

Disease diagnosis and prevention strategies in aquaculture including vaccination

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Scope of presentation

- Disease diagnosis
 - sample collection
 - sample preparation
 - diagnostic procedures
- Disease prevention strategies
 - good farm management
 - disease surveillance
 - vaccination



Level of diagnosis		
Level	Site	Activity
1	Field	Observation of animal and environment Gross clinical examination
2	Lab	Parasitology Bacteriology Mycology Histopathology
3	Lab	Virology Electron microscopy Molecular biology Immunology

Sample collection

Sample size needed for 95% confidence detection																
Population Size	Target prevalence (%)															
	2	5	10	15	20	25	26	29	32	35	40	46	53	64	78	95
50	48	39	22	15	12	10	10	9	8	7	6	5	4	3	2	1
100	78	45	25	17	13	10	10	9	8	7	6	5	4	3	2	1
250	112	55	27	18	14	11	10	9	8	7	6	5	4	3	2	1
500	129	56	28	19	14	11	10	9	8	7	6	5	4	3	2	1
1,000	138	57	29	19	14	11	10	9	8	7	6	5	4	3	2	1
1,500	142	58	29	19	14	11	10	9	8	7	6	5	4	3	2	1
2,000	143	58	29	19	14	11	10	9	8	7	6	5	4	3	2	1
4,000	146	58	29	19	14	11	10	9	8	7	6	5	4	3	2	1
10,000	149	59	29	19	14	11	10	9	8	7	6	5	4	3	2	1
≥100,000	149	60	29	19	14	11	10	9	8	7	6	5	4	3	2	1

Sample preparation for diagnostic laboratory

- Sampling fish as alive
- Sample size is complied to the assume disease prevalence
- Pack in plastic bags/polystyrene boxes
- Supply enough oxygen
- Keep cool for long transportation
- Salt or sedative agents may be applied such MS222 and clove extract

Supporting information

- gross observation
- mortality rate
- feed records
- stocking records
- environmental parameter
- history and origin of fish population
- reasons of submitting the samples
- owner or contact name, address and phone number
- date of sample collection

Disease diagnosis

- Parasitic diseases
- Bacterial diseases
- Fungal diseases
- Viral diseases

Parasitic diseases

Major clinical signs:

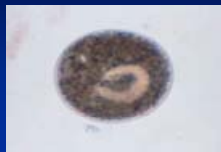
- Skin haemorrhage
- Flaring operculums
- Restless swimming, flashing or rubbing movement
- Distinct lesions: “rust slime”, “cotton wool”, red pin spots, white spot scattering
- Low-moderate mortality associated

Haemorrhage, “cotton wool” lesion



Parasite: *Epistylis* sp.

white spot scattering



Parasite: *Ichthyophthirius* sp



rust slime, velvet



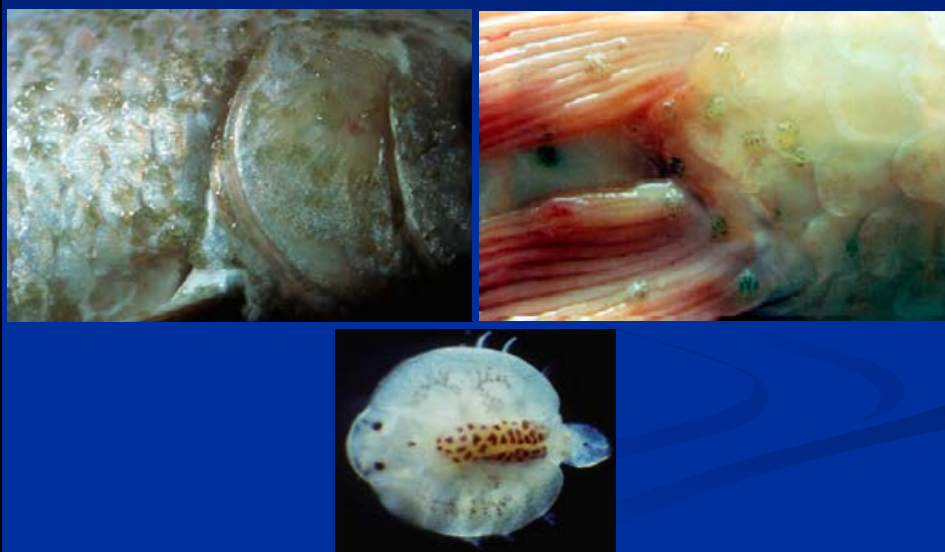
infested with skin/gill flukes,
“red pin” spot lesion



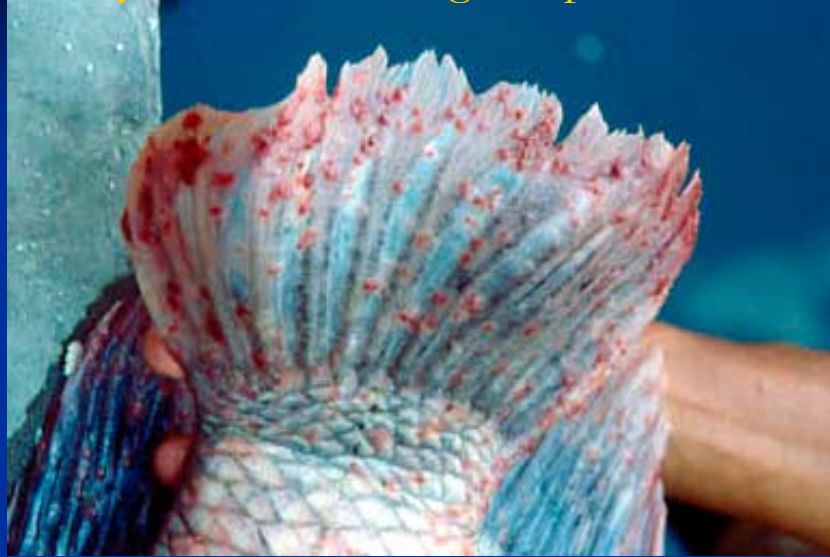
Parasite: *Lernaea* sp.



Parasite: *Argulus* sp.



heavily infested with *Argulus* sp.



Isopods



Bacterial diseases

Major clinical signs:

- abdominal distension (dropsy)
- exophthalmia (pop-eye)
- scale protrusion
- haemorrhagic lesion on skin, fin, eye, and internal organs
- enlargement of internal organs
- discolouration of skin and internal organs
- abscess or granuloma on skin or internal organs

dropsy



exophthalmia



<http://www.jbl.de/onlinehospitaluk/perpicture/index.php>



<http://www.uoguelph.ca/~pwoo/S.b.fig8.jpg>

scale protrusion, dropsy



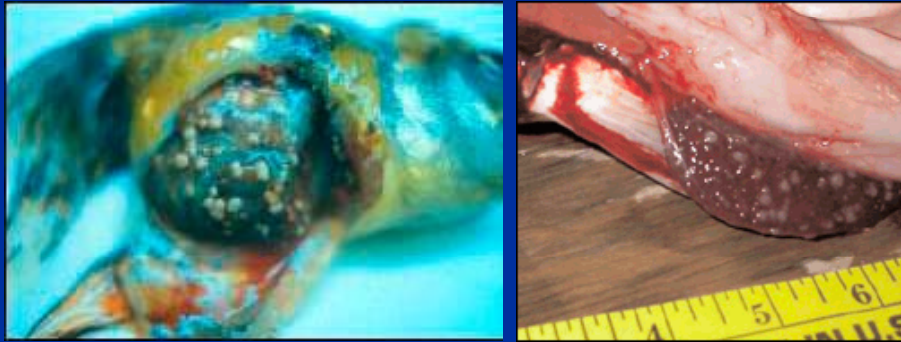
rotten gills



Haemorrhage on skin, eyes, & internal organs



granuloma, white nodules



Swimming pool granuloma
in human associated with
aquaculture practice



Fungal diseases

- External fungal disease: *Achlya*, *Saprolegnia*
 - visible to naked eye
 - mainly infect superficial area
 - often found associated with other health problems such as skin damage or low immunity
 - inducing sporulation is required for genus identification

Infection with *Achlya*, *Saprolegnia*



Source: <http://www.jbl.de/onlinehospitaluk/perpicture/index.php>

Fungal diseases (cont')

- Internal fungal disease: *Aphanomyces invadans* (EUS)
 - loss of appetite, lethargy, floating near water surface
 - distinct dermal ulcer
 - may lose some body parts
 - mortality is usually high
 - presumptive diagnosis: squash preparation of muscle
 - confirmatory: histopathology

Infection with *Aphanomyces invadans*



Infection with *Aphanomyces invadans* (EUS)



Viral diseases

- Presumptive diagnosis: visual examination (some)
- Confirmatory: virology

Lymphocytes



Koi Herpes Virus (KHV)

- White blotchy skin, ulcer, haemorrhage
- Pale, rotten gills with excessive mucous
- High mortality

Koi Herpes Virus (KHV)



Disease prevention strategies

- Good farm management
- Disease surveillance and monitoring
- Vaccination

Good farm management

- screen seed/broodstock for any infectious agents before introducing into the farms
- control water source and quality to ensure optimum growing of their animals and reduce exposure to pathogens
- maintain stocking densities at optimum level
- allow time to fallow sites between crops and apply disinfectant to pond/tank and equipment
- apply strict hygiene practices including disinfection procedures
- avoid equipment movement between different areas of the farms
- improve nutrition and diet, and store them in cool, dry, and well-ventilated place to prevent rancid and molding
- closely monitor the stocks to ensure early detection of disease problem



Disease surveillance

Farm level

- Aim to assess the health status of fish stocks
- Sample size should be a representative of population
- Regular monitoring



Vaccination

General principles of fish vaccination

- What is a vaccine?

Vaccines are preparations of antigens derived from pathogenic organisms, rendered non-pathogenic by various means, which will stimulate the immune system in such a way as to increase the resistance to disease from subsequent infection by a pathogen

What does a vaccine do?

- Stimulate the immune system in such a way to increase the resistance to disease from subsequent infection by a pathogen
- Vaccination is a protection against disease, not necessarily infection
- Thus, vaccine protect against outbreak of disease but not necessarily carrier

Vaccine availability

Anti-bacterial vaccine

- Commercial vaccine
 - *Yersinia ruckeri* (Enteric redmouth disease)
 - *Vibrio anguillarum*
 - *V. ordalii*
 - *V. salmonicida*
 - *Aeromonas salmonicida* (Furunculosis)
 - *Streptococcus iniae* (for Asian seabass)

Anti-bacterial vaccine (cont')

- Need vaccine to
 - *Renibacterium salmoninarum* (BKD)
 - *Piscirickettsia salmonis* (salmonid rickettsial septicemia)
 - *Edwardsiella ictaluri* (Enteric septicemia of cat fish)
 - *Enterococcus seriolicida* (Enterococcal septicemia)
 - *Pasteurella piscida* (Pasteurellosis)
 - *Streptococcus* sp. (for Tilapia)

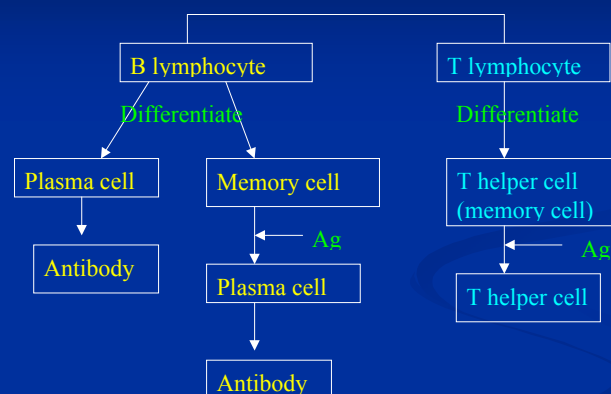
Anti-viral vaccine

- There was one in 1981 to *Rhabdovirus carpio* (Spring viraemia of carp) but no longer available
- No other vaccines available for other viral diseases
- Infection in young fish cause high mortality
- Immersion method is often fail for viral vaccine
- DNA vaccine seems to be promising and safe (IPN vaccine is now available in Norway)

Immune mechanism of fish

- Immunity
 - Innate system (non-specific immunity)
 - Adaptive system (specific and non-specific immunity)

Humoral immunity



Environment affect on fish immune mechanism

- Natural environmental stress factors:-
 - season, temperature, salinity, photoperiod
- Social stress factors:-
 - crowding, hierarchy
- Artificial environmental stress factors:-
 - pollutant such as acid rain, heavy metals & organic compound

Factors affect on vaccinated fish

- Internal factors
 - age
 - genetic and species
- External factors
 - route of application
 - Ag (vaccine) dose
 - temperature
 - diet

Administration of vaccines

- The administration strategies depend on:
 - fish species
 - fish size (2-20g)
 - husbandry
 - disease against
 - optimum point within the growth cycle
 - timing of any expected disease challenge

Vaccine administration

Direct immersion

- Widely practised in small fish where it is an easy and highly cost effective method
- Antigen is uptaken over the surface & gills
- The standardisation of dose centre around “dilution rate” rather than any finite definition of Ag load

Standard parameters for immersion vaccination

Dilution of vaccine	Application time	Quantity of fish
1:10	Dip small batches 30-60 sec	100 kg
1:500	Add to holding water for 1 h.	35-50 kg
1:300	Add to holding water for 3-6 h.	200,000
Size of fish	Species	Duration of protection
0.5-2.0 g	salmonids	< 4 months
2.0-20 g	salmonids	9-12 months

Spray administration

Spray administration is a variant of the direct immersion method, where the fish is larger than the preferred sized for immersion and individual handling for infection is not considered an option

Peroral administration

Oral vaccine are more expensive per dose than their immersion or injectable equivalent and confer a lower degree of protection.

The antigens are transported across the gut wall to lymphoid tissues in sufficient quantity to stimulate a solid humoral response.

Choice of adjuvant is also difficult and feeding rate need to be adjusted

Injection

Injection is the method yielding strongest immune response and can delivery Ag with a certain amount

Vaccination is carried out either by hand or machine. Fish needs to be anaesthetised and passed to a machine or handling by hand.

Anaesthetics for fish

Anaesthetic	Dose
Benzocaine (Ethyl-4-amino-benzoate)	50 mg/l
Chlorbutol	1 g/l
Hydroxyquinaldine	10 mg/l
Metomidate	0.2-0.8 mg/l
Methyl pentynol	0.5-3.0 ml/l
MS-222 (Tricaine methanesulphonate)	50-100 mg/l
2-Phenoxyethanol	0.3 ml/l
Quinaldine	50 mg/l
Tertiary amyl alcohol	1-10 ml/l
Urethane	100 mg/l

Risk factors

- Fish carrying the homologous disease at a subclinical level
- Loss of appetite/Loss of growth
- Immunotolerance (vaccinate at too early stage of fish)
- Fungal attacks

Practical constraints

- Protection period do not last to cover the entire production cycle
- Vaccination scheme cannot be well planned with non-seasonal diseases
- Vaccine cost does not match to the fish value

Conclusion

- Diagnosis will serve as a tool to assess the fish health status and play a major role in disease control
- Prevention including farm management, disease surveillance, and vaccination scheme minimise the disease outbreak

Thank you