Recent Developments in Disease Diagnosis and Management in Aquaculture



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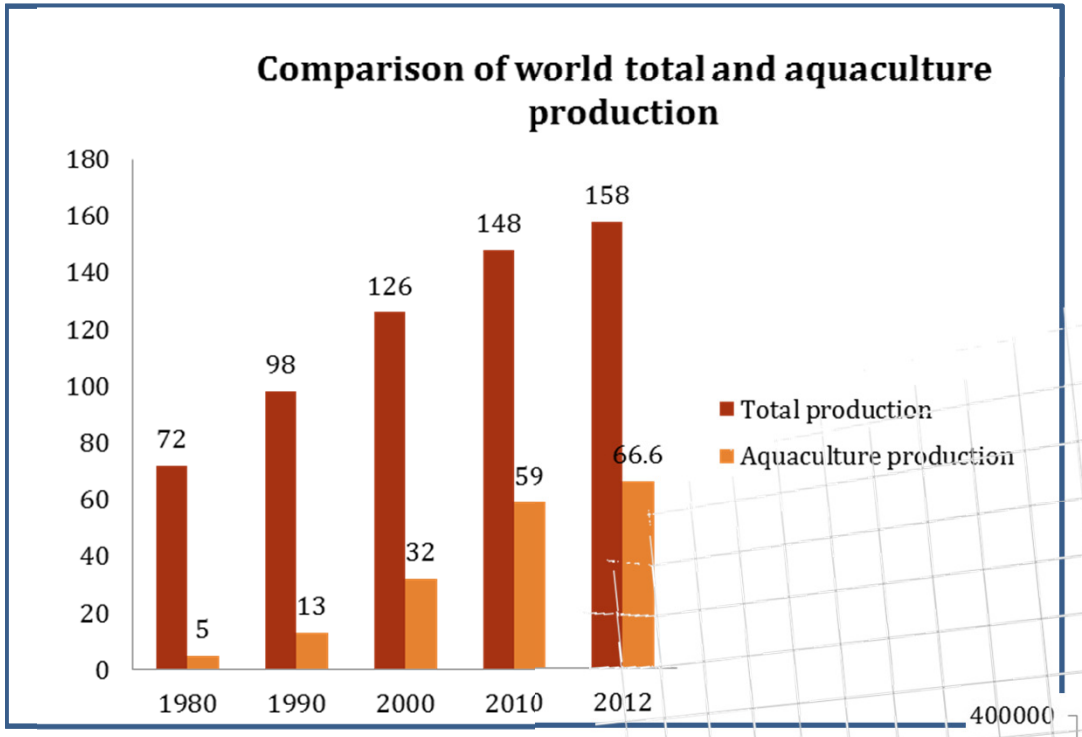
Introduction

- World food fish aquaculture production expanded at an average annual rate of 6.2 percent in the period 2000–2012 from 32.4 million to 66.6 million tonnes

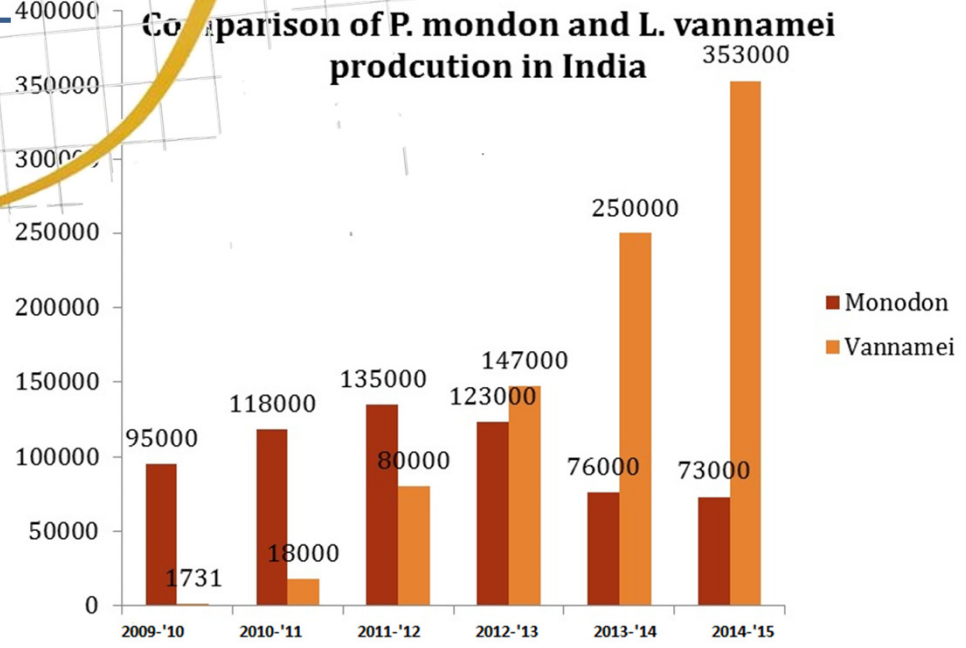
- World aquaculture production is dominated by the Asia- Pacific region which contributes 87% of the total production.

- Increase in aquaculture production has reduced the pressure on capture fisheries and overfishing

- Aquaculture contributes to increase in the foreign exchange revenues and also farmer's livelihood.



{ SOFIA, 2014 }



{ MPEDA, 2015 }

Contd.,

- Diversification, expansion and intensification of aquaculture practices has led to the emergence of many new pathogens.
- Disease has had a major impact on the aquaculture industries
- Since 1981, a succession of new viral pathogens has emerged in Asia causing mass mortalities and threatening the economic sustainability of the industry
- Many known Finfish virus and bacterial diseases are reported mainly from European aquaculture industries
- Shrimp viral and bacterial diseases dominates asia-pacific region

Emerging Diseases in Aquaculture

Finfish Diseases

- Pancreas disease
- Heart and skeletal muscle inflammation
- Infectious salmon anaemia
- Koi Herpes Virus
- BetanodaVirus

Shrimp Diseases

- Early Mortality Syndrome (EMS)
- Infectious Mionecrosis Virus (IMNV)
- YHV
- TSV
- Baculoviral midgut gland necrosis virus (BMNV)
- White gut and white faeces

Disease Outbreaks……. reasons

- The transboundary movement of live fishes and their products
- Intensive aquaculture practices
- Introduction of exotic species for aquaculture
- Expansion of trading of ornamental fishes
- Disease interaction and pathogen exchange in wild and farmed fishes
- Accidental escape of cultured fishes into the native environment and *vice versa*

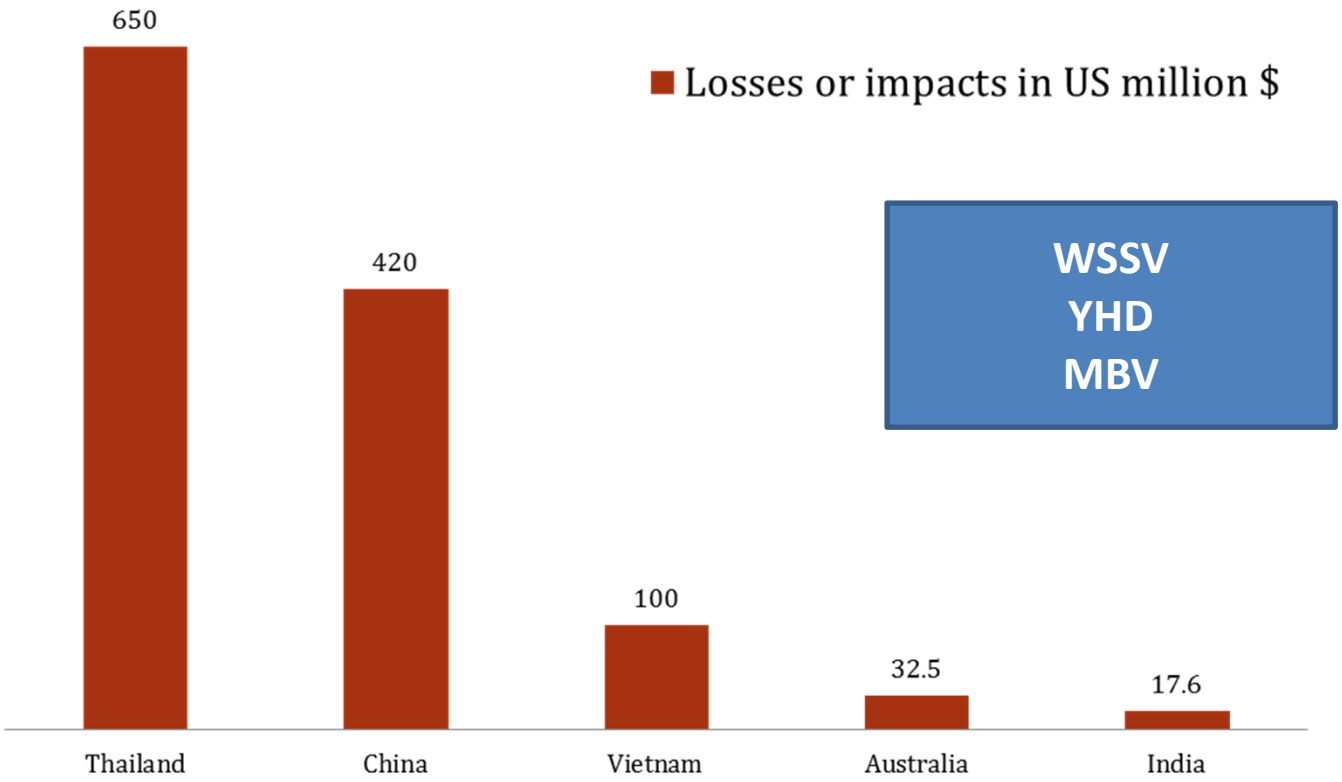
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- Not following recommended biosecurity practices for culture
- Not aware of emerging diseases in aquaculture
- Poor understanding and ill-use of specific pathogen free (SPF) animals
- Along with these global warming and climate changes

Importance of diseases

- Loss of fishes due to morbidity and mortality
- Increase in use of drugs for disease treatment
- Loss of valuable Ornamental fishes/breeding stocks
- Economic loss to the farmers
- Loss of farmers confidence in farming
- Spread of disease to natural environment and vice versa
- Impairs international trading of aquatic animals
- Indirectly affects the public health like antibiotic resistance

Loss due to shrimp diseases in 1992-94



M.G. Bondad et al. *2005*

Fishery Technology 50 (2013) 80 - 86

Economic Losses due to Disease Incidences in Shrimp Farms of India

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2006-08 in 1142 shrimp farms
Losses - 48717 metric t of shrimp valued at Rs. 1022.10 crores, and employment of 2.15 million man days

Surveillance of fish diseases in NORDIC countries (Northern Europe and the North Atlantic)
Viral haemorrhagic septicaemia virus (VHSV)
Infectious haematopoietic necrosis virus (IHNV)
infectious pancreatic necrosis virus (IPNV)
Viral Nervous Necrosis (VNN)
Spring Viraemia of carp (SVC)
Infectious salmon anaemia (ISA)
Bacterial kidney disease (BKD)
Furunculosis
Gyrodactylus salaris

National Surveillance Programme For Aquatic Animal Diseases

- **Funded by NFDB, Hyderabad, 2014**
- **Role of CIFE, Mumbai- Screening of the shrimps from different farms of Maharashtra and Gujarat for the presence of emerging as well as endemic viral diseases using PCR method**
    - **WSSV**
    - **MBV**
    - **Hepatopancreatic parvovirus (HPV)**
    - **Infectious mionecrosisi virus (IMNV)**
    - **Infectious Hypodermal and Haematopoietic Necrosis (IHHNV)**
    - **YHV**

- Accurate diagnostic methods play an inevitable role in health management
- Early detection and treatment of diseases are important to reduce their impact on aquaculture
- Disease diagnosis allow us to separate the infected stocks from uninfected ones
- Provide management options for control
- Efficient control and eradication of diseases
- To cope with the emergence of new diseases



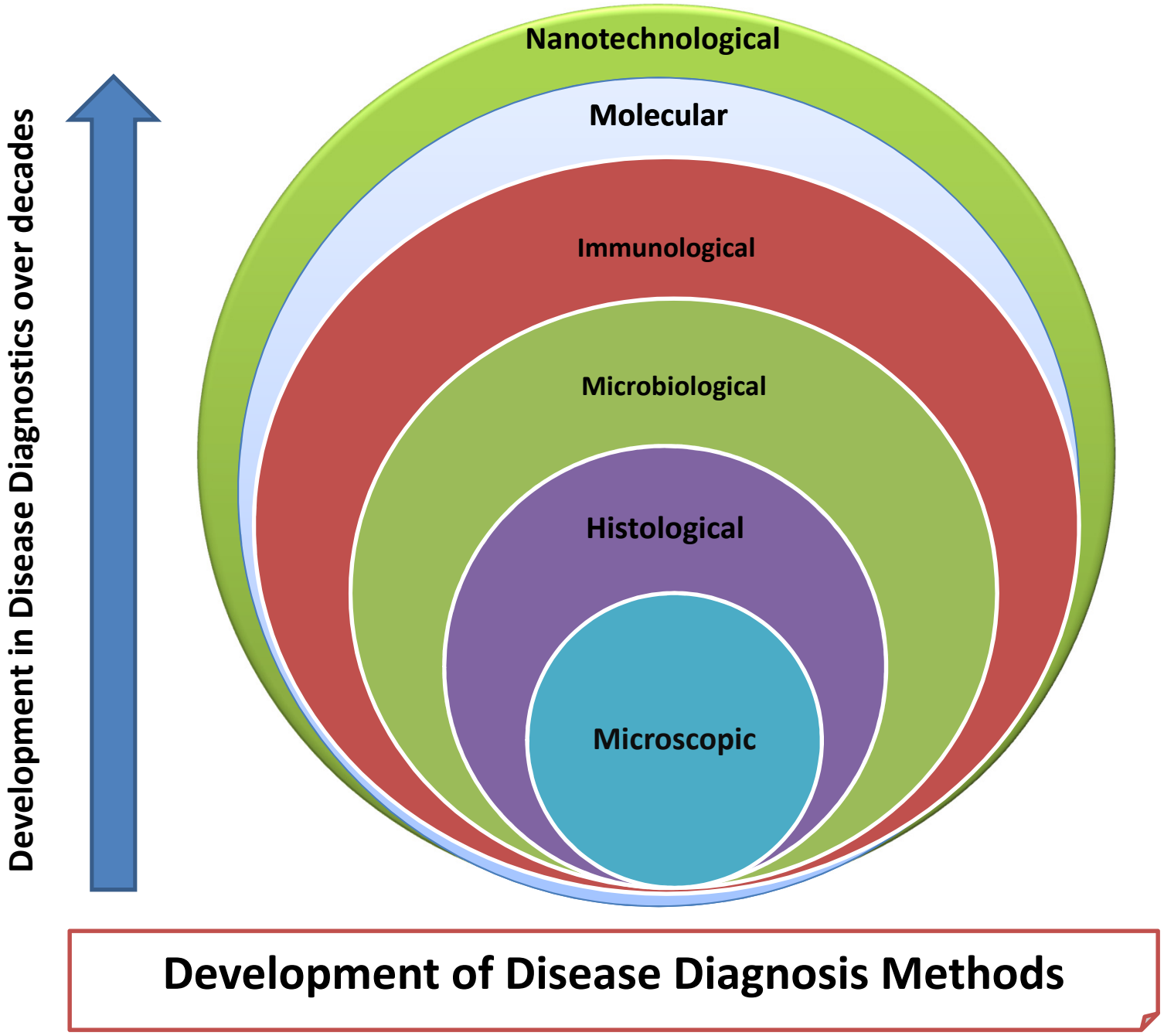
Disease diagnosis

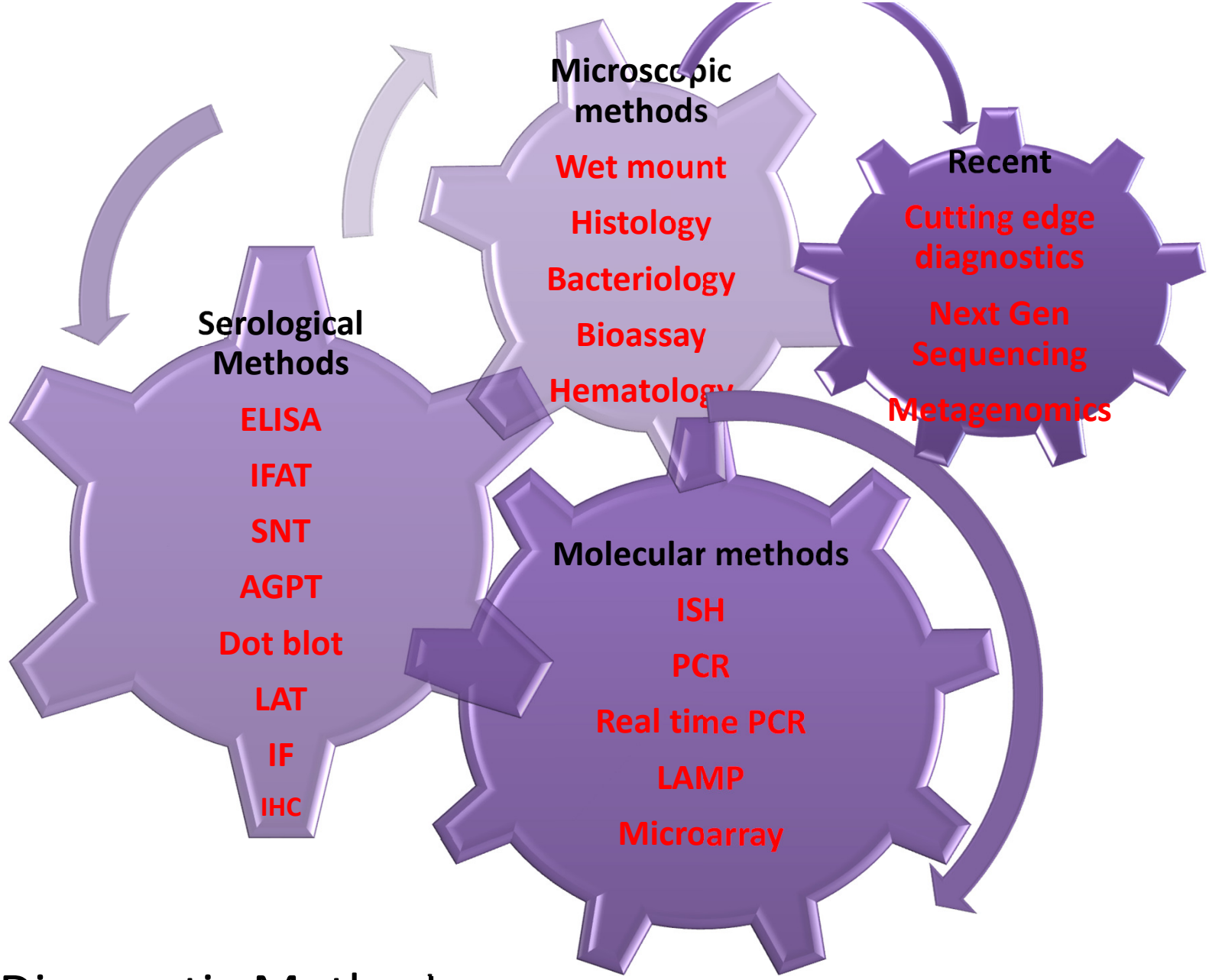
- The performance of a test is described by its precision (lack of random error, measured by the repeatability of a test) and validity (lack of systematic error)

- Validity is described by two measures
  - Sensitivity
  - Specificity

Gold standards

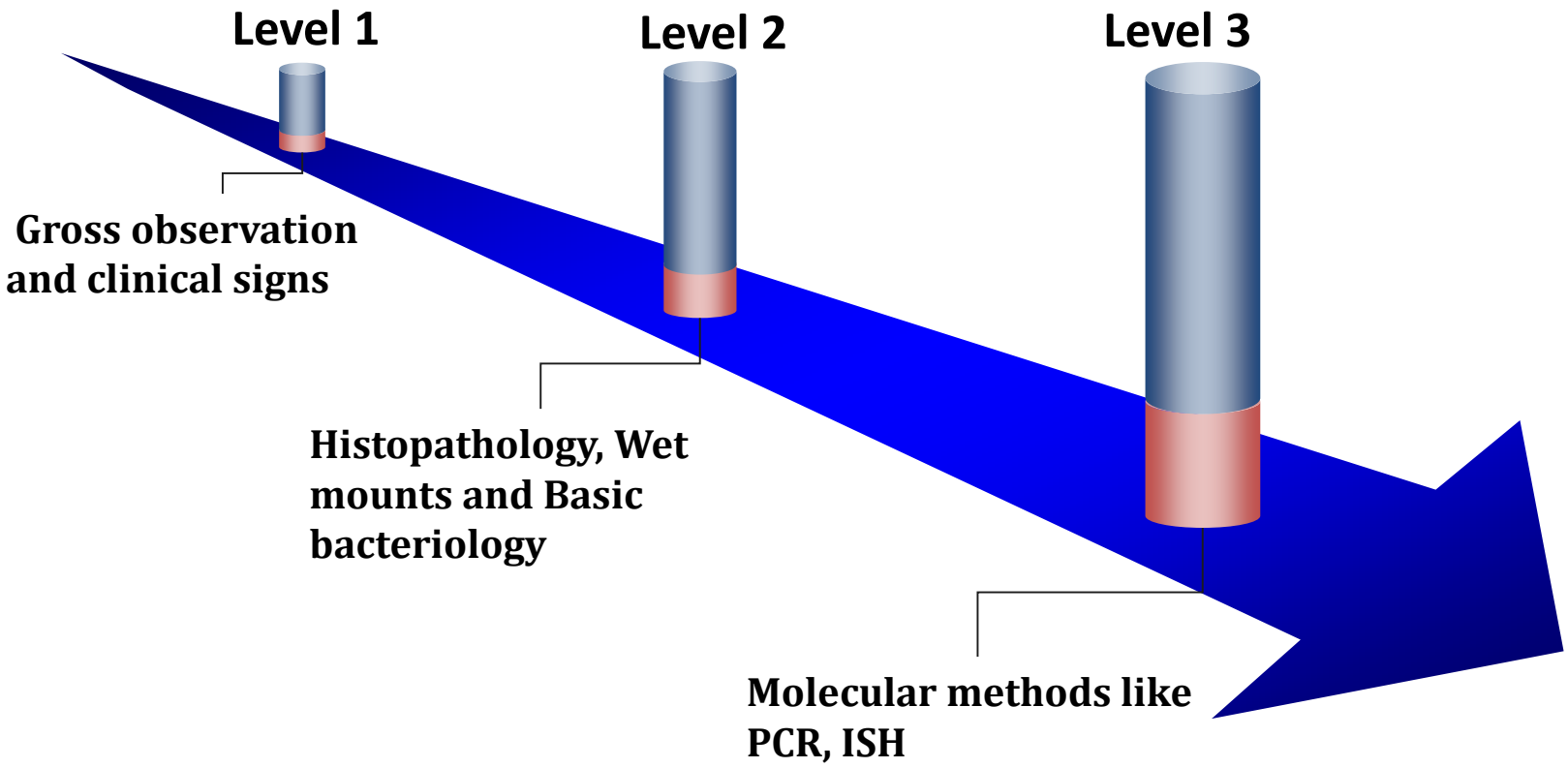
- Gold standard is used to describe the best test or combination of tests that is available for a particular disease.
- Evaluating the sensitivity and specificity using a gold standard is determining the relative sensitivity and specificity, compared with another test
- Gold standard method is used to evaluate new methods of diagnosing disease


Development in Disease Diagnostics over decades
Nanotechnological
Molecular
Immunological
Microbiological
Histological
Microscopic
Development of Disease Diagnosis Methods



Diagnostic Methods

Different levels in disease diagnosis



Contd.,

- Level 1- Mostly applied in hatcheries where examination of all the seeds are not possible
- Based on phototaxis or swimming patter the health status of seeds are checked
- Level 2 – used in decision making process in hatcheries. Basic bacteriological load and wet mount is checked for the presence of pathogens
- Level 3 – PCR screening of brooders for the presence of viral pathogens and seeds screening by the farmers before stocking

Classical methods

- **Case History, Gross Signs and Behavior – source of seeds, disease outbreak history and type of feed used, water source et.,**
- **Direct microscopy - Stained tissue smears, Whole mount or Wet mount of tissues examined in Phase or Bright field microscope for the presence of pathogens**
- **Histopathology – tissue sections are used for screening cellular changes due to pathogens**
- **Basic bacteriology/mycological methods – moribund animals are used for the isolation of bacteria or fungal agents.**

Contd.,

Bioassay

- **In bioassay methods, the animals are exposed to carriers of virus or other disease causing agents**

Hematology

- **Compared to animal and human science the use of haematology and clinical chemistry for fish disease diagnosis is uncommon**
- **The main problem - establishing reference range**
- **For blood parasite screening and stress enzymes status - haematology is used**

Serological Methods

Serological methods are the immunological methods that are based on the specific interactions of antigen-antibodies

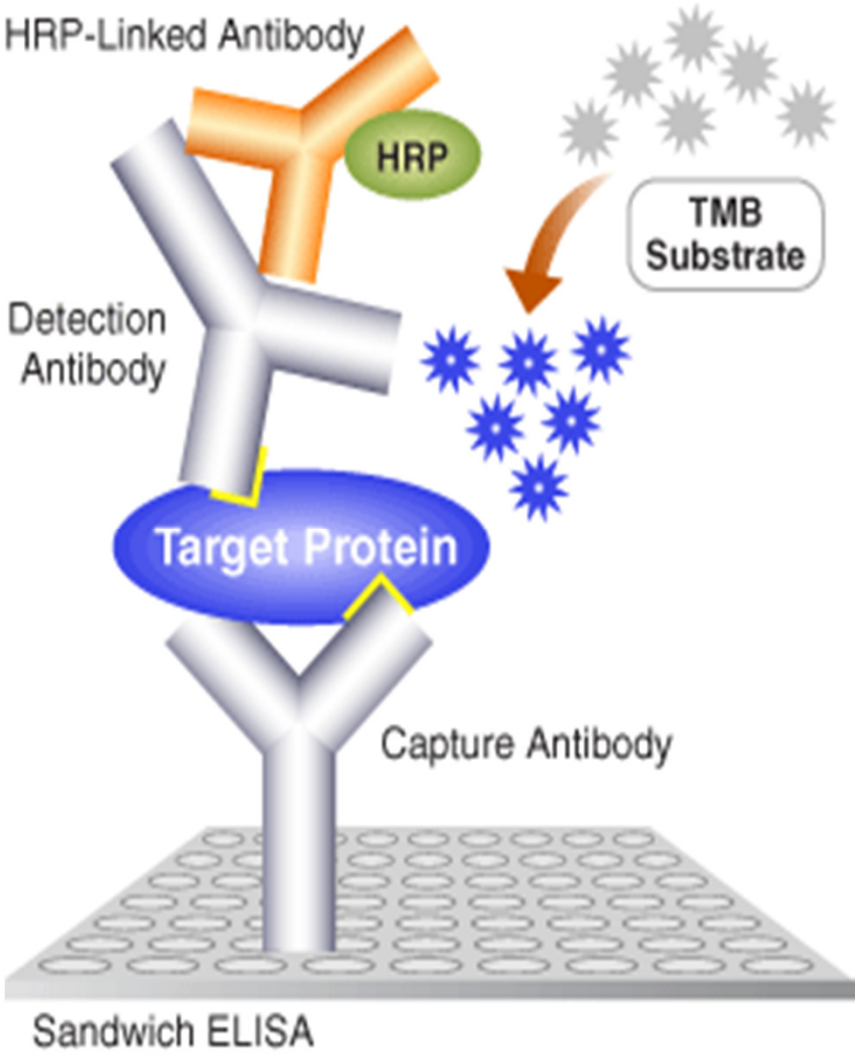
The basic principle of these tests are the detection of unknown agents (eg.antigen) by using known agent (eg. antibody)

Some of the commonly used serological methods are

1. Agar Gel Precipitation Test
2. Agglutination Test
3. ELISA
4. Dot blot
5. Latex Agglutination Test and
6. Fluorescent Antibody Test

Contd.,

- IF = immunofluorescence
- IHC - Immunohistochemistry
- FAT - fluorescent antibody test,
- ELISA
- Serum Neutralization test
- All the above methods are commonly used in fish viral disease diagnosis



Recombinant Technology

- Cloning of specific genes of pathogens in a suitable vector and subsequent expression of the protein
- These recombinant proteins can be used for diagnostic purpose without the need for pathogens
- Marker vaccine development
- Recombinant proeins used as antigens, immunogens or vaccines, immunomodulators, growth factors, hormones and enzymes.
- prokaryotic (bacteria cells), mammalian cells, yeast, insect cells and transgenic plants systems are used for expression

Surface Display technique

- Microbial cell surface displays are used in vaccine and antibody development

- Displays the functional proteins, such as cellulases or lipases, are genetically fused to an anchor protein and expressed on the cell surface

- *Saccharomyces cerevisiae*

- The surface display of haemolysin from *Vibrio harveyi* on the yeast cells and their potential applications as live vaccine in marine fish has already been demonstrated

In situ Hybridization

- Developed in early 1990s (For IHHNV diagnosis)
- Using gene probes
- Traditionally radioactive tags were used
- Non-radioactive tags like DIG-labeled gene probes developed later
- Commercial kits are available for IHHNV, HPV, BP, MBV and WSSV diagnosis

PCR

- Polymerase chain reaction is a DNA amplification method
- PCR is highly reliable methods for disease diagnosis due its high sensitivity and specificity
- PCR can detect up to 10 viral copies per reaction
- In India, PCR is the most common method for the disease screening of shrimps
- Nested PCR, Reverse transcriptase PCR

Real time PCR

- A robust and high throughput quantification technique which can detect even a single copy of genome

- Quantification of pathogen is based on the theory that there is a quantitative relationship between the input target DNA and the amount of PCR product generated at any given cycle number

- Detects the accumulation of amplicon at every cycle and the data are then measured at the exponential phase of the PCR.

- Advantages in detection and quantification of pathogens owing to its increased sensitivity, reproducibility and quantitative accuracy apart from the decreased hands-on-time

- less chances of contamination

Microarray

- Potential to diagnose clinical conditions by detecting gene expression patterns associated with disease states in either biopsy samples or peripheral blood cells

- The principle aim of using microarray technology as a gene expression profiling tool is to answer the fundamental questions of biology such as “when, where and to what magnitude genes of interest are expressed.

- Analyses the changes in the multigene patterns of expression

- Specific microbial gene amplification by either a broad-range or a multiplex PCR prior to microarray analysis enhances test sensitivity

- In addition to bacterial and myco-bacterial organisms, microarrays following broad-range PCR amplification have been used to detect and identify fungal, parasitic, and viral pathogens

Loop Mediated Isothermal Amplification (LAMP)

- LAMP amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions.
- The method employs a *Bst* polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA.
- Lamp can amplify a few copies of DNA to 10^9

Contd.,

•The detection limit (DL) using the LAMP method is up to 1 fg, when compared to 10 fg by nested polymerase chain reaction (PCR) (Kono *et al.*, 2004).

•Has an increased sensitivity due to the hybridisation of primers to 6 distinct regions.

•LAMP-LFD (LAMP- Lateral Flow Dipstick) is one of the modifications of the LAMP technique which involves the combination of LAMP and FITC nucleic-acid detection lateral flow dipstick.

•This technique has been proven to be efficient in detection of 10 copies of WSSV/µl.

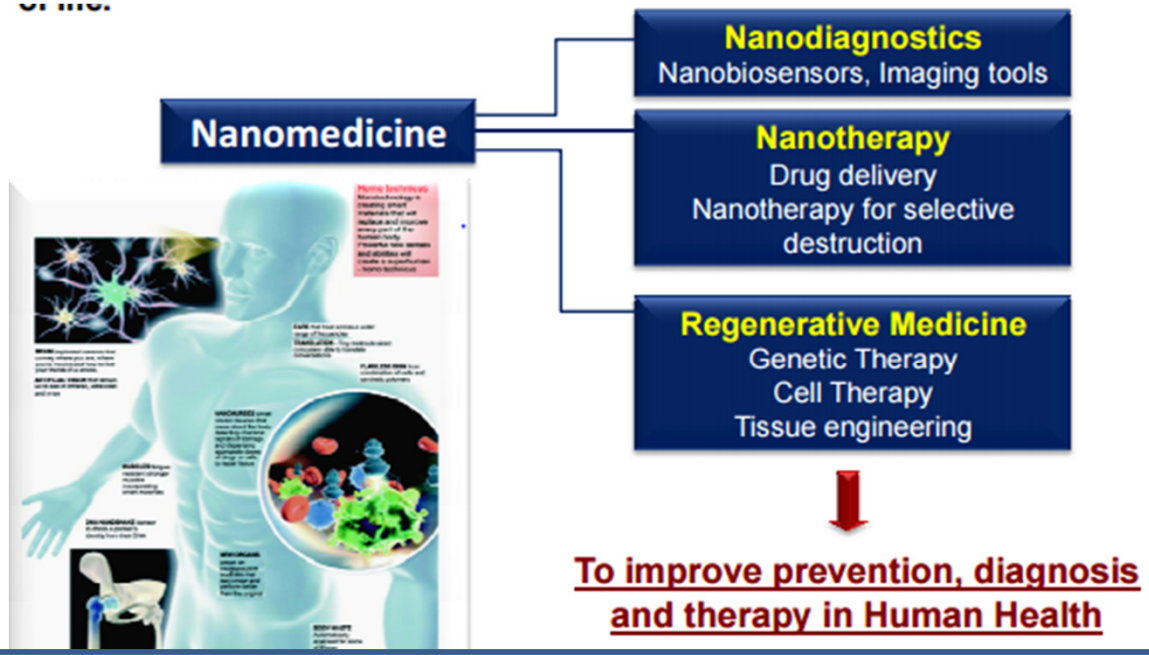
Cell Culture

- Cell lines inoculated with at least two serial tenfold dilutions of the samples
- The cell lines monitored by microscopy every day for the occurrence of CPE
- Mainly for viral disease diagnosis
- Not common due to the restricted availability of cell lines

Nano diagnostics

- Nano medicine uses nano-sized tools for the diagnosis, prevention and treatment of diseases and also to gain increased understanding of the complex underlying pathophysiology of the disease.

- In aquaculture, nanotechnology is emerging as inevitable in the field of diagnostics and therapy (drug delivery) and environmental management.



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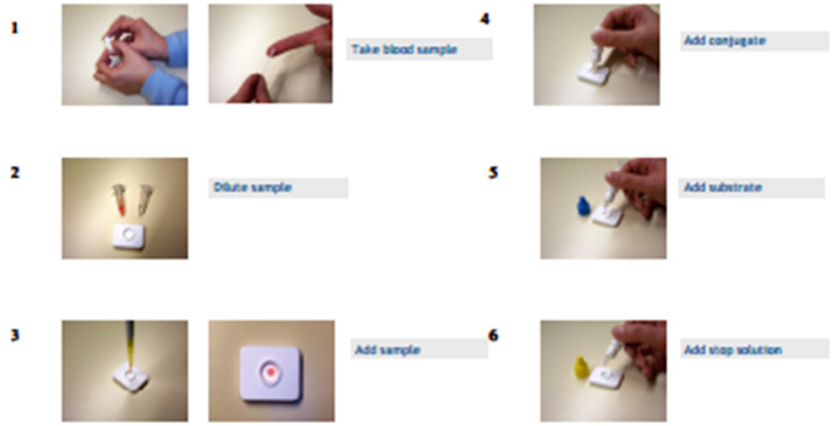
- Nano-based early detection of diseases and their markers if any - targeted therapeutic strategy development
- DNA-coated gold nanoparticles (NPs) i-PCR / Nano-i-PCR can be used for developing tests more sensitive than ELISA with 1-3 orders of higher sensitivity
- "Smart" dynamic nano platforms have the potential to change the way diseases are diagnosed, treated, and prevented
- A bio conjugated nanoparticle based bioassay for in situ pathogen quantification can detect a single bacterium within 20 min
- Early diagnosis of infectious agents

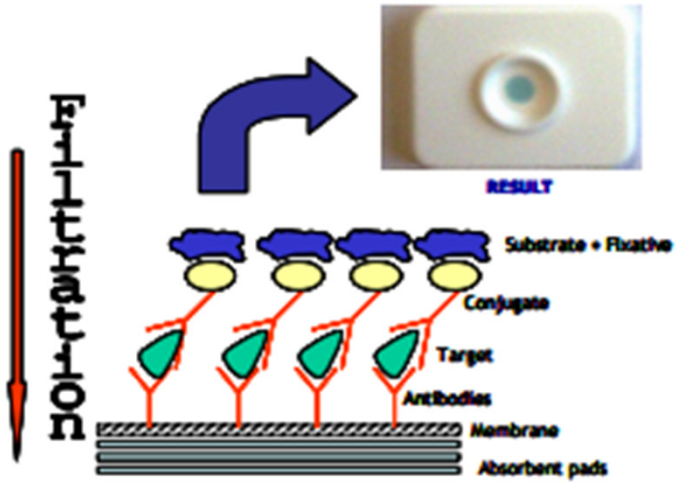
Onfarm diagnsotics

- Inexpensive, rapid, reliable and simple to use diagnostic methods with high specificity and sensitivity are required
- Distant location of the farms and rapid spoilage of aquatic animals, quick spread of disease in the system demands the need for OFD kits
- OFD also can be used for fish disease surveillance
- OFD helps in emergency decision making and health maintenance of cultured fishes

BLUSPOT diagnostic

- Sample flows through a membrane that has been coated with a specific MAb against the pathogen of interest by vertical filtration.
- This concentrates the pathogen on the membrane to improve sensitivity of the reaction.
- Antigen- antibody complexes detected by a secondary antibody labeled with an enzyme.
- The enzyme reaction with the substrate gives a colour in case of positive reaction
- The test is highly rapid, results are obtained in 5 min.





(Adams and Thompson,2011)

Cutting edge diagnostics

- Current methods are mostly adapted form Human and veterinary diagnostic methods
- NEXT GEN sequencing for pathogen identification
- Metagenomic approach for etiological agent identification
- Cefas (Centre for Environment, Fisheries and Aquaculture, UK) developed a new DNA-based diagnostic technology linked to data reporting by smartphone for WSV and other diseases
- mobile qRT-PCR platform (Smart Cycler) for salmonid disease detection

OPEN ACCESS Freely available online

PLOS one

Heart and Skeletal Muscle Inflammation of Farmed Salmon Is Associated with Infection with a Novel Reovirus

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Abstract

Atlantic salmon (*Salmo salar L.*) mariculture has been associated with epidemics of infectious diseases that threaten not only local production, but also wild fish coming into close proximity to marine pens and fish escaping from them. Heart and skeletal muscle inflammation (HSMI) is a frequently fatal disease of farmed Atlantic salmon. First recognized in one farm in Norway in 1999[1], HSMI was subsequently implicated in outbreaks in other farms in Norway and the United Kingdom[2]. Although pathology and disease transmission studies indicated an infectious basis, efforts to identify an agent were unsuccessful. Here we provide evidence that HSMI is associated with infection with piscine reovirus (PRV). PRV is a novel reovirus identified by unbiased high throughput DNA sequencing and a bioinformatics program focused on nucleotide

Inter-Research
Diseases of Aquatic Organisms

You are at: Inter-Research > DAO > v105 > n3 > p237-242

DAO 105:237-242 (2013) - doi:10.3354/dao02628

Metagenomic identification of a nodavirus and a circular ssDNA virus in semi-purified viral nucleic acids from the hepatopancreas of healthy *Farfantepenaeus duorarum* shrimp

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ABSTRACT: Fisheries and aquaculture are impacted sporadically by newly emerged viral diseases. In most cases, searches for a causative pathogen only occur after a serious disease has emerged. As random shotgun sequencing (metagenomics) offers opportunities to identify novel viruses preemptively, the method was tested on nucleic acids extracted from the hepatopancreas of 12 healthy northern pink shrimp *Farfantepenaeus duorarum* captured from the Gulf of Mexico. Among the sequences, a nodavirus (*Farfantepenaeus duorarum* nodavirus, *Fd*NV) and a virus with similarities to circoviruses and cycloviruses that possess circular single-stranded DNA (ssDNA) genomes, were identified. The *Fd*NV genome sequence was most closely related phylogenetically to nodaviruses causing white tail disease in *Macrobrachium*

Disease diagnosis service providers

- Private laboratories plays a mojor role in shrimp disease diagnostis
- Institutional laboratories also offers disease diagnostic services to the farmers

Laboratory proficiency testing

- Proficiency testing is important to test and improve the diagnostic capability of aquatic animal disease laboratories
- Recently, NACA has taken the initiative to perform the LPT in Asia Pacific region including India
- Ring test – Inter laboratory calibration
- Allows to evaluate either the performance of testing laboratories, to characterize material or to validate a test method.

Contd.,

- **The purpose of the Ring Test**
  - ❖ to determine proficiency
  - ❖ to help improve performance
  - ❖ to determine if the test methods in use are reliable and reproducible.
  - ❖ to assure that the results provided are accurate, sensitive and specific.
- Laboratory proficiency testing by ring test is done routinely by Dr. Lightner's lab for shrimp diseases in the American region

Blue Book

- Compilation of minimal standards and methodologies determined to be most appropriate for detecting the presence of specific pathogens when carrying out health inspections on aquatic animals

- The Blue Book includes three sections: Diagnostic Methods, Procedures for Aquatic Animal Health Inspections, and Quality Assurance

OiE Reference Laboratories

1. White spot disease
2. Taura syndrome
3. Spherical baculovirosis (Penaeus *monodon type* baculovirus)
4. Infectious myonecrosis
5. Infectious hypodermal and haematopoietic necrosis

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WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

Yellow head disease

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White tail disease

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White spot disease

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Spring viraemia of carp

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Koi herpes virus disease

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The Aquatic Animals Health Standards Commission

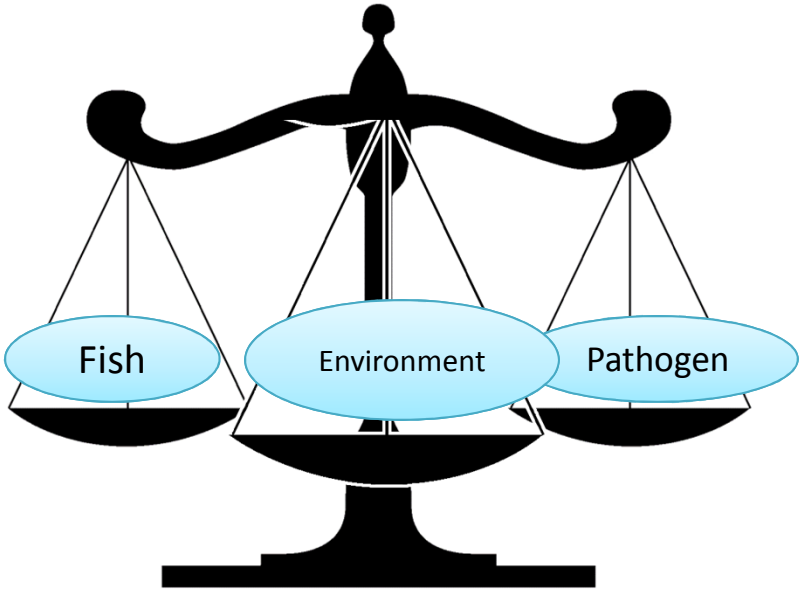
- The Aquatic Animals Health Standards Commission (the Aquatic Animals Commission) is responsible for ensuring that the *Aquatic Animal Health Code* (the *Aquatic Code*) and *Manual of Diagnostic Tests for Aquatic Animals* (the *Aquatic Manual*) reflect current scientific information

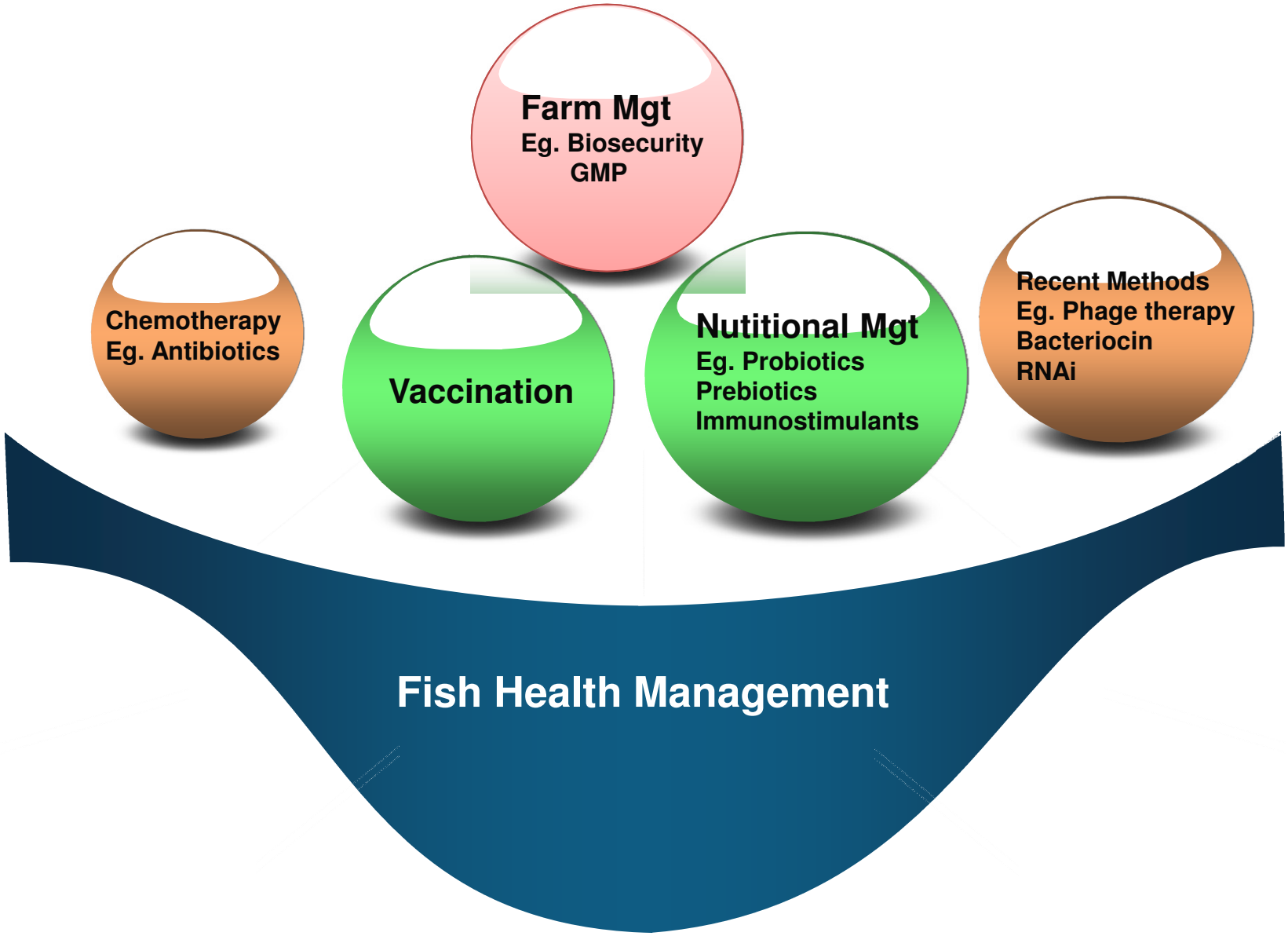
- Standards for the improvement of aquatic animal health worldwide, including standards for safe international trade in aquatic animals (amphibians, crustaceans, molluscs and fish) and their products

Health Management

- For good health management, the fish, pathogen and environment balance must all be taken into account

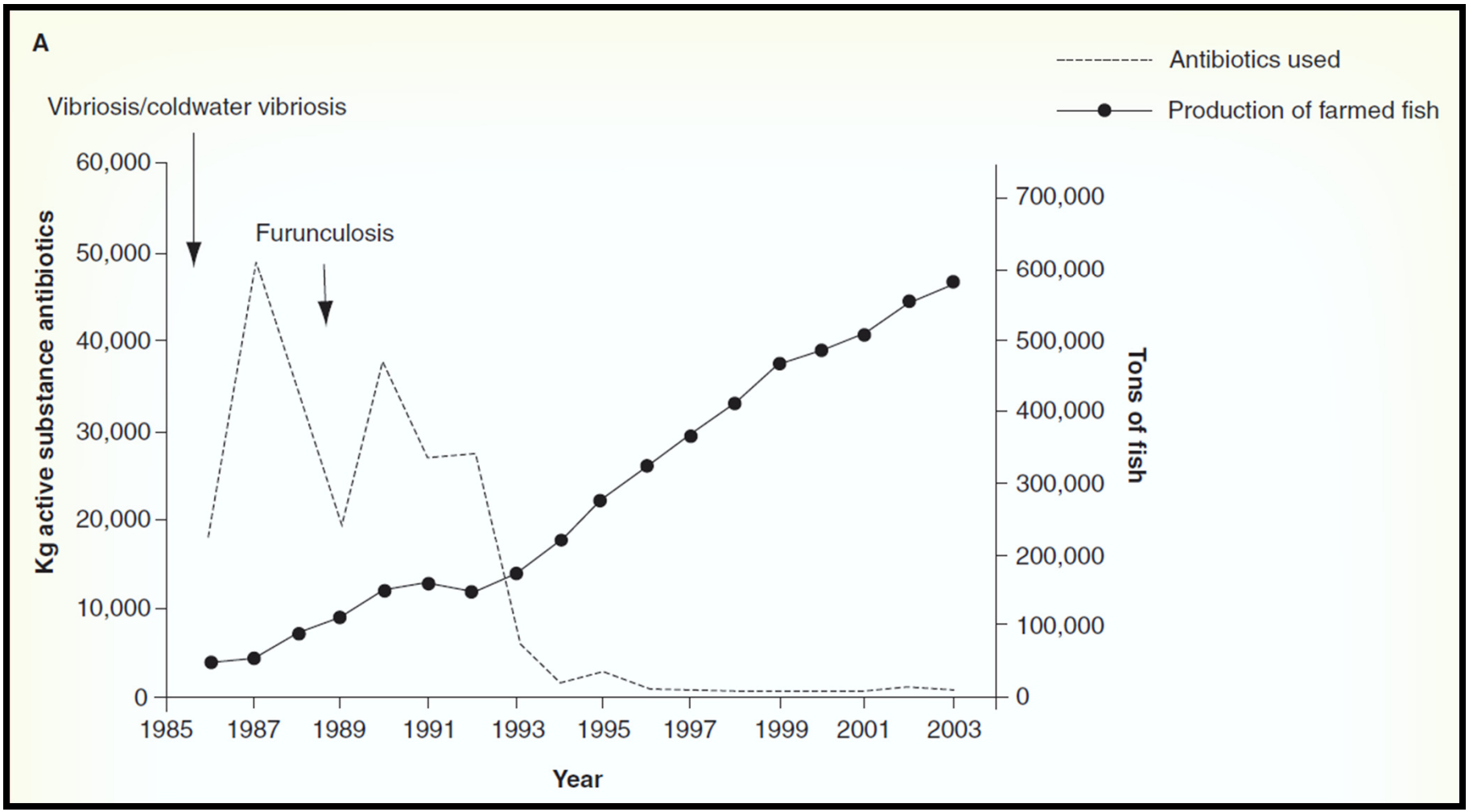
- Farmers need a holistic approach to health and should aim for high tolerant fish, optimum environmental conditions and minimal disease challenge




Farm Mgt
Eg. Biosecurity
GMP
Chemotherapy
Eg. Antibiotics
Vaccination
Nutitional Mgt
Eg. Probiotics
Prebiotics
Immunostimulants
Recent Methods
Eg. Phage therapy
Bacteriocin
RNAi
Fish Health Management

Vaccination

- Important antibiotics are becoming obsolete due to antibiotic resistance
- Vaccine play a major role in preventing the antibiotic abuse
- Worldwide, vaccines have been developed and commercially available to treat at least 18 bacterial infections and 8 viral infections



{ Sommerset *et al.*, 2005 }

Use of antibiotics and production of salmonid fish in Norway

Probitoics

- Probiotics are live micro-organisms that confer a health benefit to the host by providing both a nutritional benefit and protection against pathogens

- Working on a principle of competitive exclusion - contain the numbers of harmful bacteria - production of inhibitory compounds - competing for essential nutrients and / or by modulating the immune responses

- *Bacillus, Enterobacter, Leuconostoc meseneroides, Lactobacillus, Lactococcus, many yeast species* and *Streptococcus*

- Feed or water - but Feed supplementation more effective than direct addition to culture pond water

Prebiotics

- Prebiotics are nondigestible, introduced into food and help bacteria and beneficial microorganisms flourish

- Most common prebiotics used in fish are
  - inulin,
  - fructooligosaccharides (FOS),
  - short-chain fructooligosaccharides (scFOS),
  - oligofructose, mannanoligosaccharides (MOS),
  - *trans*-galactooligosaccharides (TOS),
  - galactooligosaccharides (GOS),
  - xylooligosaccharides (XOS),
  - Arabinoxylooligosaccharides (AXOS),
  - isomaltooligosaccharides (IMO)

- *Synbiotics* are nutritional supplements combining a mixture of probiotics and prebiotics in a form of synergism, which beneficially affect the host by improving the survival and implementation of live microbial dietary supplements in the GI tract of the host

Immunostimulants

- An immunostimulant is defined as a substance which enhances the innate or non-specific immune response by interacting directly with cells of the immune system and activating them.

- Immunostimulants can be grouped under different agents based on the source, such as bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines

- Beta-glucans, polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, Chitin and Chitosan, muramyl dipeptide, Vitamin C, E, Plant extracts, Cytokines- Leukotriene, Interferon and yeast derivatives

- There is no memory component developed and duration of the immune response is over a short period

Antibiotics

- Antibiotics are routinely applied in bath treatments or mixed with feed to prevent (prophylactic use) and treat (therapeutic use) bacterial infections.

- Oxytetracycline and chloramphenicol

- Teixobactin - A new class of antibiotic capable of, killing "without detectable resistance" discovered in 2015 (Ling., *et al*. 2015)

- Methods to boost the efficacy of antibiotics also explored like, using other antibiotics in conjunction; inhibiting resistance genes with molecules and using synergistic nonantibiotics

Phage therapy

- Phages are viruses that invade bacterial cells and disrupt their metabolism
- Two major infection cycles are the lytic (or virulent) and lysogenic cycles
- Lytic phages replicate inside the host cell and progeny viruses are released causing cell lysis. This capacity to destroy host bacteria sets the stage for the use of lytic phages as therapeutic or prophylactic agents.
- A method using naturally occurring lytic phages, or their products, as bioagents for the treatment of bacterial infectious diseases is called bacteriophage therapy or phage therapy

Bacteriocin

- Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria, bacteriocins attack bacteria by focusing on the plasma membrane
- Have a lower potential for resistance than antibiotics
- Strengths include hardiness against ultraviolet and heat and, unlike other antimicrobial peptides, they are not toxic to mammalian cells
- Lactic acid bacteria produce a bacteriocin, nisin A, which has been used in food preservation

Egg Yolk Antibodies (IgY)

- Principle and passive immunization
- The productivity of antibodies from yolk is nearly 18 times greater than that from rabbits, based on the weight of antibody produced per animal
- Chicken antibodies have been developed and found efficient in diagnosis and prevention of viral infection like White spot syndrome in shrimp and cray fish, and fish pathogens like *E.tarda*, *Y.ruckeri*, *Vibrio spp*.

Nanotechnology

- Use of nanoparticles in drug delivery system and vaccination
- Antibacterial surfaces in the aquaculture system
- Nano delivery of veterinary products in fish food using porous nanostructures, and nanosensors for detecting pathogens in the water.
- Water purification for removing microbes, organic chemicals and metals
- Nano biosensor probe that integrates a biological component, such as a whole bacterium or a biological product (e.g., an enzyme or antibody) with an electronic component to yield a measurable signal

Contd.,

- ‘Smart membranes’ is an innovation to remove nitrates from water
- The antimicrobial properties of NMs such as nano titania and nano silver can be exploited to reduce the build-up of bacteria in the aquaculture system.
- Antibacterial coatings on the sides of fish tanks and pipe work to prevent biofouling.

Bio-barcode assay

- Bio-barcode assay is a recently developed technique for the detection of nucleic acid and protein targets.
- Bio barcode assay, an ultrasensitive method
- Can detect the DNA with zeptomolar sensitivity (10^{-21} M or 10 copies of DNA in a sample of 30 ml) making it as sensitive as PCR but avoiding the need for enzymes.
- With regard to proteins, this test reaches attomolar sensitivity (10^{-18} M), corresponding to a sensitivity one million times higher than that of classic ELISA tests.
- Thus the applications of nanotechnology in aquaculture is immense

RNAi

- Post translational silencing of targeted genes using dsRNA
- Basic antiviral defence mechanism of many vertebrates and invertebraes
- Gene silencing by *in vitro* transcribed, bacterial expressed and chemically synthesised siRNAs
- Gene silencing by vector based RNAi constructs
- Delivery through injection, oral and bath treatment
- RNAi technology has been explored for their potential use as antiviral tool in many fish and shrimp viral diseases

Genomic tools

- Selective breeding for disease resistant fishes
- Marker assisted selection of wsv resistant shrimps Indo-Norwegian project

Farm management practices

- **Standard Operating Procedures (SOPs)**
- **Hazard Analysis Critical Control Point (HACCP) analysis**
- **Biosecurity measures**
- **Good Management Practices (GMPs)**

Need of the hour

- Registration of all aquaculture facilities
- Import risk analysis for the introduction of new exotic species
- Studies on Economic losses due to diseases
- National List of Reportable Diseases of Aquatic Animals
- Surveillance projects for fish viral and bacterial disease
- Disinfection and disposal procedure for disease infected farms
- Projects for fish vaccine development and commercialization
- Quarantine facilities for fish import and export
- Capacity development for disease diagnosis and health management
- Accredited disease diagnostic laboratories at district/state level

FIND..... FIGHT..... FOLLOW

THANKYOU