Viral Disease in Aquaculture
A Rotavirus, B Adenovirus, C Norovirus, D Astrovirus
Viruses at Same Magnification Allowing Comparisons
What is a Virus

Infectious Agents composed of Nucleic Acid
With a Protein Coat (Capsid)
Only Visible by Electron Microscopy
Size between 10 and 200 nm
Infectious Form Inert: No Growth – No Respiration
Can Enter Living Plant, Animal or Bacterial Cells
Have Evolutionary History
Responsible for Lateral Gene Transfer
What Viruses Aren’t

Not a Bacterium
Not Independent
Cannot Replicate Without a Living Cell
Antibiotics Do Not Kill Them
Viral Appearance

Capsid, Core and Genetic Material (DNA/RNA)
Capsid: Outer Shell of the Virus That Encloses Genetic Material
Make-up of Capsid Determines Immune Response
Capsid Made of Many Identical Proteins
Protein Core Within Capsid Protects Genetic Material
Additional Coverings Called The Envelope
Viruses Comes in Various Forms: Rods, Filaments Spheres, Cubes Crystals
Typical Viral Shapes

Rods

Spheres

Icosahedral
Role of Viral RNA / DNA

Supplies Code for Capsid Proteins - Enzymes to Replicate more Virions

Information Included Allows Newly-Built Virions to Lyse Cell

Result: Host Cell Destroyed, Hundreds of New Virions Release

*Bottom Line* – Viruses Exist to Make More Viruses – Most Are Harmful Replication Means Host Cell Death
# DNA Virus Classification

<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Examples (common names)</th>
<th>Group</th>
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<tbody>
<tr>
<td>Adenoviridae</td>
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<td>Anelloviridae</td>
<td>Torque Teno Virus</td>
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<td>Reoviridae</td>
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<td>Poliovirus</td>
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<td>Caliciviridae</td>
<td>Norwalk Virus</td>
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<td>Togaviridae</td>
<td>Rubella</td>
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<td>Flaviviridae</td>
<td>Hepatitis C</td>
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<td>Orthomyxoviridae</td>
<td>Influenza</td>
<td>V</td>
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<td>Paramyxoviridae</td>
<td>Measles, Mumps</td>
<td>V</td>
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<td>Bunyaviridae</td>
<td>Hantavirus</td>
<td>V</td>
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<td>Rhabdoviridae</td>
<td>Rabies Virus</td>
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<td>Filoviridae</td>
<td>Ebola, Marburg</td>
<td>V</td>
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<tr>
<td>Astroviridae</td>
<td>Astrovirus</td>
<td>IV</td>
</tr>
<tr>
<td>Retroviridae</td>
<td>HIV</td>
<td>VI</td>
</tr>
</tbody>
</table>
Baltimore Classification Viruses

**Group I:** double-stranded DNA

**Group II:** single-stranded DNA

**Group III:** double-stranded RNA

**Group IV:** positive-single-stranded RNA

**Group V:** negative-single-stranded RNA

**Group VI:** single-stranded RNA with *reverse transcriptase*

**Group VII:** double-stranded DNA with *reverse transcriptase*
Reverse Transcriptase

DNA Replicated by DNA Dependent DNA Polymerase
If Genome RNA – How to Get DNA from RNA?

Need Reverse Transcriptase –
An RNA Dependent DNA Polymerase

Used by Reserve-Transcribing Viruses – Such as HIV Human Immunodeficiency Virus

Commercial Reverse Transcriptase Hugely Important in Molecular Biology eg RNA – PCR

Several Classes of Anti-Viral Drugs (HIV) – Are Reverse Transcriptase Inhibitors
Bacteriophage

Genetic Material can be

- ss & ds RNA
- ss & ds DNA

Classification Unresolved

Most Common & Diverse Entities in Biosphere

Seawater: $9 \times 10^8$ Virions/ml

Lytic Cycle or Lysogenic

Used as Alternative to Antibiotics - Europe
Phage Injecting Genetic Material
Lysis
or
Lysogeny

- Phage λ attaches to bacteria
  - Injected phage DNA may either integrate or replicate
  - Linear phage DNA circularizes
- Replication Pathway (lytic cycle)
  - Prophage is integrated into bacterial chromosome
- Integration Pathway (lysogenic cycle)
  - Integrated DNA
- Excision of phage DNA
  - New virions are formed (assembly)
- Replication of phage DNA
Bacteriophage: Lytic or Lysogenic Cycle

Lytic Cycle:
- Model T4 Bacteriophage
- Phage Attaches to Bacterial Cell
- Injects Genetic Material
- Replicates Using Host Machinery
- Bacterial Cell Lyses
- Releasing New Phage
- Lytic Phage More Suitable for Phage Therapy
Bacteriophage Lytic Cycle
Lysogenic Cycle

Phage Attach to Bacterium
  Inject Genetic Material into Host
Integrates Genome into Host DNA – Now Prophage
Lies Dormant – Replicating with Host DNA
  May Be a Plasmid
When Conditions Deteriorate – Massive Replication of Prophage – Lysis of Host
*Vibrio cholerae* – Prophage turns Harmless Strain of *Vibrio* into Virulent One That Causes Cholera
T1 Bacteriophage Attached to an *E. coli* Cell Injecting Their Genetic Material
Lysis of Bacterial Cell Releasing Dozens of New T4 Bacteriophage
Viruses of Aquaculture

Identification of Viral Species Requires Expensive Equipment and Highly Trained Personnel

Viral Infections of Fish

- Infectious Salmon Anemia Virus (ISAV)
- Infectious Pancreatic Necrosis (IPN)
- Viral Hemorrhagic Septicemia (VHS)
- Infectious Hematopoietic Necrosis (IHN)
- Channel Catfish Virus Disease (CCVD)
Infectious Salmon Anemia Virus
First Time Outbreaks

- 1984
- 2000
- '96 2009
- 2001
- 2007
- '98 2009
- 2009
Prevalence of ISAV Cases Chile

Numero de Centros
Meses
Virulentos
HPR0

Meses:
- jul07
- sep07
- nov07
- ene08
- mar08
- may08
- jul08
- sep08
- nov08
- ene09
- mar09
- may09
- jul09
- sep09
- nov09
- ene10
- mar10
- jul10
- sep10
- nov10
- ene11
- mar11

Numero de Centros:
- 3
- 4
- 2
- 3
- 3
- 3
- 3
- 6
- 7
- 8
- 13
- 17
- 24
- 21
- 22
- 19
- 4
- 5
- 2
- 2
- 4
- 2
- 1
- 1
- 1
- 3
- 1
- 4
- 2
- 2
- 2
- 1
- 2
ISAV in Chile 2007 - 2008

First ISA Outbreak Occurred June 2007
Atlantic Salmon Seawater Farm site in
Central Chiloé – Region X – Following
Recovery Outbreak of Piscirickettsiosis.

ISAV was most similar to isolates from Norway.
Acquired Mutations in Envelope Proteins
Predominant Pathogenic Type was
ISAV HPR7b until March 2010

Direct Costs of the Chile ISA Outbreak
(Q3 2007 – Q4 2008) Estimated at $434M
ISAV HPR0 Characteristics

ISAV without any deletion/insertion in HPR is designated HPR0 to indicate “full-length HPR”

All ISAV isolated to date have deletions in HPR relative to HPR0. HPR0 is considered the putative ancestral virus.
Infectious Pancreatic Necrosis (IPN)

What: Viral Infection of Salmonids
   (Salmon, Trout and Char)
Time: Acute
Result: High Mortality of Fry and Fingerlings
   Rare in Larger Fish
History: Isolated in Pacific NW in 1960s
   Wiped-out Brook Trout Oregon: 1971-73
Size: Only 65 nM in Diameter
   Smallest of All Fish Viruses
Infectious Pancreatic Necrosis Virus

- Single Capsid Shell, Icosahedral Symmetry
- No Envelope, ds-RNA

- Fairly Stable – Resistant to Chemicals
- Variable Resistance to Freezing

- Remains Infectious 3 Months in Water

- Targets Pancreas & Hematopoietic Tissues
- Kidney and Spleen
IPN – Disease Process

Who: All Salmonids, Other Marine Fish

Reservoirs: Carriers, Once a Carrier Always a Carrier, Virions Shed in Feces & Urine

Transmission:
Horizontal – by Water, Carriers, or Fry
Vertical – Infected Adults to Progeny
Experimental – by Virions in Feed - IP injection

Pathogenesis: Entry via Gills, Digestive Tract, Skin Lesions

Environmental: Mortality Reduced at Lower Temperatures
IPN – Pathology – What Do We See?
IPN: Detection, Diagnosis & Control

Isolation: Check Whole Fry, Kidney, Spleen, Pyloric Caeca, Fluids All Good Indicators

Presumptive Tests: Epizootic Evidence
Diagnostic PCR for Infected Cells

Definitive Tests: Serology – Fluorescent Monoclonal Antibody Test (FMAT)

Control: Destroy Virus in Water, Employ Only Virus-Free Stock – Destroying Any Infected Stock
A Vaccine Now Exists
Fish Severely Affected by IPNV

Atlantic Salmon – *Salmo salar*
Brook Trout – *Salvelinus fontinalis*
Brown Trout – *Salmo trutta*
Zebrafish – *Brachydanio rerio*
Chinook Salmon – *Oncorhynchus tshawytscha*
Chum Salmon – *Oncorhynchus keta*
Coho Salmon – *Oncorhynchus kisutch*
Pink Salmon – *Oncorhynchus gorbuscha*
Sockeye Salmon – *Oncorhynchus nerka*
Rainbow Trout – *Oncorhynchus mykiss*
Yellowtail Amberjack – *Seriola lalandi*
Species Susceptible to IPNV

Menhadden – *Brevoortia tyrannus*
Grayling – *Thymallus thymallus*
Pacific Halibut – *Hippoglossus stenolepis*
Japanese Amberjack – *Seriola quinqueradiata*
Loach – *Misgurnus anguillicaudatus*
Striped Snakehead – *Channa striatus*
Summer Flounder – *Paralichthys dentatus*
Southern Flounder – *Paralichthys lethostigma*
Turbot – *Psetta maxima*
Goldfish – *Carassius auratus*
Redfin Perch – *Perca fluviatilis*
Yellowfin Bream – *Acanthopagrus australis*
Carp – *Cyprinus carpio*
Common Scallop - *Pecten maximus*
Asymptomatic Carriers

Coalfish – *Pollachius virens*
Carp – *Cyprinus carpio*
Goldfish – *Carrasius auratus*
Minnow – *Phoxinus phoxinus*
Pike – *Esox lucius*
Lamprey – *Lampetra fluviatilis*
Spanish Barbel – *Barbus graellsi*
White Sucker - *Catostomus commersoni*
Heron – *Ardea cinerea*
Crayfish – *Astacus astacus*
Shore Crab – *Carcinus maenas*
Viral Hemorrhagic Septicemia (VHS)
Viral Hemorrhagic Septicemia (VHS)

What: Viral Disease of Salmonids – Rainbows

*Oncorhynchus mykiss* Vulnerable 59-66°F

When: Recognized in Denmark in 1949

Isolated from Pacific Coast in 1989

Size: Rhabdovirus, Bullet-Shaped (one rounded end)

185 x 65 nm, Lipoprotein Envelope, ss-RNA

Constitution: Sensitive to Ether, Chloroform

Heat, Acid, Resistant to Freeze-Drying
Infectious Hematopoietic Necrosis Virus (IHNV)

Who: *Oncorhynchus nerka, O. tshawytscha, O. mykiss*

but Cohos (*O. kisutch*) Resistant

When: ‘50’s in Oregon Hatcheries – Between 1970-1980

100 million Mortalities – Adults 70% Mortality

Young with 90-95% Mortality

What: Bullet Shaped Rhabdovirus, ss-RNA, Heat and pH Sensitive, Spiked Surface Glycoproteins
Channel Catfish Viral Disease (CCVD)

Clinical Signs: Feed Poorly, Swim Erratically, Float Dropsy, Petechial Hemorrhages Fin Base, Pale Liver, Enlarged Spleen

Transmission: Vertical Via Gametes, Through Water From Fish, Birds, Effluent, Equipment

Diagnosis: Suspect When High Mortality in Fry & Fingerlings Above 70° F – Not Official Diagnosis
Channel Catfish Viral Disease (CCVD)

Who: Contagious Herpes Virus Affecting Only Channel Catfish Less Than 4 Months Old

Where: Occurs in SE United States, California, Honduras

When: Discovered 1968, Acute Hemorrhagia High Mortality

What: Enveloped Capsid, Icosahedral Symmetry Capsid with 162 Self Assembling Captomeres Sensitive to Freeze-Thaw, Acid, Ether
Cannel Catfish Viral Disease
Lymphosystis Virus Disease

Most Common Fish Disease

Marine, Freshwater and Ornamentals All Susceptible

Lesions in Connective Tissue Skin & Fins

Lesions – Hypertrophied Cells up to 1,000x Normal Size

No Cure, Usually not Fatal – But Hard to Sell
Shrimp Viruses
Common Shrimp Viruses

White Spot Symptom Virus WSSV
  1992 Wipes-out Much Asian Production
  1999 Devastates Shrimp Latin America
Taura Syndrome Virus – TSV
White Tail Virus – WTV
Yellow Head Virus – YHV
Infectious Hypodermal Hematopoietic Virus – IHHNV
Monodon Slow Growth Virus – MSGV
Infectious Myonecrosis Virus – IMV
Necrotizing Hepatopancreatitis Virus – NHV
Aquacultured Shrimp

Sample Any Shrimp Pond in SE Asia

88% Shrimp are Infected with Virus
53% Infected with 2-3 Viruses

Survival After Years of Exposure
    Returned to a More Normal Level

Does This Indicate Resistance or Tolerance?

Resistance – Shows No Sign of Pathogen
    However, Virus Detected in Tissues

Conclusion: Something Other Than Resistance
Taura Syndrome Virus

Affects Shrimp – Identified Ecuador 1992 – Disease Caused Catastrophic Losses

Spread Rapidly to All Shrimp Farms in the Americas Via Shipments Infected Post-Larvae & Brood-Stock

Symptoms: Reddening of Tail Fan – Visible Necrosis in Cuticle

Remain Infectious in Feces of Birds that have Eaten Infected Shrimp
<table>
<thead>
<tr>
<th>Shrimp</th>
<th>Fish</th>
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<tbody>
<tr>
<td>- No clear response to viruses</td>
<td>- Specific response to viruses</td>
</tr>
<tr>
<td>- Survivors remain infected</td>
<td>- Survivors often don’t remain infected</td>
</tr>
<tr>
<td>- Pathogen persists</td>
<td>- Pathogen removed from body</td>
</tr>
<tr>
<td>- Survivors infectious to others</td>
<td>- May or may not be infectious to others</td>
</tr>
<tr>
<td>- Tolerance is a normal situation</td>
<td>- Tolerance not normal</td>
</tr>
<tr>
<td>- No antibodies</td>
<td>- Antibodies present</td>
</tr>
<tr>
<td>- Multiple active viral infections are normal</td>
<td>- Usually only one virus at a time</td>
</tr>
</tbody>
</table>
Defense Against Viruses

First Line: Skin - Mucous Membranes - also Line Gastrointestinal & Respiratory Spaces
Skin is Tough – Stomach Acidity a Disinfectant

Second Line: After Virus Enters Tissues – Phagocytes Consume Them – Accumulation of Phagocytes Known as Puss

Third Line: Antibodies Best Defense Against Viruses But They Are Specific
A Particular Virus Stimulates Production of a Particular Antibody
Aquatic Diagnostic Lab Challenges

Surveillance & Monitoring Requires Many Animals
Reliable Detection Challenging Without Clinically Sick Animals

Effective Monitoring Requires Quantitative Methods
For Pathogen Load in Animals or Environment

Need Cost-Effective, Fast, Sensitive & Specific Methods
Allowing Unbiased Pathogen Detection
Conclusions

Aquaculture Will be Principal Source of Animal Protein

Aquatic Animal Disease is Part and Parcel of Aquaculture

Intensification of Aquaculture Will Result in Stocks Becoming Infected.

Unbiased Pathogen Detection Essential for Effective Disease Control

Improved Diagnostic & Surveillance Efforts will Result In Discovery of Emerging Diseases.

Nucleic Acid Assays Well Suited for Pathogen Detection in Aquatic Animal Populations
Iridovirus

Iridovirus Enlarged Spleen
Swine Flu
Herpes Virus
Smallpox Virus
Tobacco Mosaic Virus
HIV (RNA Virus)
Ebola (RNA Virus)
H1N1 Influenza Virus (RNA Virus)