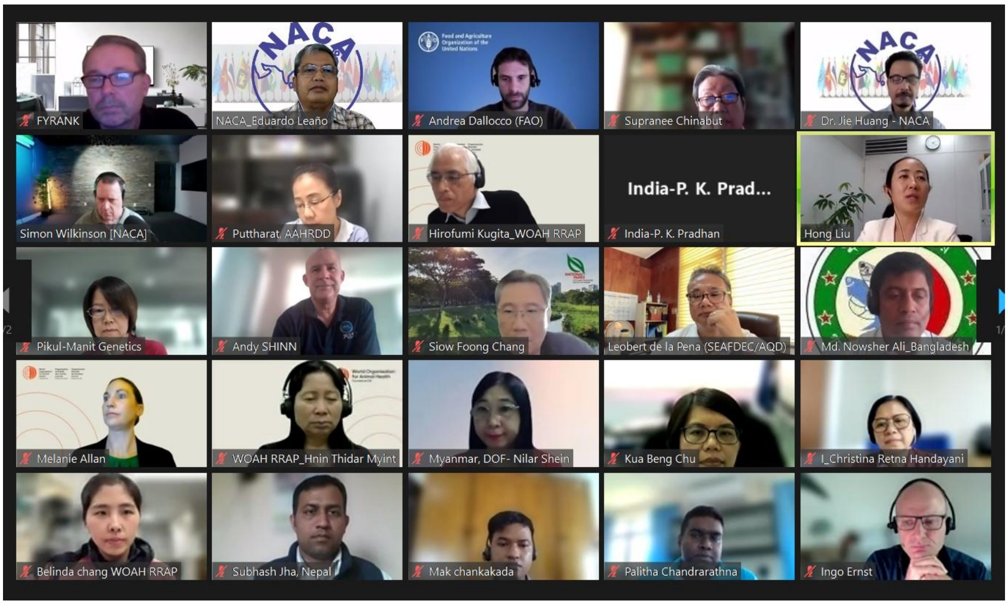
Reference: NACA 2022. Twenty First Meeting of the Asia Regional Advisory Group on Aquatic Animal Health: Report of the Meeting. Published by the Network of Aquaculture Centres in Asia-Pacific, Bangkok, Thailand.

ABBREVIATIONS AND ACRONYMS

AAD	Aquatic animal disease
AAH	Aquatic animal health
AAHRDD	Aquatic Animal Health Research and Development Division, Department of Fisheries, Thailand
AAHSC	Aquatic Animal Health Standards Commission of the WOAH
AG	Asia Regional Advisory Group on Aquatic Animal Health (NACA)
AGM	Advisory Group Meeting
AHPND	Acute hepatopancreatic necrosis disease
AMR	Antimicrobial resistance
AMU	Antimicrobial use/usage
AP	Asia-Pacific
CA	Competent authority
CEV	Carp edema virus
CN	Concept note
CMNV	Covert mortality nodavirus
COFI/SCA	Committee on Fisheries, Sub-Committee on Aquaculture (FAO)
COVID-19	Corona virus disease 2019
DIV1	Decapod iridescent virus 1
EHP	Hepatopancreatic microporidiosis caused by *Enterocytozoon hepatopenaei*
EUS	Epizootic ulcerative syndrome (Infection with *Aphanomyces invadans*)
FAO (HQ)	Food and Agricultural Organization of the United Nations (Headquarters)
GS	General Session of the WOAH Delegates
ICTV	International Committee on Taxonomy of Viruses
IHHNV	Infectious hypodermal and haematopoietic necrosis virus
IHNV	Infectious haematopoietic necrosis virus
IMN	Infectious myonecrosis
IMNV	Infectious myonecrosis virus
ISAV	Infectious salmon anaemia virus
ISKNV	Infectious spleen and kidney nodavirus
KHV	Koi herpesvirus
LMBV	Largemouth bass virus
MrNV	*Macrobrachium rosenbergii* nodavirus
MrGV	*Macrobrachium rosenbergii* golda virus
MSU	Mississippi State University
NAAHS	National Aquatic Animal Health Strategy
NACA	Network of Aquaculture Centres in Asia-Pacific
NHP	Necrotising hepatopancreatitis
NORAD	Norwegian Agency for Development Cooperation
NSAAH	National strategy for aquatic animal health
NVI	Norwegian Veterinary Institute
PCR	Polymerase chain reaction
PL	Post larvae
PMP/AB	Progressive management pathway for improving aquaculture biosecurity
PT	Proficiency testing
QAAD	Quarterly Aquatic Animal Disease
RL	Regional laboratories (WOAH)
RSIV	Red seabream iridovirus
SEAFDEC-AQD	Southeast Asian Fisheries Development Center, Aquaculture Department
SPF	Specific pathogen free

SHIV	Shrimp hemocyte iridescent virus
SVCV	Spring viraemia of carp virus
TG	Technical Guidelines (Asia Regional Technical Guidelines on Health Management for the Responsible Movement of Live Aquatic Animals)
TiLV	Tilapia lake virus
TiPV	Tilapia parvovirus
TRBIV	Turbot reddish body iridovirus
TWG	Technical working group
VCMD	Viral covert mortality disease
VHS	Viral haemorrhagic septicaemia
VHSV	Viral haemorrhagic septicaemia virus
VNN	Viral nervous necrosis
WAHIS	World Animal Health Information System
WOAH	World Organisation for Animal Health (founded as OIE)
WOAH PVS	WOAH Performance of Veterinary Services (tool)
WOAH-RRAP	WOAH Regional Representation in Asia and the Pacific, Tokyo, Japan
WSD	White spot disease
YHV	Yellow head virus
YSFRI	Yellow Sea Fisheries Research Institute, P.R. China

The 21st Asia Regional Advisory Group on Aquatic Animal Health.



Participants of the virtual AGM 21 composed of AG members and co-opted members from FAO (Rome, Italy), WOAH HQ (Paris, France), WOAH-RRAP (Tokyo, Japan), WOAH-AAHSC (Paris, France), SEAFDEC AQD (Iloilo, Philippines), AAHRDD (Bangkok, Thailand), P.R. China, Australia, Singapore, Thailand, the private sectors (PHARMAQ, Inve), NVI (Ås, Norway), and NACA Secretariat. Observers from NACA member countries and territories were also invited, and governments represented include: Australia, Bangladesh, Cambodia, Hong Kong SAR, India, Indonesia, Malaysia, Myanmar, Nepal, Philippines and Sri Lanka.

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OPENING SESSION

The 21st Meeting of the Asia Regional Advisory Group on Aquatic Animal Health (AGM-21) was convened in Bangkok, Thailand on the 17-18 November 2022. Due to the continuing travel restrictions brought by the COVID-19 pandemic, AGM 21 was again held virtually via the Zoom platform. Originally attended by only AG members, co-opted members and few observers, the meeting was again participated by NACA member country representatives. NACA member countries and territories represented include Australia, Bangladesh, Cambodia, Hong Kong SAR, India, Indonesia, Malaysia, Myanmar, Nepal, Philippines and Sri Lanka.

The meeting was opened by **Dr. Eduardo Leaño**, Senior Programme Officer of NACA and Technical Secretary of the AG. Welcome message was given by **Dr. Jie Huang**, Director General of NACA. Dr. Huang emphasized the aquaculture production of the AP region through sustainable development, which is a critical requirement for food security and livelihood. The collaboration among NACA, FAO, WOAH and other relevant organizations in the region has set-up successful mechanisms to face challenges brought about by aquatic animal diseases and biosecurity risks in the past decade. Previous and current members of the AG were thanked for their contribution to this mechanism since its establishment in 2002.

After brief self-introduction by all the participants, Chairperson as well as Vice-Chairperson were selected, and who will serve the AG for two years. **Dr. Andy Shinn** was selected as the Chairperson while **Dr. Leobert dela Peña** as Vice-Chairperson. Dr. Shinn took over in the facilitation of the AGM 21 and moved for the adoption of the Provisional Agenda (**Annex A**). The complete list of participants is attached as **Annex B**.

SESSION 1: PROGRESS REPORT FROM NACA'S ASIA REGIONAL AQUATIC ANIMAL HEALTH PROGRAMME

Dr. Eduardo Leaño presented the progress report of NACA's Asia Regional Aquatic Animal Health Programme since the previous AGM 20 which was held virtually on 4-5 November 2021. Key points discussed during the AGM 20 include:

- Strategic Plan on Aquatic Animal Health Management. There is always a need to focus resources on some important aquatic animal health management issues, thus, a strategic plan is important for NACA in order to address specific issues and problems.
- AMR Surveillance in Aquaculture. Gaps have already been identified including the problem on interpretation of results from the different AMR activities undertaken in the region. As such, the Regional Guidelines for AMR Surveillance in Aquaculture will serve as a harmonized guidelines/protocols for undertaking AMR surveillance in aquaculture in the region.
- AMU in Aquaculture. How to precisely monitor the volume of antibiotics used by the farms is still a big challenge in the assessment of AMU in aquaculture farms. The Guidelines being prepared by FAO-RAP and WOAH on antimicrobial use at the farm level will somehow address this challenge.

- Regional Disease Reporting. Reporting is still an issue for aquatic animal diseases in the region, and the importance of transparency and reporting of emerging diseases is again highlighted. Disease reporting continues to be an important source of information which is readily available and does help in creating transparency and building trust among trading partners.
- Aquaculture Biosecurity. Countries in the region should actively participate and collaborate in the different activities related to the implementation of PMP/AB, especially on farm-level biosecurity which will pave way to better aquatic animal health management and disease prevention. The quantitative biosecurity assessment tool being developed by NVI, once finalized, should be used for farm-level biosecurity assessment in line with the self-assessment requirement under the PMP/AB.
- WOAH AAH Strategy. The Strategy is important and should be promoted by countries in the region, in line with their respective NAAHS for better aquatic animal health management and consequently prevention of disease outbreaks. It is crucial to receive feedbacks from member countries so that WOAH can identify what will not work, and would know the needs of the country especially in terms of support from WOAH.
- WOAH Regional Collaboration Framework. Countries are encouraged to support regional activities on AAH, and to continuously give updates on important issues on AAH management that need to be addressed.

Report of the meeting (e-copy) was widely circulated among NACA member countries and partner organizations, and published at NACA website for free download.

The regional aquatic animal disease reporting requires all Members to submit monthly data as soon as available to WOAH-RRAP and NACA to ensure the timeliness of the disease information. Updated reports are regularly published in dedicated pages at NACA and WOAH-RRAP websites.

NACA continues to support the WOAH Regional Collaboration Framework on Aquatic Animal Health in the Asia-Pacific. During the 3rd Steering Committee meeting held in December 2021, NACA presented progress on the aquaculture biosecurity project which is implemented under the Framework, as well as updates on NACA's AAH Programme. The aquaculture biosecurity project was completed in June 2022, and the final report was submitted to WOAH-RRAP. The report contains:

- Recommended farm-level aquaculture biosecurity measures applicable for small-scale farmers;
- Farm-level awareness materials from participating countries, related to aquaculture biosecurity implementation;
- Case study on hybrid catfish farmers in Thailand on the implementation of farm-level biosecurity measures; and,
- National aquaculture industry biosecurity survey: An assessment of enterprise level biosecurity in Australia.

NACA has started implementation of FAO funded project on the Development of Regional and National Aquaculture Biosecurity Strategy through the PMP/AB for Asia-Pacific since March 2022. A follow-up workshop (Phase 1) will be held on 28-29 November wherein a national and regional SWOT analysis will be launched, which will provide critical information for developing national and regional strategies for PMP/AB (Phase 2). NACA (Dr. Leaño) also collaborated with WOAH as member of the *Ad hoc* group on Technical References for Aquatic Animals, and attended the final meeting in WOAH Headquarters on 30-31 August 2022. The technical reference document listing antimicrobial agents of veterinary importance for aquatic species was finalized. The document will be included as an Annex of the AMR Document being prepared by WOAH.

A virtual training course on Mariculture Technologies for the Asia-Pacific region was conducted by NACA in collaboration with the Yellow Sea Fisheries Research Institute on 19-23 September 2022. The training course aimed at strengthening human resources and building capacity in management and technology for mariculture. NACA (Dr. Leaño) was also invited as a resource person during the Regional Capacity Building Program on Application of Biotechnological Tools for Management of Aquatic Genetic (Module 2: Aquatic Animal Diseases and Biosecurity) held virtually in Lucknow, India on 4-12 January 2022.

In support of the different AAH activities in the region and in the world, NACA actively participated in several meetings and workshops:

- Tilapia Health: Quo Vadis? Organized by FAO on 1-3 December 2021;
- FHS Webinar 4: Small and Terrible! Significant Bacterial Diseases in Aquaculture. Organized by Fish Health Section of AFS on 8 December 2021;
- Shrimp 2022. Organized by INFOFISH on 7-11 June in Putrajaya, Malaysia (hybrid event);
- Quadripartite AMR Global Consultation. Organized by FAO, WOAH, WHO and UNEP on 5-7 July 2022;
- 11th Symposium on Diseases in Asian Aquaculture (DAA 11). Co-organized by FHS-AFS and the Department of Fisheries Malaysia on 23-26 August 2022, Kuching, Sarawak, Malaysia;
- Regional Convention of the Philippine Society for Microbiology (Visayas Chapter). Organized by PSM Visayas on 29-30 October 2022 in Guimaras, Philippines.

Discussion

- The volume of antibiotics used by farmers in the aquaculture sector is still one of the biggest challenges in the assessment of AMU and AMR, as there is still no estimates on how much antibiotics are actually used especially at the farm level. This is because AMU in aquaculture in the AP region still lacks proper veterinary prescription, and no records are available on how much antibiotics are sold (by veterinary stores) or used (by farmers themselves). Access to antibiotics by aquaculture farmers is also easy, as they can purchase any kind of antibiotics from veterinary stores or suppliers (even without prescription). Moreover, farmers are also not aware on how much (and sometimes what kind of) antibiotics are actually used in their farms.
- The Guidelines that is currently being developed by FAO and WOAH on the assessment of AMU at the farm level will hopefully address the abovementioned problem on estimation of

AMU. Farm level AMU includes all the aquaculture production sectors from broodstock, hatchery, nursery and grow-out.

- WOAH requires countries to report country level AMU for both terrestrial and aquatic animals which can be a starting point in looking at the data that WOAH has collected. However, the total AMU data represents agriculture in general, and it is hard to divide its usage among the sectors like cattle, pigs and aquatic animals. On WOAH report on AMU in aquatic animals, usually they only mention on what types of antimicrobials are used but no information on the volume of actual usage at the farm level.
- On the status of the ASEAN guidelines on AMR surveillance which is being prepared by Singapore, they are still coordinating it with FAO-RAP and will keep the group updated once it is finalized and released.

RECOMMENDATIONS

- AG recommended NACA to continue its important aquatic animal health management works in the region in collaboration with partner organisations, especially on current issues like AMU/AMR, biosecurity and emerging diseases.
- AG recommended that member governments should continue to support important projects on AAH that are being implemented and planned for the region.

SESSION 2: UPDATES FROM WOAH AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Dr. Ingo Ernst gave a presentation on the progress of the Aquatic Animal Health Standards Commission's (AAHSC) work to develop new and revised standards for the WOAH Aquatic Animal Health Code and WOAH Manual of Diagnostic Tests of Aquatic Animals. Dr. Ernst highlighted some of the ongoing work discussed at the Commission's September 2022 meeting and draft standards included in the meeting report.

For the Commission's ongoing work, Dr Ernst highlighted some of the key activities that may be of most interest to members.

New Aquatic Code standards to be developed. The commission has considered the development of new standards for application of compartmentalisation, trade of ornamental aquatic animals, and trade of genetic material. These new standards were identified as priorities in the WOAH Aquatic Animal Health Strategy. The commission has considered approaches for developing these standards and work has commenced on all three. A questionnaire was provided to members in the Sep 2022 Commission report to seek early input on experiences with compartmentalisation.

Listed diseases. The Commission considered the report of its ad hoc group on susceptibility of species to RSIV. The ad hoc group had recommended that the three genogroups of the species ISKNV (i.e. RSIV, TRBIV and ISKNV) be listed. The commission conducted an assessment against the listing criteria which concluded that the species ISKNV (including its three genogroups RSIV, TRBIV and ISKNV) meets the listing criteria. The assessment was provided to members for comments.

Aquatic Code chapter for TiLV. Following the listing of TiLV in May 2022, a new Aquatic Code chapter for this disease has been developed and provided to members for comments.

Aquatic Manual. The Commission is continuing to progressively update the scientific information in all Aquatic Manual chapters and to reformat them into a new template. The revised chapters have clear guidance on recommended tests for surveillance, information on their validation status, consistent case definitions, and updated scientific information. Substantially revised chapters have been provided to members for comments for the first time; these include:

- 2.2.2. Infection with *Aphanomyces astaci* (crayfish plague)
- 2.2.5. Infection with infectious myonecrosis virus
- 2.2.7. Infection with taura syndrome
- 2.2.8. Infection with white spot syndrome virus

In addition to these chapters, other revised chapters that had been previously circulated had been updated based on member comments and circulated with the new revisions highlighted. These include:

- 2.2.0. General information (diseases of crustaceans)
- 2.2.1. Acute hepatopancreatic necrosis disease
- 2.2.3. Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)
- 2.2.4. Infection with infectious hypodermal and haematopoietic necrosis virus
- 2.3.2. Infection with epizootic haematopoietic necrosis virus

Dr Ernst also noted that there are no WOAH reference laboratories for several diseases and that applications from suitably qualified laboratories were encouraged. These diseases include:

- Infection with *Aphanomyces invadans* (EUS)
- Infection with *Batrachochytrium dendrobatidis*
- Infection with *Batrochochytrium salamandrivorans*
- Infection with infectious myonecrosis virus
- Infection with *Perkinsus marinus*
- Infection with Tilapia lake virus
- Infection with *Xenohaliotis californiensis*

DISCUSSION

- On WOAH-listed aquatic animal diseases with no existing reference laboratories, WOAH can approach a specific (qualified) laboratory directly and ask them if they are interested to apply as a reference laboratory. Another approach is to refer any good candidate laboratory to WOAH (which will be happy to receive such information) or interested laboratories can apply directly to WOAH via their delegate. One of the prerequisites is that the laboratory should be accredited with ISO17025 quality management system, which excludes research laboratories that usually lack such accreditation.
- In regard to the above, setting up a network for laboratories might be necessary for better communication and sharing of information. The network can also facilitate setting up of

twinning programs to improve performance of certain laboratories so that they can build up their capacity to become a reference laboratory in the future.

- On delisting of diseases, this was not discussed in the most recent WOAH meetings but there has been some suggestions in the past like for IHHNV, and the conclusion was, it still met the criteria for listing. WOAH provided a detailed assessment for this particular disease back to the member countries and the main issue raised was on its impact to the industry. WOAH asked the countries to send further information on this disease for consideration of the Commission.
- It is the responsibility of WOAH members to undertake surveillance and reporting of most WOAH-listed diseases. Although it became an SOP for some countries, delisting of diseases which no longer have an impact in major aquaculture species should still be pursued.
- The WOAH Aquatic Code has a set of criteria for listing diseases which can be applied to delisting diseases, should a disease be found to no longer meet the criteria. However, the real issue is, there may be a difference in views between the countries that have and those that don't have the particular disease. For countries that do have the disease, it may be a burden because of production and management impacts as well as potential trade impacts. For countries that don't have the disease, they may want to put measures in place to ensure that they don't get the disease.
- The principal idea of the Aquatic Code and Aquatic Manual is to facilitate safe trade, they are meant to provide a standard approach among countries that they can use not only to manage disease risks but also to still trade.
- If the disease no longer meets the criteria for listing, it should be removed from the list. There is a need to focus on more important diseases that really need to be managed to protect industry productivity for those countries that do not have the disease, and also to provide a mechanism for those countries that do have the disease to achieve zone or compartment freedom and trade without the disease, and have that level of market access.
- On disease listing, WOAH is currently proposing to list ISKNV species which include RSIV, ISKNV and TRBIV genotypes. Comments from member countries are requested whether the three genogroups differ enough epidemiologically that they can be listed separately, or whether they can be listed as just ISKNV. This would have an impact particularly on reporting and disease status. The Commission has asked the WOAH Information Department whether they can collect information separately for the three genogroups if it is listed as one disease.
- If the virus species ISKNV is considered for listing, RSIV would be removed from the list because the species ISKNV would include the RSIV genotype.
- Regarding CMNV which is an emerging disease, the virus can be detected in a wide range of hosts including crustaceans, fish and even echinoderms (starfishes). Symptomatic cases of this disease might be common in the susceptible species. Once this disease satisfies the WOAH criteria for emerging disease and molecular detection is considered as standard, disease cases might exist everywhere.
- The main purpose of notification is understanding the distribution of the pathogen, so that we know where it is occurring, and this will help us to understand the risk that it may cause and on what measures may or may not be warranted in terms of disease control. For the

purpose of notification, it is warranted to notify authorities if the virus is detected in aquatic animals, just like CMNV which can be quite pathogenic and cause productivity issues in certain hosts. More investigation by other research teams is needed to assess the pathogenicity of CMNV.

- In India, currently they are not sure how to report ISKNV if ever it is detected in any samples, thus the inclusion of this genotype to the list of diseases will help a lot in the proper reporting of the disease or the presence of the pathogen. Regarding CEV, it is considered as an emerging disease, but there are still no report whether mortalities are associated with the disease. On IHHNV which is still included in their national surveillance programme, they expressed support to re-evaluate the disease based on its impact to the industry, and whether it can be considered for delisting. Lastly, laboratory proficiency testing is very important and they hope that NACA, WOAH and other partner organizations can undertake another cycle of proficiency testing for important aquatic animal pathogens in the region.

RECOMMENDATIONS

- AG recommended that member countries should nominate diseases that they think no longer meet the WOAH listing criteria together with evidences, just like the ones provided for IHHNV. Upon submission, WOAH will look at it thoroughly and properly assess whether the disease can be delisted or not.
- AG recommended that investigations and research studies on emerging aquatic animal disease problems should be continuously pursued, preferably by several research teams in different countries in the region.

SESSION 3: AQUACULTURE BIOSECURITY

3.1 THE PROGRESSIVE MANAGEMENT PATHWAY FOR IMPROVING AQUACULTURE BIOSECURITY (PMP/AB): UPDATE FROM NOVEMBER 2022 PRESENTATION; ACTIVITIES OF RELEVANCE TO ASIA

Dr. Andrea Dall'Occo (on behalf of Dr. Melba Reantaso) presented updates regarding the Progressive Management Pathway for Improving Aquaculture Biosecurity (PMP/AB) from the November 2021 presentation (AGM-20) and other activities of relevance to Asia. The presentation informed the meeting of 2022 expectations as reported during AGM-20.

The **Progressive Management Pathway for Improving Aquaculture Biosecurity (PMP/AB)** has the following updates: (1) The PMP/AB Technical Working Group was reconvened in 2020, with three members from the Asian region; (2) The first in-person meeting was held in Gaeta, Italy in June 2021 which discussed a number of PMP/AB toolkits and status of pilot testing.

PMP/AB toolkits currently in development include the following:

- risk assessment using the value chain approach – this is currently being developed as an e-learning tool under the FAO E-learning Academy. A request for training on risk assessment by Thailand on behalf of ASEAN is being considered.
- emergency preparedness – a guidance document 'Aquatic organisms mass mortality events: guidance on effective preparedness, response, recovery and review' is being finalised; a number of case studies on emergency preparedness are included. As part of emergency preparedness and contingency planning, FAO has published what is called as 'Disease Strategy Manuals' which are technical documents that support contingency planning. Three have been published (IMNV, AHPND, TiLV) as below and three more are in preparation (EUS, WSSV, DIV1).
  - IMNV: https://www.fao.org/documents/card/en/c/ca6052en/
  - AHPND: https://www.fao.org/publications/card/fr/c/CB2119EN/
  - TiLV: https://www.fao.org/documents/card/fr/c/cb7293en/
- 12-point active aquatic disease surveillance checklist (https://onlinelibrary.wiley.com/doi/full/10.1111/raq.12530) intended as an educational tool for a multi-disciplinary team in developing countries. Several virtual courses were undertaken during the last two years which benefitted the following Asian countries: India, Malaysia, the Philippines, and Viet Nam
- economics of aquatic biosecurity – during last AGM, we presented some information on the case studies. In January 2023, a round table meeting will be held to review and finalise the draft cost-benefit analysis framework, its application to the four case studies and eventually development for practicioners; an academic paper is also being planned.

Pilot testing of PMP/AB has commenced using the initial steps in Stage 1 (i.e. assessment, gap and SWOT analysis, formation of PMP/AB TWG or Task Force at national level), in several countries in Asia including Bangladesh (Fish Innovation Lab c/o MSU/USAID Project), China (FAO Regular Programme funds), Indonesia and Viet Nam (FAO Project GCP/GLO/352/NOR), and Sri Lanka (FAO Project GCP/GLO/086/ROK). At the regional level, FAO is supporting the development of the NACA Regional Biosecurity Strategy; a virtual workshop was held on 28-29 November 2022. NACA member countries are completing the FAO self-assessment survey on capacity and performance on health management and biosecurity and SWOT analysis; a regional workshop to finalise the strategy is being planned for March 2023.

PMP/AB-related publications include the following: (1) Chapter 17 of the Shrimp Book (edited by Victoria Alday): The PMP/AB: Relevance and Potential Application to the Shrimp Aquaculture Sector); (2) A new Progressive Management Pathway for improving seaweed biosecurity (https://www.nature.com/articles/s41467-022-34783-8); (3) Policy Brief: Safeguarding the future of the global seaweed aquaculture industry (https://inweh.unu.edu/wp-content/uploads/2016/09/unu-seaweed-aquaculture-policy.pdf). In preparation are the following: (1) The Progressive Management Pathway for Improving Aquaculture Biosecurity (PMP/AB): Guidelines for Application (FAO Fisheries and Aquaculture Technical Paper No. 689); (2) Improving aquaculture biosecurity through a novel progressive management pathway (PMP/AB)

Virtual International Technical Seminar: Tilapia health: quo vadis?

A virtual International Technical Seminar - Tilapia health: quo vadis? organized by FAO and INFOFISH, successfully held from 1–3 December 2021, with four Keynote Presentations and twenty-five expert presentations for a total of forty experts - shared their knowledge, industry experience, and perspectives on challenges and possible solutions. An Expert Panel session concluded the event where six experts gave their perspectives on future food security, lessons from small-scale businesses, support for further sectoral expansion from governance authorities can provide, investing in fish as a nutritious food commodity and tilapia as a potential candidate, safe trading of live tilapia and products and on the part of FAO, how it can assist the tilapia sector. The event, with simultaneous five language interpretation (Arabic, Chinese, English, French and Spanish) generated great attendance; actual attendance for all days reached more than 1 700 from at least 100 countries and with a good gender balance. The event was supported by two FAO projects that focused on Tilapia lake virus (TiLV), namely: (i) GCP/RAF/510/MUL: Enhancing capacity/risk reduction of emerging TiLV to African tilapia aquaculture, and (ii) TCP/INT/3707 Strengthening biosecurity (policy and farm-level) governance to deal with TiLV.

The Special Issue on Tilapia health: quo vadis is published at Reviews in Aquaculture journal (https://onlinelibrary.wiley.com/toc/17535131/2023/15/S1) contains the following papers: (1) From Africa to the world—The journey of Nile tilapia; (2) The future of intensive tilapia production and the circular bioeconomy without effluents: Biofloc Technology, Recirculation Aquaculture Systems, Bio-RAS, Partitioned Aquaculture Systems, and Integrated Multitrophic Aquaculture; (3) How value addition by utilization of tilapia processing by-products can improve human nutrition and livelihood; (4) Strategies to enhance tilapia immunity to improve their health in aquaculture; (5) Improving tilapia biosecurity through a value chain approach; (6) A global review of problematic and pathogenic parasites of farmed tilapia; (7) Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance; (8) From the basics to emerging diagnostic technologies: What is on the horizon for tilapia disease diagnostics?

Antimicrobial Resistance in Aquaculture

FAO's work on AMR in aquaculture is in partnership with the FAO Reference Centers on AMR and Aquaculture Biosecurity. Four of them have been recently designated after passing a rigorous selection process. These include two research institutes in China, namely the Pearl River Fisheries Research Institute and the Yellow Sea Fisheries Research Institute both under the Chinese Academy of Fisheries Sciences; the Nitte University in India; and the Mississippi State University inn the USA. The designation is for a period of four years. Ongoing work include co-organization of webinars; an e-learning module on Introduction to AMR in aquaculture led by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS is part of UK FAO Reference Center on AMR); AMR Risk Profile for Vibrio parahaemolyticus and development of methodology for AMU survey and AMR surveillance led by Nitte University, All centers will be involved in capacity building of FAO member countries.

- Relevant publications have been released authored and/or co-authored by FAO officers:
    - AMR in aquaculture: global analysis of literature and national action plans (https://onlinelibrary.wiley.com/doi/full/10.1111/raq.12741)
    - Antimicrobial resistance in fish pathogens and alternative risk mitigation strategies (https://onlinelibrary.wiley.com/doi/10.1111/raq.12715?af=R)
- Relevant publications in progress:
    - Responsible Management of Bacterial diseases in Aquaculture (FAO non-series publication)
    - RAQ-04-22-0113.R2 - Review of Alternatives to Antibiotic Use in Aquaculture (accepted, in production)

Fish-Vet Dialogue

The first Fish-Vet Dialogue: Exploring Collaboration on Managing Health of Aquatic Organisms organized by FAO and the World Organisation for Animal Health (WOAH) with the support of the Norwegian Agency for Development Cooperation (NORAD) and the Norwegian Veterinary Institute (NVI) was held virtually, from 7-9 June 2021. The conference was attended by 137 experts from 35 countries representing governance authorities, intergovernmental organizations, academia, research institutions and the private sector. Ten country presentations were delivered on organizational arrangements for managing aquatic health and biosecurity (China, Peru, United Kingdom, Viet Nam, and Zambia) and on disease investigations and emergency response (Ghana, Chile, Norway, Thailand, and The Kingdom of Saudi Arabia [KSA]). The preliminary findings of a survey of country organizational arrangements for managing the health of aquatic organisms and aquaculture biosecurity, including with respect to the direct and indirect impacts of the COVID-19 pandemic, were also presented and contributed to conference deliberations. The conference successfully achieved its objectives in providing a platform to inform respective mandates, share experiences and identify areas for collaboration concerning aquaculture biosecurity, health management of aquatic organisms and/or trade-related matters as a basis for generating recommendations for further development of aquatic animal health and biosecurity systems. The dialogue underlined several key aspects for expanding wider scope of collaboration through engagement with stakeholders and harnessing public-private sector partnerships, achieving One Health goals, scientific collaboration and transparency in support of biosecurity and trade. The dialogue also emphasized the importance of self-regulatory biosecurity, looking at biosecurity regulations, the level of science required to warrant action on disease outbreaks and sharing scientific research to assist one another. The meeting discussed that while there is no strong scientific evidence of COVID-19 virus transmission through frozen aquaculture products, a better understanding and risk assessment of virus transmissions will be achieved through more peer-reviewed studies. The importance of transparency and import risk analysis in strengthening biosecurity and the need for further collaboration with competent authorities was highlighted. The findings of the Fish-Vet Dialogue can be described into into words: collaboration, trust, transparency and public-private partnerships (PPP). These aspects are essential for effective aquatic animal health and biosecurity management. Collaboration across government agencies is the minimum requirement and policies or regulations are unlikely to succeed unless developed together with stakeholders, through PPPs. Trust and transparency are also required at all levels of management to support biosecurity and trade. Furthermore, the One Health approach should be taken to promote regional and international cooperation to improve biosecurity along the aquaculture value chain and reduce the risks of disease outbreaks. The second Fish-Vet Dialogue (Fish-Vet Plus) is being planned for 2023 or 2024.

The presentation was ended with a brief description of FAO's new strategic framework contained in what is called as Four Betters: Better Production, Better Nutrition, Better Life and Better Environment. Implementation of this strategic framework is through the Priority Programme Areas (PPAs). The work of the Fisheries and Aquaculture Division anchors on the Blue Transformation PPA – being implemented under three thematic areas: Sustainable Fisheries, Sustainable Aquaculture and Sustainable Value Chain and Trade. The Food Safety, Nutrition and Health Team by Dr M Reantaso is under the third thematic area and has strong relevance to PPAs on One Health, 'Safe Food' and 'Climate Change'.

Discussion

- The PMP/AB has recently been applied to the seaweed industry and their paper (with Dr. Elizabeth Cottier-Cook as the lead author) is already published in Nature Communications (https://doi.org/10.1038/s41467-022-34783-8). The paper proposes a novel PMP for improving seaweed biosecurity in response to the increasing incidences of infectious diseases and pest outbreaks in the seaweed aquaculture industry. The inclusion of seaweeds in PMP/AB is an important move in the overall implementation of the program.
- Surveillance is one of the key components/elements of a National Aquatic Organism Health Strategy that has long been promoted by FAO and NACA since early 2000. Active surveillance has recently been the focus of FAO work using the 12-point checklist for surveillance of diseases of aquatic organisms: a novel approach to assist multidisciplinary teams in developing countries (https://onlinelibrary.wiley.com/doi/full/10.1111/raq.12530) based on requests from members (e.g. several countries in Africa and the Philippines on TiLV and Malawi and Zambia on EUS).
- Passive surveillance is a kind of ordinary work and daily food for most of FAO projects, which means it is usually included in all the projects that are being implemented in the region. Active surveillance is highlighted because of the projects being carried out in the field like in Malawi for surveillance of EUS.
- There is definitely an overlap in the works that WOAH and FAO does in the aquatic space, and there has been a long relationship and strong collaboration between these two organizations in terms of implementation of aquatic animal health projects.
- FAO's work is focussed on technical assistance to member (whatever is, capacity building, development of policy and knowledge products). The recently concluded FAO/NACA Global Conference on Aquaculture (2022) encouraged members to provide full support to the WOAH Aquatic Animal Health Strategy. WOAH and NACA are members of the PMP/AB Technical Working Group, while WOAH PVS-Aquatic is considered as one of the PMP/AB tools as part of the gap analysis/assessment process.

- FAO will be collaborating again with WOAH in the organization of the Fish-Vet Dialogue II (tentatively before end of the year or early 2024) and hope that AG will also promote the PMP/AB which is becoming an FAO corporate label:
  - https://www.fao.org/antimicrobial-resistance/resources/tools/fao-pmp-amr/es/ - PMP/AMR
  - https://www.fao.org/family-farming/detail/en/c/1618705/ -PMP/Bees
  - https://www.youtube.com/watch?v=sSL1Eu3wp5Q PMP/Terrestrial Animal Biosecurity

RECOMMENDATIONS

- AG recommended that surveillance (both passive and active) should be continued by every member countries especially for important aquatic animal diseases.
- AG recommended that strong collaboration among partner organizations (like FAO and WOAH) should continue and further strengthened towards addressing important issues on aquatic animal health management in the region.

3.2 FARM-LEVEL AQUACULTURE BIOSECURITY

Dr. Andy Shinn summarised some of the key points regarding biosecurity along the shrimp value chains by focusing on seven key areas including shrimp, the water, the feeds being used, the systems being used, the biosecurity of the people working in shrimp production, the risk of infections spread by equipment, and by risks by air looking at key considerations from broodstock, through larval production to grow-out.

Biosecurity considerations in broodstock and multiplication centres

Starting with the biosecurity of shrimp broodstock and breeding centres, it is important to highlight the importance of genetic selection in breeding for resistance against key pathogens. Shrimp reared in biosecure facilities demonstrating freedom of specific pathogens are Specific Pathogen Free (SPF). If these shrimp, however, are then transferred to non-secure facilities and are exposed to pathogens, then the shrimp can develop disease and there can be mortalities. Specific Pathogen Resistant (SPR) stocks are those that have been selected and bred for their resistance to specific pathogens. This means that if the shrimp are exposed to the pathogen, they still might get infected, but they are able to limit the infection so that mortalities do not result. The third is Specific Tolerant Shrimp (SPT) – again these are selectively bred to tolerate specific pathogens. If the shrimp are then exposed to a pathogen, they may or may not get infected but the level of disease or the level of mortality manifested by this population or line of shrimp is likely to be lower than you would see in normal shrimp stocks.

There are risks associated with feeding live or fresh diets to broodstock. These diets may consist of polychaetes, squid, mussels, etc. While only the mantle of squid is typically used and prepared as a frozen product, the risks of contamination with pathogens causing disease in shrimp are low. For polychaetes, typically the whole worm is used and it is important to be cognisant of the potential risks of infection in non-SPF supplies from various viral and bacterial pathogens and from the microsporidian *Enterocytozoon hepatopenaei* (EHP). There are companies that supply live SPF polychaetes and others that supply frozen polychaetes. The lower performance of broodstock consuming a frozen product in terms of the number of spawnings per female, fecundity, egg hatch rate, and the percentage surviving to the zoea stage, that is commonly reported is an important consideration for producers deciding to use fresh/live polychaetes. Where SPF polychaetes are not available or not an option, then there is a need to lower the biosecurity risks associated with the vertical transmission of diseases agents, by reducing or eliminating the polychaetes component of broodstock diets.

In broodstock management, certain practices such as eyestalk ablation in shrimp production can affect the infection chances and the robustness of adult shrimp and their ability to cope with disease challenges. A study by Zacarias *et al*. (2021) looked at the effect of eyestalk ablation on the robustness of their offspring. Two disease challenge trials, one for Acute Hepatopancreatic Necrosis Disease (AHPND) and one for whitespot virus, challenged shrimp from either ablated or non-ablated mothers. In both trials, the offspring from non-ablated females had greater rates of survival, significantly so when challenged with an isolate of Vibrio parahaemolyticus causing AHPND.
Looking at the water supplied to broodstock centres and to hatcheries, it is important to ensure this is sterilized, is biosecure, and this can be achieved using ozone and UV. Air that is drawn in is exposed to an electric current which separates the oxygen atoms which then reassemble as $O3$ or ozone which is then mixed with the incoming water supply. Ozone acts by oxidizing cellular membranes destroying pathogens. The ozone treated water then passes through a UV system which destroys any residual ozone and any toxic hypobromous acid and bromamines that are produced. The added value of ozone is that dissolved waste material can partially be oxidized by ozone with toxic nitrogen compounds being oxidized to $NO3$. A protein skimmer can be used to remove protein compounds and other organic compounds from the water. An alternative approach is to use electrolysed seawater where hypochlorite is produced by electrolysis. This can be used to treat large volume of both incoming and wastewater. The system needs to be used in conjunction with charcoal filters to remove any residual chlorine. For some major pathogens like EHP, much work is still needed to define the minimum doses required to ensure their effective sterilisation. Another approach to ensure the biosecurity of the incoming water that is used is to employ ultrafiltration. Systems are now available that can retain particles as small as 0.03 microns – so effectively filtering out bacteria and EHP spores.

Biosecurity considerations in the hatchery and nursery phases of production
Looking at biosecurity strategies employed in the hatchery, a key consideration is the microbial management of the culture environment. On site practices needs to drive the management of the system so that a balance between r versus K strategists is achieved. R strategists are species living in unstable and unpredictable environments and can undergo rapid reproduction to stabilise themselves; they can be dangerous opportunistic pathogens. K strategists by comparison are species living in stable environments – they typically have low or slower growth and are generally harmless. Achieving a balance between these two determines the risk for bacterial interference although it should be stressed that this can be unpredictable. Even with disinfection measures in place and probiotics being applied, there are still risks of disease – and in the hatchery these can be

Vibrio species responsible for luminescent vibriosis, zoea-2 syndrome, bolitas and AHPND, etc., so having the correct system design and biosecurity practices in operation are crucial.

The important thing to stress is that biosecurity is not only about reducing the risks of pathogen entry or about killing bacteria but – even more importantly – it is about creating a stable microbiome ecosystem that reduces the probability of pathogen populations developing. If you achieve a total kill of bacteria, then you create an open environment for other pathogens entering the system.

A recent study undertaken by Heyse et al. (2021) found that the phytoplankton community is an important driver of the rearing water microbiome. From their study, source tracking analysis revealed that 37% of all bacteria in the hatchery rearing water were introduced either by the live or dry feeds, or during water exchanges. The contribution of the microbiome from the algae was the largest, followed by that of the Artemia, the exchange water and – to a low extent- the dry feeds. This demonstrates the crucial role those peripheral microbiomes play in maintaining a health-promoting microbial balance. This study also provides key knowledge for us to use in working towards systems with better balance, including how bacteria can be managed with probiotics, water exchanges together with RAS (recirculating aquaculture system) and biofloc, and the need for biosecure live feeds. Algae can, therefore, have a major impact on the microbiome, and this needs to be addressed. The way that algae is being produced, notably in Asia, is typically in open cultivation systems which means that they are at a high risk of contamination. By moving the culture of algae into photobioreactors, where culture conditions are carefully controlled, means that algae can be produced and fed in a biosecure way. Thinking about Artemia as a live feed and how technological advances mean that this can also be produced in a biosecure way. Although there are claims of total Artemia substitution, hatcheries cannot eliminate the use of Artemia without affecting hatchery output including the overall quality and robustness of the post larvae (PL) being produced.

The empty shells of Artemia can be a perfect substrate for Vibrio. To facilitate their removal, Artemia cysts coated with specific iron particles are used in conjunction with magnets positioned in static or dynamic, self-cleaning tools, such as the those in the INVE SEP-Art range. The shells respond to the magnets which can collect >99% of the empty cyst shells or cyst shells in general, including those that are unhatched. The removal of the shells results in a cleaner source of nutritionally superior instar 1 Artemia for use in hatcheries. During the hatching and culture of Artemia, there are also specific products that can be used to lower the number of Vibrio and ensure good water quality (e.g., Sanocare® ACE PRO). Product use can result in similar rates of hatching but significant reductions in the total number of bacteria that critically result in a knockdown, keeping Vibrio at low levels.

Biosecurity considerations in grow-out

From disease challenge trials, understanding the tolerance of different sized post larvae to key pathogens like Vibrio parahaemolyticus causing AHPND can provide key insight into farming practices that can reduce the impacts from disease.

In one in-house trial, a single population of shrimp was followed through their development with sub-samples challenged with different doses of bacteria to define LC50 doses needed to kill different stages of PL. From the 600+ tests that were conducted, it was found that almost 80 to 100 times more bacteria were needed to kill 50% of a population of PL33 than that needed to kill 50% of a population of either PL 16 or PL24. While there is no surprise that larger sized shrimp need larger doses of bacteria to kill them, the large jump in the doses required between PL 24 and PL 33 is interesting and can be used in decisions regarding the best time to transfer PL to ponds.

In good biosecurity and health care management, the importance of regular health screening of stocks and being able to recognize the early signs of disease cannot be stressed enough. Biofouling, the presence of bolitas, condition of the hepatopancreas, shell necrosis, gut chromatophores, gut content and faecal condition, etc., can provide critical insights into the health of the stock the culture systems and ongoing husbandry practices. If deviations to the normal, expected condition are seen then it is important that the relevant health or investigative tests are conducted. If these are overlooked, ignored, dismissed or there is procrastination in decision making then not only can it result in impacts to the health and survival of stocks but may result in irrevocable situations regardless of the subsequent intervention. It is of course absolutely critical that a health assessment on stocks are made before they are transferred into ponds.

In recent years, disease problems caused by Vibrio spp. and viruses have emerged as major constraints in aquaculture production. The application of antibiotics to culture ponds is not only expensive but also detrimental, in that it can result in the selection of bacteria that are drug-resistant or more virulent, and it can result in drug residues in reared animals ready for consumption.

Probiotics can be a valid alternative to the prophylactic application of chemicals, notably antibiotics and biocides. Beneficial bacteria, which compete with bacterial pathogens for nutrients, space and/or inhibit the growth of pathogens, can be applied to the water or to the feed. These probiotic strains are not therapeutic agents but will alter directly or indirectly the composition of the microbial community in the rearing environment and the shrimp intestinal tract. One key message here is in the importance of creating stable microbiomes which helps in biosecurity.

Many bacterial strains are commonly used as probiotics in aquaculture. There is, however, much doubt on the efficacy and safety of certain probiotics on the market which results from the use of ineffective bacterial species and too low number of bacteria and from unrealistic claims, or from a lack of scientific evidence and poor-quality control during production of the finished product, or from inappropriate delivery methods leading to contamination or reduced performance. The development of suitable probiotics is not a simple task and requires empirical and fundamental research, full-scale trials, as well as the development of appropriate monitoring tools and controlled production. The key message is that well selected probiotics can assist in the stability and the enhancement of the gut microflora and the environment in competing against hazardous species.
Within the context of biosecurity, Recirculating Aquaculture Systems (RAS), as managed systems, can assist in creating more stable microbiomes. Pilot trials have demonstrated that RAS can result

in significantly higher biomasses of shrimp being harvested but with 30% lower running costs than traditional systems. The RAS system resulted in better water quality and removal of solids. The systems had higher bacteria loads from the start with a more stable microbial community throughout the culture period. More work with RAS systems is needed to fine tune them for use on a commercial scale.

It is also important to consider that the number of Vibrio will increase during shrimp transportation, and these should not be added to the pond when stocking. So, the Vibrio in the transport water can be controlled using specific products during this transport phase therefore reducing the risk of Vibrio being introduced into the ponds while keeping the robustness of PLs maximal.
At the production level, microbial management can be facilitated using protein skimmers to remove dissolved organics and, using biofilters in recirculation systems. In integrated farming, there is a move to reduce the number of production ponds and to increase the area dedicated for on-site water management / purification. In general terms, the lower substrate to bacteria ratio leads to greater microbial stability. There are now state-of-the-art tools available to monitor the bacterial microbiome (e.g., Heyse et al., 2021) permitting the development of approaches to their management.

The importance of good system health in biosecurity should also be emphasized. On almost all production sites, the focus of biosecurity effort tends to be on ensuring the health and security of the ponds or the tanks – essentially the heart of the system. However, it is also important to consider all the supply networks feeding into and out of these systems – the pipework, aerators, airlines etc. If these are not maintained and cleaned between each production cycle, then these can also result in health problems – they can have mussel build-up in poorly filtered systems, formation of biofilms, bryozoans, etc., and we do have products within the industry to address these to make sure that systems are regularly disinfected.

Air quality also needs to be considered in our biosecurity assessment. The spread of pathogens by air through aerosols is also frequently neglected and yet the spread of pathogens by air is no big surprise especially in these COVID-19 challenged times and yet there is a real lack of data demonstrating this as an infection. Using Vibrio parahaemolyticus causing AHPND a model, a series of in-house trials were conducted within a carefully controlled, licenced site, were able to demonstrate that bacteria can be cast great distances and that infections from infected ponds or wastewater reservoirs, if not appropriately treated, can serve as infection points potentially seeding new infections in neighbouring ponds. Similarly, the use of air pumps in broodstock centres, hatcheries and nurseries should also be fitted with air filters on their intake pipes to reduce the introduction of air borne pathogens into culture systems.

General disinfection and good husbandry practices

In addition to the biosecurity points that have been made, there is also a need for robust cleaning SOPs, monitoring of pathogens, staff training, regular biosecurity audits, and the implementation of good sanitary practices which may include the careful and judicious selection of approved disinfectants, biocides, and chemotherapy agents. The choice and regime will be dependent on several factors, including the size of the culture unit to be treated, efficacy, cost, the local environmental conditions, and therapeutic safety margins, etc.

References

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Discussion

- On aerosol transmission (of bacterial pathogen), the same principle may apply for viruses. Looking deeper into available literatures, there is very little empirical data within the spread of aquatic pathogen. But when you look at the bacteria from oceans being carried inland, it is hundreds of kilometers that they can be potentially spread. The idea on this aerosol study is to provide farmers with empirical data that bacteria can be spread, thus there is a need to carefully consider where to put reservoirs and wastewater, how to separate ponds, and then there are things that can be done to interfere and try to stop the spread of infections in ponds/farms.
- On farm-level biosecurity practices that address aerosol contamination, some farmers install aerosol barriers specifically positioned at the back of the aerators and adjusted depending on the direction of the wind and the size of the culture pond. Other measures to reduce or prevent the spread of pathogen through aerosols are being looked at.
- There are other ways that bacterial pathogen can be spread, thus care should be taken to make sure that any infection cannot be carried or spread even by humans. Example is *Vibrio parahaemolyticus* which can survive on hands for 90 minutes, and just washing hands with water will not eliminate the pathogen. Soaps or alcohols should be used for hand disinfection, and the use of gloves is highly recommended.
- Thermotheraphy for virus infection can be used as an approach to achieve SPF condition in shrimps. Studies (P.R. China) has been published on the use of this method for DIV1 in shrimps, wherein infected shrimps were exposed to 36^{o}C, and after two weeks, the virus is completely eliminated from infected shrimps. This therapy is also being tested for other viruses for elimination of viral pathogens.
- For disinfection purposes, bleach (chlorine) is usually used. It was also found that hydrogen peroxide (H_2O_2) can also be used for *V. parahaemolyticus* with less toxicity to the shrimp larvae and with less residue in the culture water. H_2O_2 can be used in hatchery and other small scale systems, but not in grow-out ponds with larger areas of production. Currently, the use of H_2O_2 is not mentioned in any WOAH standards, although this chemical is approved for use in both human and animal health.
- The challenge in the application of biosecurity measures comes with the size of the culture system. For broodstock, hatcheries and nurseries which are small and fully closed systems, any biosecurity measures can be implemented without much of a problem. The real

challenge is in the outside grow-out ponds, especially for large ponds (e.g. in Ecuador where ponds can go as large as 10 has) which lack homogeneous environment and with different pockets of infections. Trying to manage such ponds will definitely create a biosecurity headache.

- Biosecurity has a lot of different aspects, and with regards to genetic improvement for resistance to different viral pathogens, there are companies that claim to have lines resistant to IHHNV, while others are trying to select lines resistant to WSD and EHP. Selective breeding programmes are undergoing in some countries for important aquaculture species, and biosecurity measures should be strictly in place in such genetic selection programmes.

Recommendation

- AG recommended that basic farm-level biosecurity measures should be continuously promoted for adoption of farmers, depending on their facilities and capacity. Biosecurity should not become another burden, especially for small-scale farmers which lack the capacity, but rather a motivation for the farmers to improve the overall farm management in order to prevent occurrence of any disease and to sustain production.

3.3 Updates on aquaculture biosecurity assessment tool and possible collaboration in the region

Dr. Saraya Tavornpanich presented updates on quantitative biosecurity assessment tool developed by the Norwegian Veterinary Institute (NVI) for assessing and quantifying biosecurity measures. The tool was initially developed to evaluate biosecurity measures of seabass farms located in Mediterranean basin, and further developed for farmed Atlantic salmon in Norway. We created online farm questionnaire consisting of self-explanatory questions structured into these 18 sections to gather data on farm general characteristics, production management, health management, disease reporting, diagnostic capacity, biosecurity practices and record keeping. Input data combined with data from other publicly available sources were analysed and the results including the overall biosecurity score, external and internal biosecurity scores averaged for all participated farms are presented in interactive, web-based application. Farmers can also access their own individual information by logging in to their page required username and password to see additional information including their responses to the questionnaire and how the questions are scored.

Modifications made to the tools in 2022 included (1) expanding the tool to cover broodstock production facilities, (2) re-defining type of facilities based on WOAH production systems, and (3) adding tool to quantify risk specific to particular pathogen.

In the earlier version, the tool focuses on hatchery and on-growing phases, in 2022 we had organized workshops with industry and subject experts to describe the broodstock facilty, to identify pathways for pathogens introduction and revise the whole questionnaire. The production of broodstock in Norway is carried out in broodstock stations consisting of sea-based facilities for adult broodstock and land-based facilities where production of roe takes place. Broodstocks are transported with a wellboat from sea-based facilities to land-based facilities or in scenarios where the land-based facility is not in proximity to the sea, there could be some form of transport by land. At the end, the broodstock end up in freshwater tanks to create suitable conditions for egg and milt or semen production. In contrast, some farmers keep the broodstock in land-based holding units supplied with seawater. Broodstock are graded and individually tagged using a PIT tag (harmless electronic ship in their bodies). The best performing and most robust fish are selected to produce a family (offspring of each crossing). Each of the resulting families is reared in separated and marked individual tanks in identical controlled feeding, sanitary and environmental condition. Broodstock are selected through systematic breeding works. A representative number (15-20) of individuals from each family are selected, tagged, phenotyped to measure weight and record characteristics and traits of the fish, genotyped to identify genes linked to a specific trait, and tested for resistance to particular diseases. Egg production takes 9-11 weeks. Ultrasound was used to determine maturation. Females are examined for signs of disease before stripping with approximately 10000 ova per female. Males may be used 4-5 times before they are killed and examined. Before fertilization, egg-fluid is taken away to minimize the risk of contamination, and the egg-fluid and milt are tested. After fertilization, the eggs are being disinfected before taken in to the incubation facilities. The first part of incubation takes place in single incubators where the fish is being held until test results are ready. When they reach eyed-egg-stadium they are being sorted for the first time. Sorting is often done a couple of times during incubation. After a period of incubation, the eggs may be sorted by hand to take out dead eggs, small-eyed eggs before delivery to the customer. Normally the eggs are disinfected before going on the truck.

The other modifications of the tool included re-defining all facilities according to WOAH four categories of production systems to open, semi-open, semi-closed, and closed systems taking into considerations of facilities location and their capacity to have control of the water, environmental conditions, animals or vectors, etc. We also developed checklists to ensure that the questionnaire encompass what listed in Norway and EU new animal health regulations on aquaculture biosecurity (akvabiosikkerhetsforskriften: FOR-2022-07-01-1300) and meet the requirements according to WOAH standards.

In brief, the new animal health regulations requires all approved aquaculture facilities to have a biosecurity plan documented and professionally assessed plan that shows how infection can enter an aquaculture facility, spread within the facility and be transferred to the environment or to other aquaculture facilities. The biosecurity plan must take into account the special features of the facility and determine which measures will reduce the biosecurity risks that have been identified. There must be a named person responsible for biosecurity for each facility, and all employees report to the person responsible in matters relating to biosecurity.

- The biosecurity plan must at least contain information on:
- Locks in and out of the facility, and routines for visitors
- Distinguish between different departments in the facility if relevant
- Equipment that is shared between several facilities
- How dead fish are picked up and handled
- Routines and equipment for washing and disinfecting equipment
- Routine for disinfection of roe

- How the transporter's documentation for washing and disinfection is verified before loading or unloading aquaculture animals in the facility.

Based on the risk basis for the individual facility, the biosecurity plan can also contain information on:

- Health status in the area
- Coordination of operations in the area
- Distance to other facilities, waterways, slaughterhouses, etc.
- Water source and water treatment, Drainage
- Health status of fish taken into the facility
- Moving fish
- Vaccination
- Traffic to and from the facility
- Health monitoring

Furthermore, we are working on the tool to quantify risk specific to particular pathogen. Pathogen-specific risk profiling is to estimate risk of specific pathogen taking into account of the facility overall biosecurity and current disease situation of the facility and surrounding environment. ISAV is used as a case study for the risk-profiling tool. Lastly, the tools are being developed for aquaculture biosecurity assessment of institutional partner countries under Fish for Development (FfD) projects. This include Ghana – fishery commission on production of Tilapia, and Colombia with veterinary competent authorities for Tilapia and rainbow trout.

Discussion

- On the biosecurity assessment tool, when it was first developed and tested in Norway, it was thought that it can be applied (with slight modification) and used in other countries with different type of aquaculture production. However, when the tool was applied in Colombia for example, a completely new app was developed wherein the interest is more on freshwater fish (rainbow trout) with huge numbers of small-scale farmers. It is also important and easier to work with their Competent Authorities (in Colombia) as they actually have very clear idea on what they want, and already have a biosecurity checklist that are already applied to different levels of production.
- On the survey of the different regulations on aquaculture biosecurity in the region, the list of recommendations for farm-level biosecurity, especially for small-scale farms, are mostly addressed in the list of questions included in the assessment tool. Thus, modification and adjustments on the tool itself, in terms of questions related to implementation of farm-level aquaculture biosecurity , will be much simple and will facilitate the adoption of the tool for important aquaculture species in the region.
- The tool contains the minimum criteria based on international standards and country regulations, as each country has a different level of requirements. But the tool itself is designed to provide a quantitative way of measuring or assessing aquaculture biosecurity implementation at the farm level.

- Other potential use of the tool can be on benchmarking where a farm can do a self-assessment, share results with other farms and see where it fits on the overall farm biosecurity management. It can also be used for risk management so that the level of biosecurity in different facilities within the area can be identified and used for an emergency response and to understand what levels of biosecurity measures are present.
- The tool can also be used for import risk assessment (IRA) as an additional support information, as it addresses several aspects of biosecurity including level of disease status and robustness of biosecurity facilities which are important for IRA. For example, Norway is focusing on Infectious salmon anaemia (ISA) as there are companies interested in exporting SPF (for ISA) broodstock, and the tool is being used to assess the level of biosecurity measures being applied at the broodstock facilities.
- Other potential application of the tool is for trading and export certification, not only at establishment level but also to compartments or zones. And how to translate this to regulatory leaders for each country is important, as there should be a link between the platform, the outcome, the regulations and the competent authorities in order for the tool to work for this purpose.
- The tool can be used for specific pathogens that can be used for the interest of partner trading countries. At present, a checklist has been prepared for Norway's partner trading countries, but the data is for a different purpose, that is for export of ISA-free broodstock.
- The tool can also be potentially applied to internal audits of compartments especially for a particular disease that the country is claiming freedom from, and eventually facilitate international trade to support disease-free recognition of the compartment.
- The tool itself, once established and through its flexibility, can be adopted for different purposes by both modifying the IT behind it as well as the list of items included in the checklists.
- The advantage of the tool itself is, it collects all the necessary data on aquaculture practices in general and farm level biosecurity in particular. One possible option in utilizing the tool is to be able to extract the information of interest depending on one's need or purpose, for example those related to risk assessment, trading, farm biosecurity, and disease prevention among others.

RECOMMENDATIONS

- AG recommended that the biosecurity assessment tool, once finalized, should be pilot tested in selected countries in the region, involving different aquaculture species and culture systems. This is to assess its applicability into the more complex structure of the aquaculture industry in the AP.
- AG recommended to explore the usability of the assessment tool for other purposes including IRA, risk management, internal audits of compartment and zones, and trading and export.

SESSION 4: THE WOAH AQUATIC ANIMAL HEALTH STRATEGY 2021-2025: IMPLEMENTATION IN THE AP REGION

Dr. Melanie Allan gave a presentation on the current implementation of the WOAH Aquatic Animal Health Strategy. WOAH acknowledges the need to build more sustainable aquatic animal health systems, and as a result, launched its first Aquatic Animal Health Strategy (AAHS) in May 2021. This Strategy will improve aquatic animal health and welfare worldwide, contributing to sustainable economic growth, poverty alleviation and food security, thereby supporting the achievement of the Sustainable Development. This Aquatic Strategy is a call to action to address some of the WOAH Community's greatest challenges in managing aquatic animal health and welfare. It will identify and coordinate actions that address the highest-priority common needs and focus resources on activities that will provide enduring impacts. Development of the strategy was a collaborative effort with extensive contributions for the Aquatic Animal Health Standards Commission and the whole WOAH community. Member Countries and partners were asked to contribute to the strategy by providing their views through a survey on: what WOAH initiatives they consider the most valuable to them; the biggest opportunities to improve aquatic animal health and welfare over the next 5-10 years; and what they consider to be the biggest threats to a sustainable growth in aquatic animal health productions. The responses provided valuable input into the development of this Strategy and through their responses, Members were able to state their needs and express their views on which activities should be considered a priority in the Strategy. The purpose of the strategy is to enable Veterinary Services or Aquatic Animal Health Services to meet both the opportunities and the challenges of the growth in aquaculture.

The strategy has 4 objectives: Standards, Capacity Building, Leadership and Resilience There are 4-6 activities under each objective and also a number of sub-activities which are implemented on a prioritised basis. Each activity has an activity lead within WOAH Headquarters and an internal strategy committee monitors the progress.

- **Objective 1 – STANDARDS:** addresses the development of new standards and the revision of existing standards, and their implementation. The activities undertaken to meet this objective aim to ensure WOAH aquatic standards are scientifically sound and fit for purpose, and that Members are supported to engage in the standard-setting process and implement standards.
- **Objective 2 – CAPACITY BUILDING:** these activities will support Members to strengthen their Aquatic Animal Health Services, regardless of the level of development of those services. The activities will address areas such as improving utilisation of the WOAH PVS Pathway, increasing transparency in disease reporting, supporting professionals, and enhancing disease management in small-scale aquaculture.
- **Objective 3 – RESILIENCE:** these activities will support Members on early detection and rapid response to disease outbreaks of regional or global concern, and prevention of transboundary spread of disease. Antimicrobial resistance and insufficient access to appropriate and effective veterinary medicinal products is also addressed under this objective.

- **Objective 4 – LEADERSHIP:** through this objective, the WOAH will continue to build its capacity to lead efforts to meet these challenges into the future. Under this objective, five areas will be targeted to strengthen the WOAH's capability to provide this leadership. The focus will be to further develop international partnerships and engagements of scientific networks.

The strategy has short medium and long term actions. WOAH HQ will oversee the implementation of the Aquatic Strategy, monitor progress and communicate achievements to the WOAH Community. An implementation plan has been developed to identify priorities and resource requirements, define timelines and assess possible obstacles to the plan's successful implementation. This will ensure that the Strategy remains relevant and that its identified priorities are being realised.

Some of the activities that have already been delivered or are underway include updating of the Aquatic Code and Aquatic Manual by the WOAH Aquatic Animal Health Standards, which is under the Activity 1.1 of the strategy. This ensures the development of new standards for both the Aquatic Code and Aquatic Manual for adoption by the World Assembly of Delegates. The new standards are prioritised and included in the Aquatic Animals Commission workplan. One new chapter (Infection with decapod iridescent virus 1) has already been finalised and five others are in progress including new chapters on Tilapia lake virus, ornamental aquatic animals, trade in genetic materials, disease outbreak management, and emergency disease preparedness.

Most of the other activities being implemented are broken up into a series of sub-activities with some taking a number of years to finalise. Activities are prioritised each year and will be implemented over the next three years. The priority for the next 12 months are:

- Training and support for WOAH delegates and Focal Points
- Emerging disease response coordination
- Development of a disease identification guide for mobile devices
- Identifying barriers to the implementation of standards.

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To address activities 1.5, (Identify barriers to the implementation of standards), 2.2 (Increase the use of the WOAH PVS Pathway) and 2.4 (Identify barriers to transparency in disease reporting) WOAH headquarters conducted a survey of aquatic animal focal points in April of this year. Preliminary analysis of the survey results has been conducted and a report is currently being prepared which will identify priority areas for improvement. Some of the areas covered in the report include: 1) Disease surveillance and national reporting; 2) Reporting to WOAH; 3) Implementation of Chapter 4.1 on biosecurity adopted in 2021; and, 4) Findings regarding capacity building.

Through the implantation of 23 activities the strategy has three main outcomes on national, regional and global level. Competent Authorities have improved aquatic animal health management in place, supporting increased aquatic animal production and reduced disease risk. Regions are supported to collaborate on aquatic animal health issues of common concern, improving the overall health, productivity and resilience of the region. WOAH provides global leadership and in partnership with the WOAH Community, builds a stronger and more resilient global aquatic animal health system.

Discussion

- On the implementation of PVS, around 20 members has already undergone aquatic PVS while more than 150 in terrestrial PVS, representing a very big gap. One of the activities in the strategy is aimed at increasing the uptake of PVS in the aquatic space. In the survey that WOAH conducted among member countries, there is quite a high number of countries which are planning to request a PVS mission in the next five years. However, not all of these will eventually translate into actual missions.
- Another issue addressed on the survey on PVS are what other barriers are preventing members from requesting a mission. Currently, results are being analysed to see where changes can be made to improve the process of application, thus, putting the tool in place to assist members based on the gaps recognized from the previous PVS missions. This area is still considered a high priority.
- The report of the WOAH survey will be published and made available publicly, and feedbacks will be sent to the countries involved in the survey. It is surely important when the people dedicate their time in completing the survey to see what actions has been taken.
- On the development of guidelines for collaborative emergency response, there is a similar activity being undertaken in the region (led by Ingo) under the WOAH-RRAP Regional Collaboration Framework, which can be included in the overall implementation of the WAOH Aquatic Strategy. This is a good example on how the AP region is progressing in such initiative.
- It is important that strategies across the region is coordinated effectively, as sometimes there is a disconnect between headquarters and regional offices of WOAH.
- On the several barriers identified in the survey, WOAH should not take full responsibility on these alone, but others (e.g. NACA, FAO, national governments, private sectors) can use these information to address the different issues of concern, in order to try to find the way out and to improve capacity for aquatic animal health management in the region.
- In terms of the lack of capacity, terrestrial animal health has always a higher profile and have more resources than aquatic animal health, but currently there is a bit of a shift at WOAH HQ, and the actions of the global community in providing resources and training especially in the region indeed made the difference.
- On the results of the survey, there is quite a high proportion of respondents that were delegated by the Focal Point, and the Observatory takes all these into account when analysing the survey results.
- India has been very transparent on disease reporting thru their national surveillance programme, example on IMNV way back in 2016 which resulted in some trade barriers. In reporting, the benefits that the member can get from reporting a disease should be emphasized, for example in preventing the spread and impact of a particular disease. The negative impacts of reporting to the country in terms of trade is one of the reasons why

people/countries are reluctant to report. Thus, there is a need for a strategy in publicizing the benefit of disease reporting to the country as well as to the global community, rather than just saying such has to be done.

- On the implementation of the new chapter on biosecurity, most countries said that they have implemented all the recommendations within the chapter. This, however, depends on interpretation as a country has many different industries, and will definitely have different meanings of full implantation vs. partial implementation or non-implementation.
- Biosecurity is not all or none, and that's how it was set-up in the new chapter which are based on the need of the business and on the risks that were identified. At the farm level, it should be relevant and cost effective, thus it could include both a quite modest or an extreme and detailed biosecurity measures.
- On training, the new surveillance chapter could be considered as a new subject as there is substantial changes in the approach which is really important especially with regard to every disease chapter.

Recommendations

- AG recommended to continue to encourage countries in the region to undertake PVS aquatic, by addressing the gaps and problems associated with the process of application which is somehow preventing member countries to initiate request to WOAH.
- AG recommended that member countries continue to support the implementation of WOAH Aquatic Strategy either through national initiative or through collaborative projects that are being implemented in the region (e.g. on emergency preparedness and response).
- AG recommended that different strategies on aquatic animal health management should be coordinated efficiently especially projects implemented by international and regional organizations like FAO, WOAH, SEAFDEC and NACA.
- AG recommended that the global community should continue to provide resources and training in order to strengthen and further promote the importance of aquatic animal health among the current and future activities of WOAH.
- AG recommended to continue to emphasize the benefits of disease reporting and transparency, especially in the prevention of spread of many transboundary aquatic animal diseases.

Session 5: Report on aquatic animal health activities of WOAH-RRAP

Dr. Hirofumi Kugita gave a presentation on the AAH activities of WOAH-RRAP including the Regional Collaboration Framework on Aquatic Animal Health. WOAH-RRAP staff on AAH (Dr. Jing Wang) left her post in June 2022, as such most of the activities of RRAP on AAH were halted. This year, Dr. Hnin Thidar Myint (former staff who worked on AAH) just joined the office and a new staff (Dr. Thitiwan) who will mainly handle AAH activities will join in December. With this, most of the activities on AAH in the region will resume.

The Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific, which was launched in 2017, is a mechanism to get strong support from regional laboratory experts as well as to coordinate aquatic animal health activities among partner organizations in the region. It was officially endorsed during the WOAH Regional Commission Meeting in 2019. Members of the Collaboration Framework include: WOAH Delegate; WOAH National Focal Point for Aquatic Animals; WOAH Collaborating Centres in the region; representatives from WOAH Aquatic Animal Health Standards Commission; and, regional partners (FAO, NACA, SEAFDEC). At present, the coordination among the partner organizations still needs to be strengthened. The Steering Committee usually meets yearly, but due to the current pandemic, no meeting was held in the past two years. The next meeting is scheduled next year (2023), wherein one of the agenda is the adoption of a new name for the Framework proposed to be AP-Net Aqua, which stands for **A**sia-**P**acific **Net**work on **Aqua**tic Animal Health.

Since its inception, the Framework has implemented several projects in the region and some of them has been completed including: Aquaculture biosecurity in small-scale farms (in collaboration with NACA); and, Evaluation of the causative agent of AHPND (in collaboration with Prof. Grace Lo of Chinese Taipei). On going project is on Regional collaboration to respond to emerging diseases of aquatic animals led by Dr. Ernst (Australia) and in collaboration with WOAH scientific network. Through the Framework, several meetings and workshops were also undertaken:

- Regional webinar on Infection with decapod iridescent virus 1 (September 2020)
- Webinar on responsible and prudent use of antimicrobials in aquatic animals (November 2020)
- WOAH virtual consultation meeting on AMU and AMR in aquaculture (June 2021)
- WOAH regional information session on PVS for aquatic (November 2021)
- PVS evaluation mission for aquatic in Indonesia (August 2022)

For 2023, there are several proposed activities and meetings including EHP epidemiology and surveillance, appropriateness of using whole genome sequencing (WGS), and comparison of WSD pen-site test. A regional training for national Focal Points for aquatic animals is planned to be held in June or July 2023 which will be hosted by the Republic of Korea. Other workshops planned are on biosecurity on small-scale farms, AHPND consultation meeting, preparedness and response for emerging aquatic animal diseases, and AMU/AMR in aquaculture.

On regional disease reporting, currently reports are being collected from member countries and publish it on both WAOH-RRAP and NACA websites. Low number of countries have been submitting report for the past years, and WOAH-RRAP and NACA has been encouraging member governments to submit disease reports. In consideration of the existing reporting system WAHIS, there is significant duplication between the regional animal disease reporting and the WAHIS reporting. In the past more than 10 years, it has been discussed on how to consolidate and streamline the regional system into the global WAHIS system. It is definitely not easy and requires funds, but currently it is under discussion with the IT department of WOAH HQ.

DISCUSSION

- The joining of Dr. Thitiwan Patanasatienkul later in the year (December) and Dr. Hnin Thidar Myint recently will definitely facilitate the resumption of important works of WOAH-RRAP on aquatic animal health in the region, in close coordination with WOAH Headquarters especially in the implementation of the WOAH Aquatic Animal Health Strategy in collaboration with different partners in the region.
- NACA also looks forward to work with the in-coming WOAH-RRAP staff (Dr. Thitiwan), as well as Dr. Hnin who has been involved in several aquatic animal health project implementation in the past.
- The Framework is considered as a gold standard and model especially for activities and strategies in establishing similar networks in other regions. This collaborative framework in the AP region is a valuable thing for other regions to look at and see how it is managed and show how people in the region can work together in a collaborative way.
- On integration of the QAAD and WOAH reporting (WAHIS), the WOAH IT Department will have a meeting in December to discuss the progress, and hoping that the integration can take place to minimize duplication in disease reporting in the AP region.
- For Indonesia, PVS aquatic was recently implemented for Indonesia in August 2023 (originally scheduled in July). Also, no disease report is still being submitted by Indonesia as the current officials still need some training in this regard.

RECOMMENDATIONS

- AG recommended that the collaborative framework should be continued and supported to improve the aquatic animal health networking in the region, and to make sure that all the activities are aligned and coordinated with the activities of the WOAH Headquarters, especially the Aquatic Animal Health Strategy.

SESSION 6: UPDATES ON PREVENTIVE AND CONTROL MEASURES ON IMPORTANT AQUATIC ANIMAL DISEASES IN P.R. CHINA

Dr. Chenxu Cai presented updates on preventive and control measures for important aquatic animal diseases in P.R. China. In general, disease prevention and control systems of aquatic animal disease were further improved in recent years. The agricultural and rural authorities under the State Council take charge of national animal disease prevention. China's aquatic animal disease prevention and control system include disease monitoring and early warning system, technical support system and professional advisory committee. The monitoring and early warning system is based on the national five-level aquatic technology extension (aquatic animal disease prevention and control) system. The technical support system mainly includes national and local aquatic-related research institutions technology system of fishery industry and universities. The professional advisory committee plays the role of providing decision-making consultation for the government to guide aquaculture disease prevention and control. Team building of aquatic animal disease prevention and control were continuously enhanced. By the end of 2021, 7765 fish veterinarians had been officially appointed.

A total of 5085 licensed Fish Veterinarians were certified. There were more than 14,000 rural fish veterinarians registered.

Laws and regulations related to aquatic animal health have been continuously improved. The country has established a core legal framework that includes the six laws along with many administrative regulations, departmental rules and supplementary laws, such as local regulations and normative documents.

Establishment of aquatic animal disease prevention and control system was further strengthened. To improve the hardware strength of the whole system, with step-wise implementation of Nationwide Capacity Building Plan on Improvement of Animal and Plant Protection (2017-2025), aquatic animal disease prevention and control system characterized by coordination and implementation has been progressively consolidated. Since 2014, Ministry of Agriculture and Rural Affairs of the People's Republic of China (MARA) has been carrying out Laboratory Proficiency Test (PT) programme for Aquatic Animal Diseases Prevention and Control System per year. In 2022, the programme included testing on 15 pathogens of aquatic animals. A total of 242 laboratories nationwide had participated in the PT, while 224 of these were assessed as acceptable. This programme strengthened the capability of diagnosis for major aquatic animal diseases.

The disease surveillance and early warning were carried out every year. MARA annually organized the National Aquatic Animal Disease Monitoring and Surveillance Programme. Surveillance is carried out through the 5 levels of Fisheries Technology Extension Stations. In 2022, 13 diseases are specifically monitored. Most provinces in China are covered in surveillance program. Major stock farms, hatchery farms, well-bred seed farms in national and provincial level are covered in surveillance program as monitoring sites. MARA annually organized Report of Aquatic Animal and Plant Diseases nationwide. More than 4,000 monitoring sites covering 2750,000 hectares of aquaculture area were set up in China with more than 3,000 frontline personnel participating. More than 140 early warning alerts against diseases and potential disasters were released through newspapers, magazines, networks and public platforms in WeChat. Besides, annual aquatic animal health in china and analysis of major aquatic animal diseases in China were compiled and published. These authoritative materials provide reference for decision-making of government departments.

MARA formally approved trial of Jiangsu province fry and fingerling quarantine in the place of origin in 2017. Carrying out the successful experience in Jiangsu, MARA expanded the trial locations year by year. In 2020, the fry and fingerling quarantine in the place of origin were expanded to nationwide. In the situation of COVID-19, we established the "national level - provincial level - prefecture level-county level–enterprise/personal" five level online training mechanism to realize resource share. We also made educational film to introduce the regulations and total process of the fry and fingerling quarantine. The film was popular with the students, and standardize the fry and fingerling quarantine.

The technical support and service capability for aquatic animal disease prevention and control was improved. The National Remote Aquatic Animal Disease Diagnosis Service Network has been operated for 10 years, which provided tools for self-service diagnosis and supporting diagnosis. Three platforms has been established, include the computer version, Wechat applet and mobile app. The network owned about 150 national and provincial experts, more than 60 self diagnosis species, knowledges about more than 180 common diseases. In the situation of COVID-19, we held the online National Aquaculture Disease Prevention and Control Expert Live Lecture. Meanwhile, we continued to organize the compilation of the Series Booklets of Aquatic Animal Epidemic Prevention and have completed the seventh volume now. In addition, we also organized and produced a series of videos on important aquatic animal diseases.

Discussion

- The annual fish disease report is published in Chinese and is available to the public.
- If a farm (under active and passive surveillance programme) is found positive to a disease, control measures are usually implemented by the CA including transportation restrictions, quarantine, and stock eradication in order to clear the infection in the farm, recover production operations, and keep basic biosecurity level in the farm.
- Active surveillance on important aquatic animal diseases is considered as a basic work, but more importantly, it can provide basic background of the farms especially hatchery farms of some major aquaculture species (e.g. rainbow trout, grass carp). This background can be used to formulate the next step or measures and continue to monitor disease status or undertake stock culling if needed.
- On early warning alerts, it is usually combined with active surveillance, and depends on the history of the region or some environmental factors. Based on the information collected, the CA will publish an early warning report on the website or some journals.

Session 7: Updates on Regional Disease Reporting and Disease List

Dr. Eduardo Leaño presented the status of aquatic animal disease reporting in the Asia-Pacific region. From January 2021, a new AAD reporting was implemented. All Members are requested to submit the monthly data as soon as available to WOAH-RRAP and NACA to ensure the timeliness of the disease information. The new AAD monthly reporting is a "rolling report" containing all the disease information from January of each year (in every report that is submitted). Updated reports are published in dedicated pages at both NACA (https://enaca.org/?id=8) and WOAH-RRAP (https://rr-asia.woah.org/en/projects/regional-aquatic-animal-disease-report-from-2021/). From the third quarter of 2021 to the second quarter of 2022, there has been further decrease in the percentage of countries that were submitting the report (compared to the same period of the previous year). Among the countries submitting the disease reports, it is worth to mention that only Chinese Taipei submits on monthly basis.

In lieu of the QAAD Reports, NACA has published quarterly news article on AAD reporting since October 2021. During the first and second quarters of 2022, reported diseases for finfish include Infection with *Aphanomyces invadans* (EUS; Australia, Bangladesh, Chinese Taipei and India), Infection with red seabream iridovirus (Chinese Taipei and India), Viral encephalopathy and retinopathy (Australia and Chinese Taipei), Grouper iridoviral disease (Chinese Taipei), Infection with carp edema virus (India), and Infection with tilapia lake virus (India and the Philippines). For crustaceans, reported diseases were Infection with white spot syndrome virus (Chinese Taipei, India, the Philippines and Thailand), Infection with yellowhead virus genotype 1 (Thailand), Infection with infectious hypodermal and haematopoietic necrosis virus (India and the Philippines), Acute hepatopancreatic necrosis disease (Chinese Taipei, the Philippines and Thailand), Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (Chinese Taipei, India, the Philippines and Thailand), and Infection with decapod iridescent virus 1 (Chinese Taipei).

For molluscs, Australia reported Infections with *Perkinsus olseni* and abalone herpesvirus, while India reported Infection with *P. olseni*. Lastly for amphibians, Australia reported Infection with *Batrachochytrium dendrobatidis* in several species of wild frogs.

Other reported diseases are:

Bangladesh:

- *Streptococcus agalactiae* (Tilapia and climbing perch)
- *Aeromonas* sp. (climbing perch and Shing catfish)

India

- Infection with Infectious spleen and kidney necrosis virus (angel fish)

Singapore:

- Infection with *Lates calcarifer birnavirus* (Asian seabass)

The low number of countries submitting the report is still a concern and it is advised that National Focal Points for Aquatics should take full responsibility in preparing the reports with proper coordination to their respective WOAH Delegate who will officially submit the report to WOAH. As highlighted during the AGM 19, member countries contribute to the control of transboundary diseases of aquatic animals by complying with their obligations to the WOAH to notify the occurrence of listed diseases and emerging diseases. Sharing of information (including disease occurrences) create awareness so that the industry and regulators can actively take the needed risk management measures including emergency preparedness and response. Disease reporting is also useful for countries are having negotiations with their trading partners/countries (e.g. export of shrimp products): importing countries usually check their disease reporting history with reference to WOAH six-monthly report and/or NACA-WOAH-FAO QAAD Reports. This transparency of disease information is very important for the country to build trust with their trading partners for export of their aquaculture products.

Discussion

- For diagnostic purposes, there are available laboratories in the region that provide positive samples or primers for important and emerging aquatic animal diseases. One of which is Biotec Thailand which provide them for free for as long as it will not be used for commercial purposes. There is also a list of laboratories providing free positive samples and primers for DIV 1 (list is available at WOAH-RRAP website through this link https://rr-asia.woah.org/wp-content/uploads/2020/08/div1-positive-control-information_combined.pdf).
- On the provision of positive controls, some laboratories are providing synthetic positive controls which do not necessarily help in the validation of diagnostic methods. Laboratory

networks and collaboration are, therefore, important to validate laboratory diagnostic methods especially for emerging diseases, as it is much better if such efforts is distributed to quite a range of laboratories rather than being done by a lone laboratory.

- One of the reasons why the number of countries submitting the quarterly disease report continue to decrease is not really a problem on the capacity of the country to undertake surveillance and diagnostics, but mainly due to the changing of guards or the responsible officer to do the task of reporting. There has been a lot of reorganization that has happened in many countries in the region, and in most cases, there is no smooth transition on handing over or properly endorsing the task of disease reporting to the incoming officer.
- Another possibility on the decrease in the number of reports is the "reporting fatigue" by some of the member countries, wherein they just get tired of undertaking their responsibility to submit quarterly reports to both the WAHIS and the former QAAD of WOAH and NACA.
- Australia has been supporting PT in the region for quite a while involving around 40 government laboratories in the participating countries and covers a panel of ten important fish and crustacean diseases. The phase 2 of the program has ended and funding is being sought out to run another phase, as PT is for a common good and very useful for a lot of laboratories which many showed improvements in their capacity and performance after each of the PT program.
- On capacity building, focus should also be given on human resources (expertise) on aquatic animal health management. In most countries in the region, experts on this area has been decreasing throughout the years. Government as well as the private sectors improve the activities, to strengthen works on aquatic animal health, as it takes time to train and to find people who have a passion to be an aquatic animal health expert.

RECOMMENDATIONS

- AG recommended that the issue on disease reporting be brought to the forthcoming WOAH meetings for Aquatic Focal Points, and discuss the problems and gaps on disease reporting and how to encourage member countries to resume submission of disease reports, which is one of their obligations as member of WOAH and as an appointed Aquatic Focal Point of their respective countries.
- AG recommended that the region should continue to bring to the table the current gaps and challenges for disease surveillance and reporting, including laboratory capacity on diagnostics, and other related resources.
- AG recommended that sponsors (like Australia) should continue proficiency testing programme especially for other methods other than molecular diagnostics, and to cover important and emerging pathogens of aquatic animals.
- AG recommended that brainstorming activities should be undertaken in order to address the problem on human resources capacity, i.e. to encourage more people to become passionately involved in the aquatic animal health world, and eventually become experts in the future.
- Based on the updates on WOAH AAHSC on the listing of TiLV in May 2022, AG recommended to include Infection with TiLV under WOAH-listed diseases for finfishes, commencing January 2023 reporting.

SESSION 8. OTHER MATTERS AND CLOSING

- Dr. Jie Huang presented a draft disease card on an emerging disease of freshwater prawn (*M. rosenbergii*), the Infection with infectious precocity virus (iIPV) (Annex D). It is also called iron-prawn or iron-shell syndrome due to its disease characteristic of having hard shell, and cause a significant stunting among infected prawns. With regard to economic impacts of the disease, recent reports from P.R. China showed that some hatchery farmers can effectively control the prevalence of the disease to a manageable level, thus production impacts is currently not significant. But if the disease occurs in the pond, it can cause a maximum of 50% production losses. The disease card will be circulated again to the AG members for comments, and assess whether it can be published and considered to be included in the regional aquatic animal disease list for reporting.
- Dr. Leaño thanked all the presenters and participants for their active participation in the meeting.
- The AGM 20 officially closed at 16:30 PM (BKK time), 18 November 2022.

ANNEX A

21ST MEETING OF ASIA REGIONAL ADVISORY GROUP ON AQUATIC ANIMAL HEALTH (AGM21) (VIRTUAL MEETING) 17-18 NOVEMBER 2022

PROVISIONAL AGENDA:

Day 1 (17 November; Thursday)

Welcome and Introduction (15 mins)

- Introduction (**Dr. Eduardo Leaño, NACA**)
- Welcome Remarks (**Dr. Jie Huang, DG NACA**)
- Self-introduction (**all participants**)
- Selection of Chairperson and Vice-Chairperson

Chairperson will take over in moderating the meeting

- Progress since AGM 20 (15 mins; **Dr. Eduardo Leaño, NACA**)
- Updates from WOAH Aquatic Animal Health Standards Commission (15 mins; **Dr. Ingo Ernst, AAHSC, WOAH**)
- Aquaculture Biosecurity (PMP/AB) and FAO's AAH Initiatives in the AP region (15 mins; **Dr. Andrea Dall'Occo, FAO**)
- The WOAH Aquatic Animal Health Strategy 2021-2025: Implementation in the AP region (15 mins; **Dr. Melanie Allan, WOAH-HQ**)
- Farm-level aquaculture biosecurity (15 mins; **Dr. Andy Shinn, INVE**)

Note: 15-20 minutes discussion and recommendations after each presentation (Country representatives are encouraged to participate actively in the discussion)

Day 2 (18 November; Friday)

- Welcome and recap of day 1 (5 mins; **NACA Secretariat**)
- Updates on aquaculture biosecurity assessment tool and possible collaboration in the region (15 mins; **Dr. Saraya Tavornpanich, NVI**)
- Updates on WOAH Regional Collaboration Framework on AAH in AP region (15 mins; **WOAH-RRAP**)
- Recent Innovations on Disease Prevention and Control in Aquatic Animals (15 mins; **Dr. Kjetil Fyrand, PHARMAQ/Zoetis**)
- Updates on preventive and control measures on important aquatic animal diseases in P.R. China (15 mins; **Dr. Dongyue Feng, NFTEC**)
- Updates on QAAD Reporting and disease list (10 mins; **Dr. Eduardo Leaño, NACA**)
- Other issues including emerging diseases in the region (if any) (10 mins)

Note: 15-20 minutes discussion and recommendations after each presentation (Country representatives are encouraged to participate actively in the discussion)

ANNEX B

List of Participants (AGM 21)

I. Advisory Group Members
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Annex C:
List of Diseases in the Asia-Pacific

Reportable Aquatic Animal Diseases
(Beginning January 2023)

1. DISEASES PREVALENT IN THE REGION	
1.1 FINFISH DISEASES	
WOAH-listed diseases	**Non WOAH-listed diseases**
1. Infection with epizootic haematopoietic necrosis virus	1.Grouper iridoviral disease
2. Infection with infectious haematopoietic necrosis virus	2.Viral encephalopathy and retinopathy
3. Infection with spring viraemia of carp virus	3.Enteric septicaemia of catfish
4. Infection with viral haemorrhagic septicaemia virus	4.Carp edema virus disease (CEVD)
5. Infection with *Aphanomyces invadans* (EUS))	
6. Infection with red seabream iridovirus	
7. Infection with koi herpesvirus	
8. Infection with tilapia lake virus	
1.2 MOLLUSC DISEASES	
WOAH-listed diseases	**Non WOAH-listed diseases**
1. Infection with *Bonamia exitiosa*	1. Infection with *Marteilioides chungmuensis*
2. Infection with *Perkinsus olseni*	2. Acute viral necrosis (in scallops)
3. Infection with abalone herpes-like virus	
4. Infection with *Xenohaliotis californiensis*	
5. Infection with *Bonamia ostreae*	
1.3 CRUSTACEAN DISEASES	
WOAH-listed diseases	**Non WOAH-listed diseases**
1. Infection with Taura syndrome virus (TSV)	1. Hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP)
2. Infection with White spot syndrome virus (WSSV)	
3. Infection with yellow head virus genotype 1	2. Viral covert mortality diseases (VCMD)
4. Infection with Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	3. *Spiroplasma eriocheiris* infection
5. Infection with Infectious myonecrosis virus (IMNV)	
6. Infection with *Macrobrachium rosenbergii* nodavirus (MrNV; White tail disease)	
7. Infection with *Hepatobacter penaei* (Necrotising hepatopancreatitis)	
8. Acute hepatopancreatic necrosis disease (AHPND)	
9. Infection with *Aphanomyces astaci* (Crayfsh plague)	
10. Infection with Decapod iridescent virus 1 (DIV1)	
1.4 AMPHIBIAN DISEASES	
WOAH-listed diseases	**Non WOAH-listed diseases**
1. Infection with *Ranavirus* species	
2. Infection with *Bachtracochytrium dendrobatidis*	
3. Infection with *Batrachochytrium salamandrivorans*	
2. DISEASES PRESUMED EXOTIC TO THE REGION	
2.1 Finfish	
WOAH-listed diseases	**Non WOAH-listed diseases**
1. Infection with HPR-deleted or HPR0 salmon anaemia virus	1. Channel catfish virus disease
2. Infection with salmon pancreas disease virus	
2. Infection with *Gyrodactylus salaris*	
2.2 Molluscs	
WOAH-listed diseases	**Non WOAH-listed diseases**
1. Infection with *Marteilia refringens*	
2. Infection with *Perkinsus marinus*	

Annex D:

ASIA REGIONAL TECHNICAL GUIDELINES – STATUS OVERVIEW (ADOPTED FROM AGM 9 REPORT)

Element of technical guidelines	Progress / status	Gaps / opportunities
1. Disease reporting *An understanding of the basic aquatic animal health situation is a pre-requisite for prioritising activities, developing national policy and identifying pathogens of national importance.*	• Regional QAAD reporting system established – participation has increased • The QAAD list has incorporated emerging diseases that were later listed by the WOAH • Many countries have established national lists for reporting purposes with appropriate supporting legislation	• Participation could improve further – some countries report irregularly • The proposed regional core utilising the WOAH's WAHID will streamline reporting and may improve participation • The exact status of individual countries with regard to adoption of national lists and supporting legislation is not know
2. Disease diagnosis *Diagnosis requires various levels of data, starting with farm- or site-level observations and progressing in technical complexity to electron microscopy, immunological and nucleic acid assays and other biomolecular methods. This means all levels of expertise, including that of the farmer and extension officer working at the pond side, make essential contributions to rapid and accurate disease diagnosis.* *Effective diagnostic capability underpins a range of programs including early detection for emergency response and substantiating disease status through surveillance and reporting.*	• Diagnostic capabilities have improved in many countries • NACA disease cards have been developed and maintained for emerging diseases • The Asia regional diagnostic manual has been developed • An Asia regional diagnostic field guide has been developed • WOAH reference laboratories • Regional reference laboratories – where no WOAH reference laboratory exists • Regional Resource Experts are available to provide specialist advice • Ad hoc laboratory proficiency testing programs have been run	• WOAH twinning programs are a means to assist laboratories to develop capabilities • The exact status of diagnostic capability in individual countries is not certain • There is limited or no access to ongoing laboratory proficiency testing programs • Some areas of specialist diagnostic expertise are lacking • Network approaches are a means draw on available diagnostic expertise
3. Health certification and Quarantine measures *The purpose of applying quarantine measures and health certification is to facilitate transboundary trade in aquatic*	• Strong progress has been made, particularly for high risk importations (e.g. importation of broodstock and seed stock) • Training has been provided through regional initiatives (e.g. AADCP project)	• The importance of supporting aquatic animal health attestations through sound aquatic animal health programs continues to be underestimated, with possible ramifications for trade

animals and their products, while minimising the risk of spreading infectious diseases	• Commercial implications for trade have driven improved certification practices • Harmonisation with WOAH model certificates has occurred	• Some inappropriate or illegal activities continue and threaten to spread trans-boundary diseases
4. Disease zoning and compartmentalisation *Zoning (and compartmentalization) allows for part of a nation's territory to be identified as free of a particular disease, rather than having to demonstrate that the entire country is free. This is particularly helpful to facilitate trade in circumstances where eradication of a disease is not feasible.Zoning is also an effective tool to restrict the spread of important pathogens and aid in their eradication.*	• Is an emerging need to meet requirements of importing countries • To facilitate trade, some countries are working toward having compartments and zones recognised	• Where common health status can be identified restrictions on trade can be reduced • Training opportunities would be beneficial • Learn from the experience of terrestrial animal industries (e.g. poultry)
5. Disease surveillance and reporting *Necessary to produce meaningful reports on a country's disease status by providing evidence to substantiate claims of absence of a particular disease and thereby support import risk analysis, justify import health certification requirements, and enable export health certification*	• Regional Resource Experts are available to provide specialist advice • Training has been provided through a number of initiatives (e.g. AADCP project) • Many published resources are available, including those of the WOAH (publications and the WOAH centre for aquatic animal epidemiology) • Collation of surveillance information has improved through participation in international reporting	• Remains a reliance on passive surveillance. Active surveillance may be beneficial but cost is often a barrier. • Methodologies to undertake effective but low-cost active surveillance would be of assistance • Epidemiological expertise is often limited • There is a need to increase surveillance of wildlife to support health status
6. Contingency planning *Important to provide a rapid and planned response for containment of a disease outbreak—thereby limiting the impact, scale and costs of the outbreak*	• Important provides a rapid and planned response for containment of a disease outbreak Some countries have advanced contingency planning with appropriate supporting legislation • Some countries have tested contingency plans through simulation exercises • Resources are available (e.g. Australia's AQUAVETPLAN,	• The exact status of contingency planning in individual countries is not certain • Training in emergency management frameworks may be useful • Support for developing contingency plans might usefully be directed at particular disease threats e.g. IMN

	FAO guidelines, WOAH links to resources)	
7. Import risk analysis *The movement of live aquatic animals involves a degree of disease risk to the importing country. Import risk analysis (IRA) is the process by which hazards associated with the movement of a particular commodity are identified and mitigative options are assessed. The results of these analyses are communicated to the authorities responsible for approving or rejecting the import.*	• Numerous resources and case studies published • The approach has been applied, particularly for some circumstances e.g. import of live *P. vannamei* • However risk analysis is not always applied, or is not applied appropriately • Regional training has been provided (e.g. AADCP project)	• There is a need to build awareness of the concepts • Training can be abstract and disengaging - should aim at trainees learning on scenarios relevant to their circumstances • This is a high priority generic need that is suited to development of a central training program
8. National strategies *The implementation of these Technical Guidelines in an effective manner requires an appropriate national administrative and legal framework, as well as sufficient expertise, manpower and infrastructure.*	• Many countries have developed national strategies • Detailed assistance has been provided to some countries (e.g. AADCP project)	• The exact status of national strategies in individual countries is not certain • The WOAH's PVS tool provides a means of assessing the progress of individual countries

Annex E: Proposed Disease Card

Diseases of Crustaceans – **Infection with Infectious Precocity Virus (iIPV)**

Infection with infectious precocity virus (iIPV) is an emerging disease in farmed giant freshwater prawn *Macrobrachium rosenbergii*, causing stunted growth due to precocity. The disease is also called iron prawn syndrome (IPS) due to the stunted prawns having hard shells (Dong et al., 2021). This disease card provides the disease information, diagnosis methods, listed experts, and reference.

Disease Agent

Infectious precocity virus (IPV) has a single-stranded positive-stranded RNA genome of about 12, 630 nt, including a poly(A) tail at the 3' end. Phylogenetic analysis of the conserved RNA-dependent RNA polymerase (RdRp) and NS3 domains showed that IPV belongs to a new genus between Jingmenvirus and *Flavivirus* in *Flaviviridae* (Dong et al., 2021).

Target Tissues

Histopathology and *in situ* hybridization showed that iIPV has significant nerve tropism (Dong et al., 2021; Wang et al., in review). The average relative abundance in the nerve relative tissues of prawns infected with IPV, including eyestalks, the brain, and the thoracic ganglion can reach about 70%. The hepatopancreas is an unsusceptible tissue of IPV with a relative abundance less than 0.01%. The average relative abundances of IPV in the muscle, stomach, and gonad are no more than 1%, respectively (Wang et al., in review). Most symptomatic prawns with typical IPS clinical signs had a high load of IPV above 3×10^5 copies/μg-RNA in eyestalk. Therefore, the test of IPV with molecular detection should use tissues containing eyestalks, brain, gills, thoracic ganglion, or pereiopods. The hepatopancreas, gonad, stomach, muscle, and compound eyes (due to pigment inhibiting amplification) are not suitable for sensitive diagnosis of iIPV. Moreover, the distal end of a pereiopod may be used as a live sampling method for IPV detection (Wang et al., in review).

Host Range

Currently known susceptible species of IPV include *M. rosenbergii* (Dong et al., 2021). IPV can be detected in *M. nipponense*, *Procambarus clarkii*, *Penaeus vannamei*, *P. monodon*, *Oratosquilla oratoria*, and *Anisops kuroiwae* (Zhao et al., 2023).

Presence in Asia-Pacific

iIPV was first reported in farmed *M. rosenbergii* in China (Dong et al., 2021). However, stunted pond-cultured *M. rosenbergii* with similar signs has been reported in other Asia-Pacific countries since 2007 (Paulraj et al., 2007).

Signs of Disease

Disease signs (Level I diagnosis) (Dong et al., 2021; Wang et al., in review)

The following disease signs can be used for presumptive diagnosis of the disease.

- Diseased *M. rosenbergii* is characterized by sexual precocity and precocity-associated growth retardation (Figure 1).
- Delayed moulting and hard shells.
- In the early stage of aquaculture, *M. rosenbergii* infected with IPV exhibit reduced feeding or no eating when the water temperature drops by a large range (such as a drop of 5 ℃).
- The affected female prawns show growth retardation and sexual precocity, i.e., carrying eggs on the abdomen or showing developed ovaries with a smaller body size.
- The affected male prawns show growth retardation and sexual precocity, i.e., having blue second pereiopods and dark body colour with a smaller body size.

- Contagious features show in the healthy population restocked to a pond with the diseased population.

Histo- and Cytopathological signs (Level II diagnoses) (Dong et al., 2021)

The following can be observed in infected prawns:

- Putative eosinophilic inclusions were observed in the perinuclear cytoplasm of some neurosecretory cells of the organ of Bellonci and the globular cells of the semi-ellipsoid in histopathological sections stained by H&E (Figure 2).
- Typical *Flavivirus*-like virions occur in cytoplasmic inclusions of infected shrimp eyestalk tissues under a transmission electron microscope. Virions of IPV exhibit as spherical with a diameter of 40 to 60 nm, with a higher electron-dense envelop (Figure 3).

Molecular Diagnostic Methods (Level III diagnoses)

Nested RT-PCR (Dong et al., 2021)

In the first step, the RT-PCR amplifies a 1038 bp amplicon. In the second step, a 395 bp amplicon is amplified. To visualize the amplicons, the RT-PCR products were analyzed in a 2% agarose gel containing GeneFinder. The extracted RNA was reverse-transcribed at 42°C for 45 min and 90°C for 5 min using a PrimeScript II first-strand cDNA synthesis kit (TaKaRa).

The first-step of the nested RT-PCR:

i) The cDNA obtained in the above steps is used as the template. The reaction system of the first-step of the nested RT-PCR is 20 µL, including: one microliter (1 µL) aliquot of cDNA template solution, 10 µL Premix *Taq* (with 0.5 U *Ex Taq*, 4 nmol dNTP, and 40 nmol $MgCl_2$) (TaKaRa), 2.5 pmol IPV-F1 (5'-GCA-CAC-TCC-CAA-CAC-GTT-TC-3'), 2.5 pmol IPV-R1 (5'-CGC-GCG-TAA-TCT-CTA-CAC-CT-3'), and nuclease-free water were added to 20 µL.

ii) The first step of the nested RT-PCR was performed at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 65 s, finally ending at 72°C for 10 min.

The second step of the nested RT-PCR:

iii) The reaction system of the second of the nested RT-PCR is 20 µL, including: one microliter (1 µL) aliquot of the 1st-step product for the template, 10 µL Premix *Taq* (with 0.5 U *Ex Taq*, 4 nmol dNTP, and 40 nmol $MgCl_2$) (TaKaRa), 2.5 pmol IPV-F1 (5'-TCC-CTA-GGC-AGG-GGA-TAC-TG-3'), 2.5 pmol IPV-R1 (5'-AGC-TAT-CCG-TGG-TGT-GGA-AC-3'), and nuclease-free water were added to 20 µL.

iv) The second step of the nested RT-PCR was performed at 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s, finally ending at 72°C for 10 min.

This diagnostic method did not cross-react to *Macrobrachium rosenbergii* nodavirus (MrNV), Tembusu virus (TMUV), yellow head virus genotype 8 (YHV-8), covert mortality nodavirus (CMNV), and the prawn RNA.

Nested RT-PCR (Zhao *et al.*, 2023)

In the first step, the RT-PCR amplifies a 754 bp amplicon. In the second step, a 395 bp amplicon is amplified. To visualize the amplicons, The PCR products were analyzed in 1 % agarose gel containing ethidium bromide. cDNA Synthesis SuperMix (AccurateBiology, China) was used to synthesize first-strand cDNA following the manufacturer's instructions.

The first-step of the nested RT-PCR:

i) The reaction system of the first step of the nested RT-PCR is 20 µL, including: 1 µL aliquot of cDNA template solution, 10 µL Premix *Taq* (with 0.5 U *Ex Taq*, 4 nmol dNTP, 40 nmol $MgCl_2$) (TaKaRa), 2.5 pmol IPV-F1 (5′- GCC-TCC-ACA-TCA-TTG-GCT-TCG-3′), 2.5 pmol IPV-R1 (5′-TCG-GGT-GTC-ATC-AAC-AAA-CTC-ATA-3′) and nuclease-free water were added to 20 µL.

ii) The first step of the nested RT-PCR was performed at 94 ℃ for 5 min, followed by 30 cycles of 94 ℃ for 30 s, 56 ℃ for 60 s, and 72 ℃ for 60 s, finally ending at 72 ℃ for 10 min.

The second step of the nested RT-PCR:

i) The reaction system of the second of the nested RT-PCR is 20 µL, including: one microliter (1 µL) aliquot of the 1st-step product for the template, 10 μL Premix *Taq* (with 0.5 U *Ex Taq*, 4 nmol dNTP, 40 nmol $MgCl_2$) (TaKaRa), 2.5 pmol IPV-F2 (5′-ACA-TCA-TTG-GCT-TCG-TAT-3′), 2.5 pmol IPV-R2 (5′-ACA-GAG-CAG-GAG-ATT-GGA-3) and nuclease-free water were added to 20 μL.

ii) The second step of the nested RT-PCR was performed with the following cycling parameters: initial denaturation at 94 ℃ for 5 min, followed by 30 cycles of 94 ℃ for 30 s, 56 ℃ for 60 s and 72 ℃ for 30 s, with a final extension at 72 ℃ for 10 min.

This diagnostic method did not cross-react to positive recombinant plasmid of infectious hypodermal and hematopoietic necrosis virus (IHHNV), white spot syndrome virus (WSSV), *Enterocytozoon hepatopenaei* (EHP).

Real-time quantitative RT-PCR (Wang et al., in review)

A TaqMan probe RT-qPCR detection technology for IPV was established using a pair of primers (IPV-F/IPV-R) targeting an amplicon of 139 bp in the IPV ORF1 gene.
The amplification can be carried out following the protocol:

i) The total volume of the one step TaqMan-RT-qPCR reaction system is 20 µL, including: 10 µL 2 × Luna Universal One-Step Reaction Mix (NEW ENGLAND BioLabs, USA), 1 µL 20 × Luna WarmStart® RT Enzyme Mix (NEW ENGLAND BioLabs, USA), 0.4 µM of IPV-F (5'-AGG-AGA-GGG-TTT-TGG-CTT-G-3'), 0.4 µM of IPV-R (5'-CTG-GAT-TGG-AAG-GGA-ACT-CTG-3'), 0.2 µM IPV-P (FAM-5'-CCG-CGA-CAC-TTA-CAA-CTG-CCC-TT-3'-TAMRA), 1 µL template RNA and 7 µL nuclease-free water.

ii) The amplification was performed at 55 ℃ for 10 min, 95°C for 1 min for initial denaturation, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s.

The detection limit of this method was as low as 1.0×10^0 copy/reaction. The TaqMan-RT-qPCR was about 13 and 1300 times more sensitive than Dong et al.'s (2021) nested RT-PCR and first-step RT-PCR. Compared with Dong et al.'s (2021) nested RT-PCR, diagnostic sensitivity (DSe) and diagnostic specificity (DSp) of TaqMan-RT-qPCR were 97.0 % and 86.1 %, respectively. In contrast, DSe and DSp of nested RT-PCR were 85.2 % and 97.2 %, respectively, compared with TaqMan-RT-qPCR. A majority of symptomatic prawns showing clinical signs of IPS had the IPV copy number of eyestalks higher than 3×10^5 copies/µg-RNA or Ct value lower than 20 (Wang et al., in review).

Real-time quantitative PCR (Zhao *et al.*, 2023)

A TaqMan probe RT-qPCR detection technology for IPV was established using a pair of primers (IPVq-F/IPVq-R). The sequences of primers are IPVq-F: 5′-GAA-GAT-GTC-ATC-GTC-CCA-GAG-TT-3′ and IPVq-R: 5′- GGA-ATG-CCC-CCT-CCG-TAT-3′. The sequence of the TaqMan probe is FAM-5'-CCC-CAA-GGT-TTT-ATT-G-3'-TAMRA.
The amplification can be carried out following the protocol:

i) Real-time PCR amplification was performed in a 16.5 μL reaction system consisting of 1.5 μL of cDNA sample and 15 μL reaction mixture containing 1 μL of each primer.

ii) The PCR profile was held at 95 ℃ for 1 min for initial denaturation, followed by 40 cycles of 95 ℃ for 15 s and 60 ℃ for 30 s. Fluorescence was collected at 60 ℃.

The limit of this method was as low as 10^1 copies. No validation information for DSe and DSp was available (Zhao *et al.*, 2023).

In situ hybridization (Dong et al., 2021)

Synthesis of RNA probe:

i) The 395 bp amplification product amplified by RT-PCR in the 2nd step was extracted and connected with PMD18-T vector (TaKaRa). The recombinant reaction was carried in a 10 μL mixture containing 5 μL Solution I, 4 μL PCR procedures and 1 μL PMD18-T vector. The recombinant plasmid was transformed into TOP10 competent *Escherichia coli* (TIANGEN). Select one clone from Luria Bertani (LB) agar supplemented with ampicillin (Amp) (Solarbio) for sequencing. Positive clone was selected to extract plasmid DNA.

ii) Using a set of primers F (5'-GTA-CCC-GGG-GAT-CCT-CTA-GAG-AT-3') and R (5'-<u>TAA-TAC-GAC-TCA-CTA-TAG-GG</u>T-TGC-ATG-CCT-GCA-GGT-CGA-CGA-T-3') possessed T7 transposon sequence (underlined) to amplify the template of the RNA probe and tail it. The reaction was synthesized with 10 μL Premix *Taq* (with 4 nmol deoxynucleoside triphosphate, 0.5 U *Ex Taq*, and 40 nmol Mg^{2+}) (TaKaRa), 1 μL DNA template and 10 pmol of each primer. The amplification procedure was performed with the following parameters: an initial denaturation at 95°C for 4 min, followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min.

iii) The digoxigenin-labelled antisense RNA probe performed in a 20 μL mixture containing 1 μg template, 2 μL dithiothreitol (100 mmol/L) (Promega), 4 μL transcription optimized 5 × buffer (200 mmol/L Tris-HCl, 30 mmol/L $MgCl_2$, 10 mmol/L spermidine, 50 mmol/L NaCl, pH 7.9) (Promega), 1 μL T7 RNA polymerase (20 U/μL) (Promega), 2 μL 10 × DIG RNA labelling mixture (Roche) and 1 μL RNase inhibitor (40 U/μL) (New

England Biolabs). The specific steps of transcription *in vitro* are as follows: incubating the mixture at 37°C for 2.5 h at first, placing it on ice for 2 min, and then digesting it with 5 U RNase-free DNase I (Thermo Fisher) at 37°C for 15 min. A SigmaSpin sequencing reaction clean-up, and postreaction clean-up columns kit (Sigma) were used to purify the probe. NanoDrop 2000 (Thermo Fisher) was used to detect the concentration and quality of the probe and then stored at -80°C.

***In situ* hybridization:**

i) The specific steps of *in situ* hybridization are as follows: dewaxing and rehydration at first, tissue sections were processed with HCl (0.2 mol/L, 20 min) and proteinase K (20 µg/mL, 30 min, 37°C) (TaKaRa). After washing with phosphate buffer containing Tween 20 (PBST), place the slides in the mixture of 500 µg/mL tRNA (Sigma), 50% formamide, 0.1% Tween 20 (Solarbio), 5 × saline citrate (SSC) (Solarbio), 1.9 g/L citric acid monohydrate and 50 µg/mL heparin sodium (Solarbio) for 4 h at 42°C to prehybridize.

ii) Hybridization was performed in the same solution mixed with 1 mg/mL DIG-labeled RNA probe at 42°C for 16 h. In order to detect probes hybridized with viral RNA, tissue sections were incubated with anti-DIG-AP Fab fragments (Roche) at 4℃ for 12 h, and then the hybridization was stained with BCIP (5-bromo-4-chloro-3-indolyl phosphate) and NBT (4-nitroblue tetrazolium chloride) (Roche). The slides were counterstained with Bismarck brown Y.

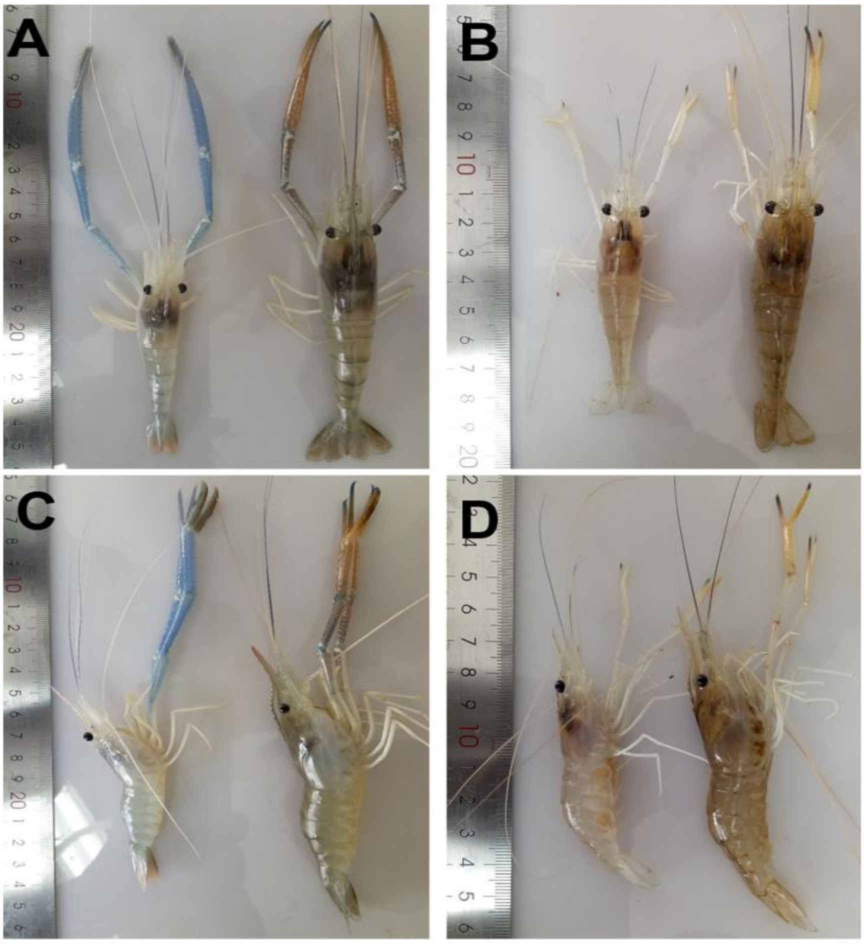

Figure 1. Gross signs of *M. rosenbergii* infected with IPV. (A and C, left) Infected male *M. rosenbergii*. (A and C, right) Control male. (B and D, left) Infected female *M. rosenbergii*. (B and D, right) Control female.

Source: (Dong et al., 2021)

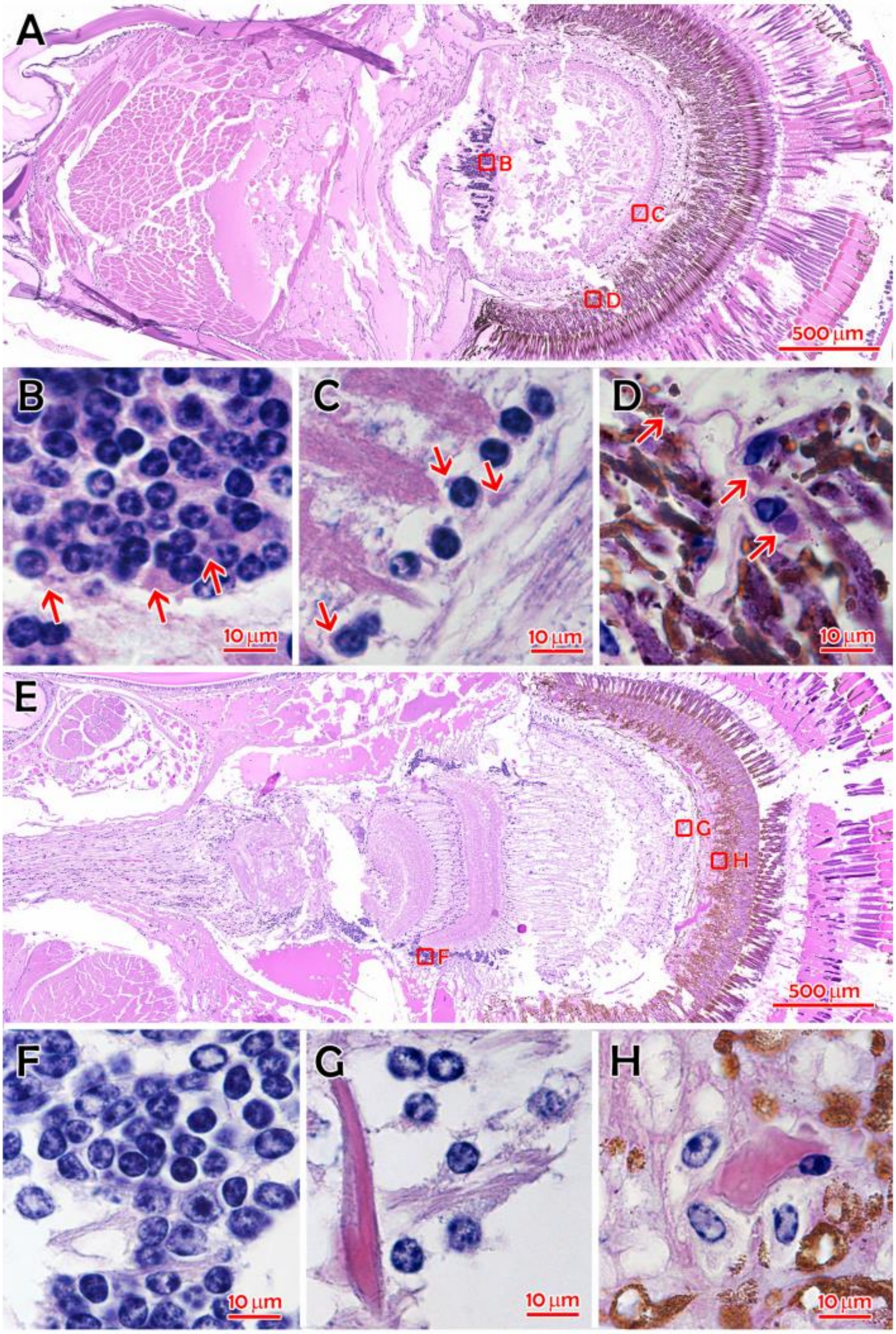

Figure 2. H&E-stained histological sections of *M. rosenbergii* tissues. (A) The overall view of a compound eye of *M. rosenbergii* infection with IPV. (B) Globuli cells in the hemiellipsoid body. (C) Cells in the lamina ganglionaris. (D) Cells in the fasciculated zone. (E) The overall view of a compound eye of healthy *M. rosenbergii*. (F) Globuli cells in the hemiellipsoid body. (G) Cells in the lamina ganglionaris. (H) Cells in the fasciculated zone. Red arrows indicate cytoplasmic inclusions. Bar in panels A and E, 500 µm; bar in panels B, C, D, F, G, and H, 10 µm.

Source: (Dong et al., 2021)

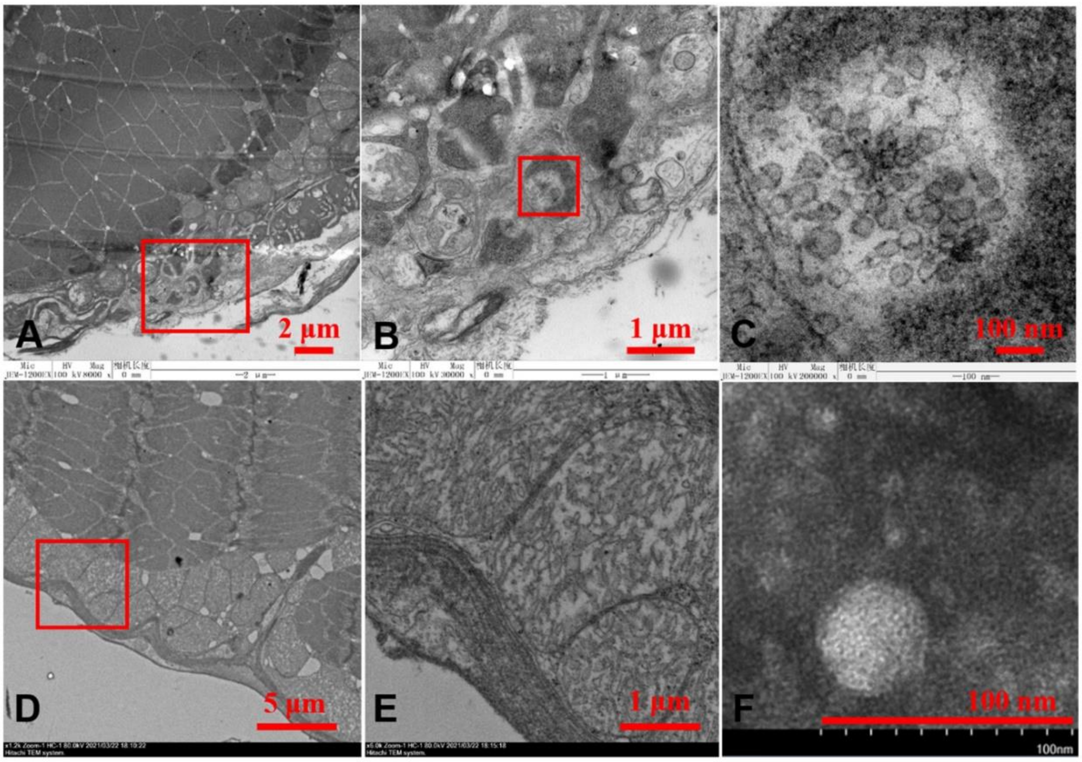

Figure 3. Transmission electron micrographs of the putative IPV particles. (A, B, and C) Spherical virions with a diameter of 40 to 60 nm in the eyestalk of *M. rosenbergii* infected with IPV. (D and E) Eyestalk from uninfected *M. rosenbergii*. (F) Purified putative IPV particles. Bar in panels A, 2 µm, Bar in panels B and E, 1 µm, bar in panels D, 5 µm, Bar in panels C and F, 100 nm.

Source: (Dong et al., 2021)

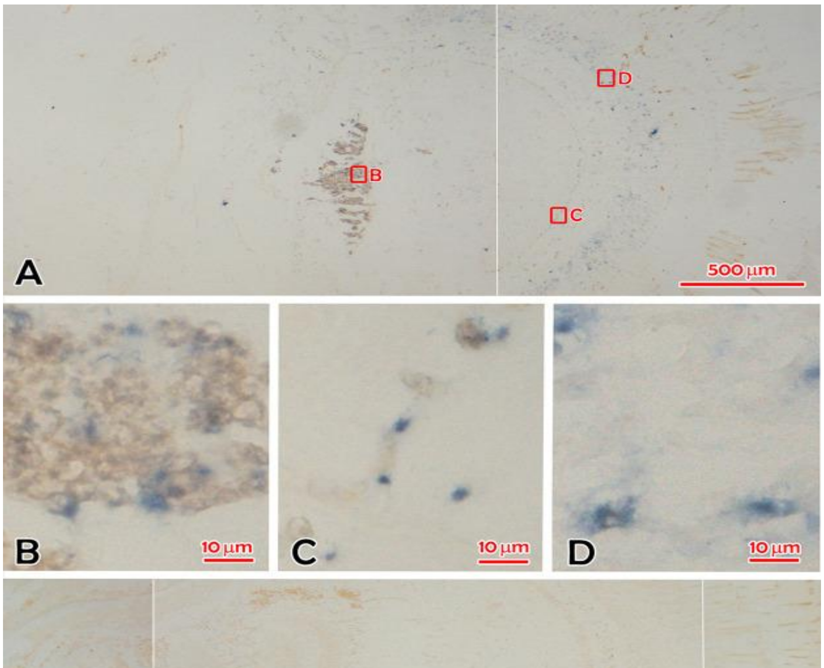

Figure 4. *In situ* hybridization (ISH) micrography of compound eyes from *M. rosenbergii*. (A) The overall view of a compound eye of *M. rosenbergii* infected with IPV. (B) Intracellular hybridization signals in globuli cells in the hemiellipsoid body. (C) Hybridization signals in cells of the lamina

ganglionaris. (D) Intracellular hybridization signals of the fasciculated zone. (E) General view hybrid section of the compound eye of healthy *M. rosenbergii*. Bar, 500 µm (A and E) and 10 µm (B, C, D).

Source: (Dong *et al.*, 2021)

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