Laboratory Detection Approaches to Fish Pathogen Surveillance in Ontario

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FISH HEALTH LAB
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1977

CANADIAN FISH HEALTH PROTECTION REGULATIONS

Department of Fisheries and Oceans

Ontario Ministry of Natural Resources

GREAT LAKES FISH HEALTH COMMITTEE

2017

National Aquatic Animal Health Program

NAAHP and CFIA

?

Great Lakes Basin

- freshwater fish
- many species
  (including Atlantic and Pacific salmon)
- wild and cultured for rehabilitation
- sportfish …and bait

… and <u>sizes</u> vary too

Samples are submitted from **stocks reared at OMNRF fish culture stations**,
and from
fall and spring **egg collections from wild fish.**

ROUTINE FISH HEALTH TESTING

Necropsy

Kidney
Heart
Liver
Spleen
pyloric caecae
gills

Bacteriology
- culture
- gill slides
- BKD IFAT stains

Virology
- tissue culture

Parasites
- microscopy

SOURCES FOR METHODS USED IN THE FISH HEALTH LAB

In the beginning: CFHPR "Manual of Compliance"

AMERICAN FISHERIES SOCIETY
Fish Health Section
"BLUE BOOK"
www.afs-fhs.org/bluebook/bluebook-index.php

FISH HEALTH SECTION

Manual of Diagnostic Tests
for Aquatic Animals
www.oie.int/international-standard-setting/aquatic-manual/

For fish health purposes, there is increasing emphasis on TEST VALIDATION and rapid molecular methods.

What do we find ?

VIRAL: **Aquareoviruses**

INFECTIOUS PANCREATIC NECROSIS VIRUS

VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

SPRING VIREMIA OF CARP

KOI HERPES VIRUS

INFECTIOUS SALMONID ANAEMIA VIRUS

BACTERIAL:

FLAVOBACTERIUM PSYCHROPHILUM	**coldwater disease**
FLAVOBACTERIUM BRANCHIOPHILUM	**bacterial gill disease**
FLAVOBACTERUM COLUMNARIS	**columnaris**
AEROMONAS SALMONICIDA	**furunculosis**
RENIBACTERIUM SALMONINARUM	**bacterial kidney disease**
YERSINIA RUCKERI	**enteric redmouth disease**

PARASITES:

MYXOBOLUS CEREBRALIS

NUCLEOSPORA SALMONIS

HETEROSPORIS

HAVE HAD

HAVE

WORRY ABOUT

BACTERIA

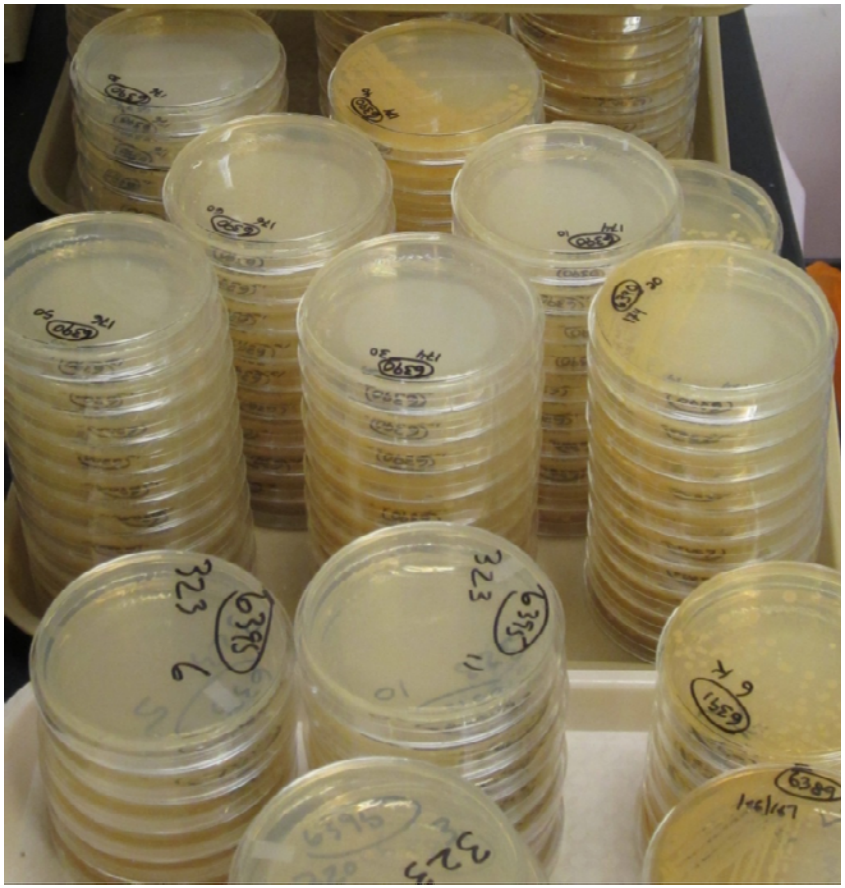
Organ samples plated on TSA after 2 days enrichment in TSB

Aeromonas salmonicida

2015-2016

14 isolations

Brook trout, wild rainbow trout, splake, white sucker, chinook salmon, coho salmon



Yersinia ruckeri

2006 - 2017

17 isolations

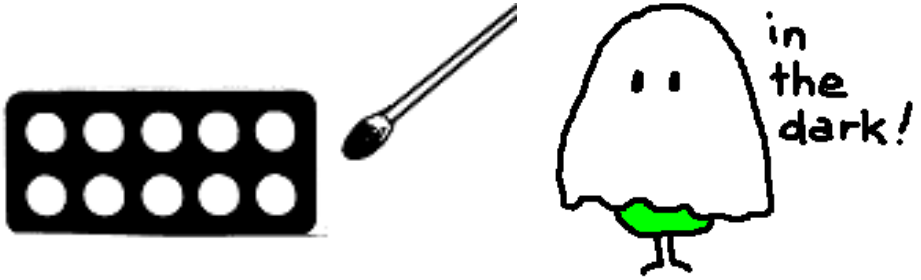
Wild lake trout, brook trout, brown trout, rainbow trout

BUT A LOT MORE FISH ARE TESTED AND FOUND TO HAVE NO PATHOGENS!

… really boring!

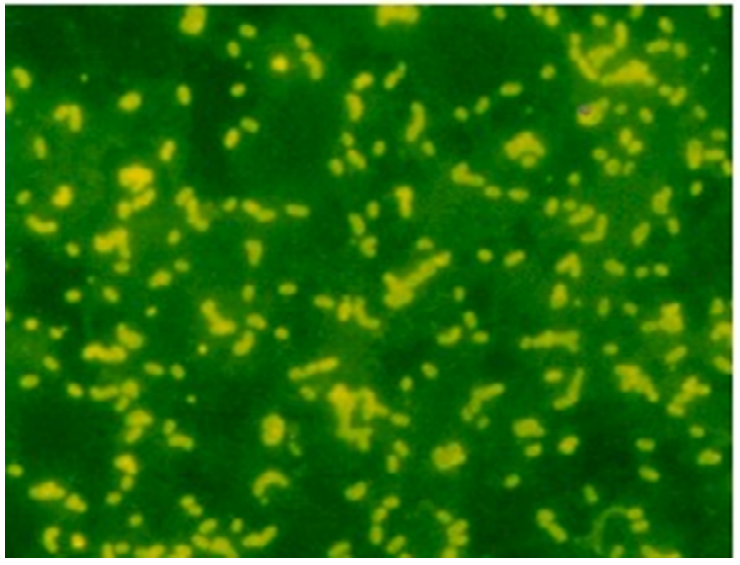
Bacterial Kidney Disease
Renibacterium salmoninarum

Fluorescent Antibody test



Culture of *Renibacterium* is feasible if you can wait 6-12 weeks (and avoid contamination).

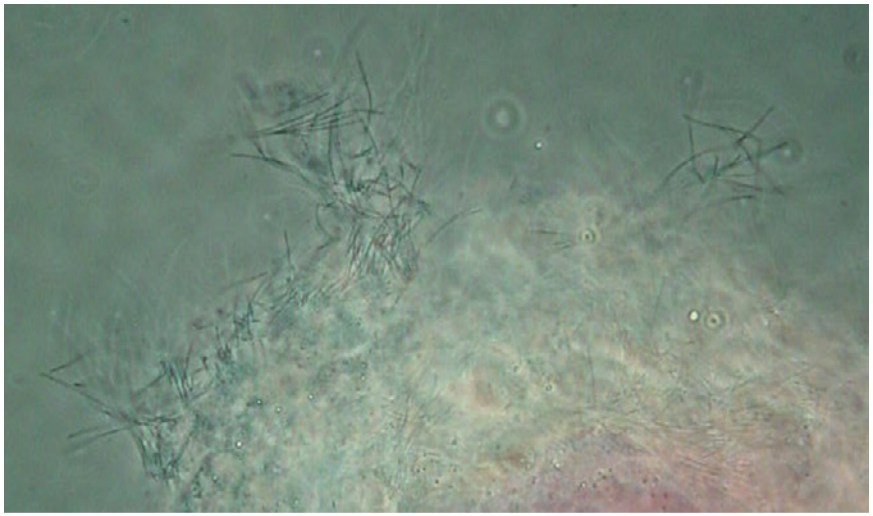
GROWING THE ORGANISM DOES ALLOW PRODUCTION OF DIAGNOSTIC ANTISERUM FOR THE IFAT TEST.



PRODUCTION-LIMITING DISEASES

It's worth culturing even easily recognized disease agents to allow study … and checking!.

THAT'S WHY WE CALL CULTURE "THE GOLD STANDARD"



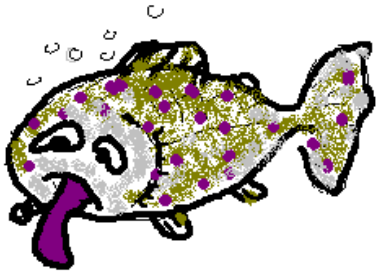
bacterial gill disease

Flavobacterium branchiophilum

Flavobacterium psychrophilum

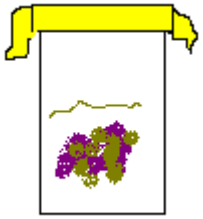
coldwater disease

Detecting VIRUSES by TISSUE CULTURE



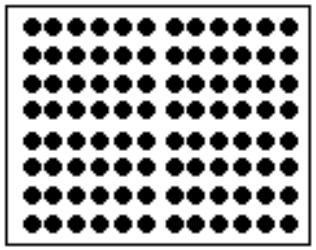
CLINICAL SIGNS ?
viremia, virus loads in organs

gill, pyloric caeca,
spleen, kidney



- macerated
- diluted
- centrifuged
- filtered

mples applied to
sh cell lines grown
tissue culture
served at 4, 10, 14, 21 days
r CYTOPATHIC EFFECT



re-pass

FISH CELL LINES

- Which to use ?
- How many ?

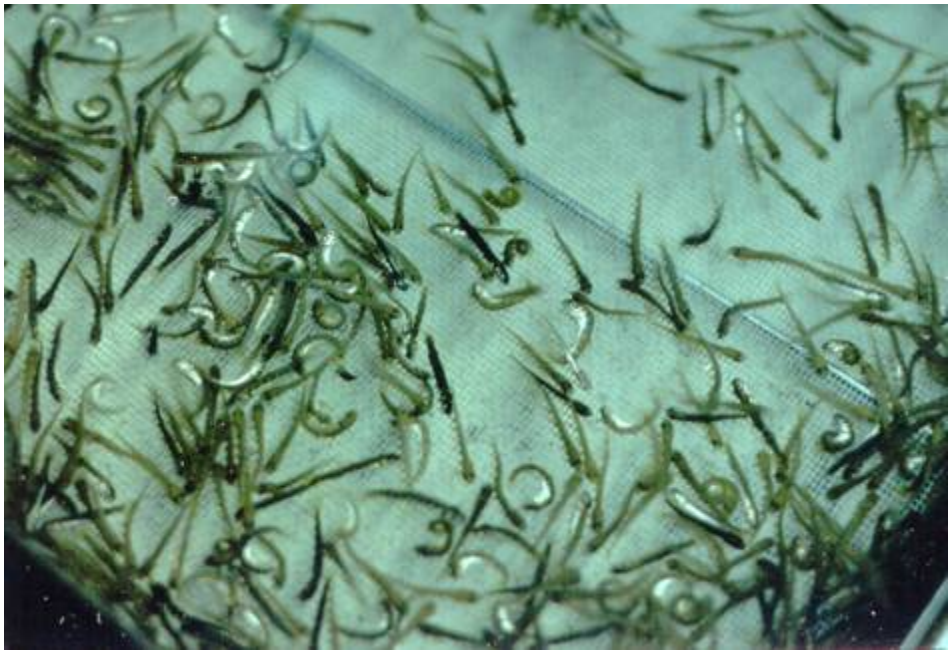
- **RTG-2** RAINBOW TROUT GONAD
- **CHSE-214** CHINOOK SALMON EMBRYO
- **FHM** FAT HEAD MINNOW
- **EPC** EPITHELIOMA PAPULOSUM CYPRINIS
- **BF-2** (bluegill fry)

With the Ontario VHSV, CPE <u>only</u> on FHM; not on RTG-2 or CHSE cells ...

→ BUT IT'S REAL ORIGIN IS FAT-HEAD !
BIODIVERSITY INSTITUTE OF ONTARIO

outine testing for fish viruses has been carried
t since 1977 … and, mostly, it was pretty boring.

**nfectious pancreatic
ecrosis virus (IPNV)
as an issue until a
atchery on surface
ater was closed in
ne early 1980's.**



1997 and again in 1999, CPE was observed, in
sue culture of a routine sample

→ **AQUAREOVIRUS**

"may you live in interesting times"

nce 2005, three significant viral pathogens have been detected in Ontario:

Viral Haemorrhagic Septicaemia virus

Great Lakes VHSV IVb

2005 Bay of Quinte, drum (and others)

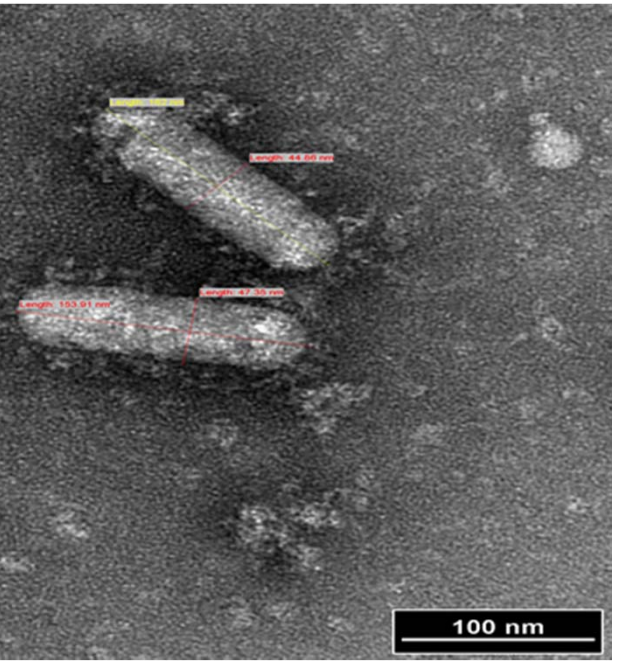
Koi Herpes virus

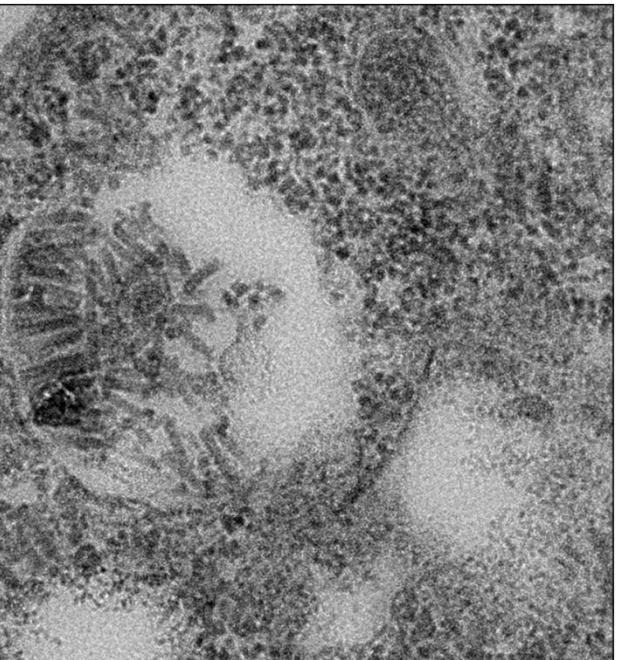
2007, 2009 - Kawartha Lakes, carp

Spring Viremia of Carp

.. and some other viruses appeared as well

"WE HAVE LIFT-OFF ! " (CPE)





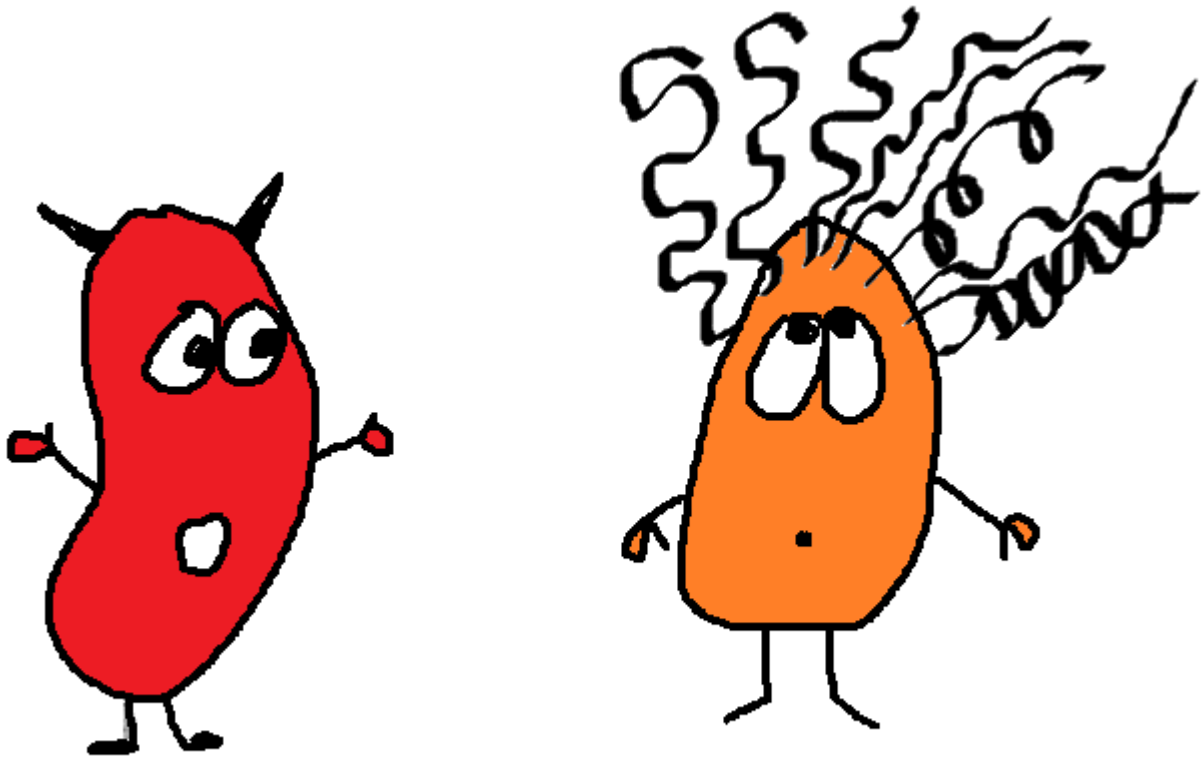
Chinook Bafinivirus

(**ba**cilliform **fi**sh **ni**do**virus**)

In the fall of 2008, a novel fish virus was detected in terminal spawning Chinook salmon in the Credit River, Ontario. No virus was found in the resulting fry, and no unusual mortalities were observed. The virus was not found in spawning Chinook in 2009 or 2010, but was again isolated in the fall 2011 and 2012 populations.

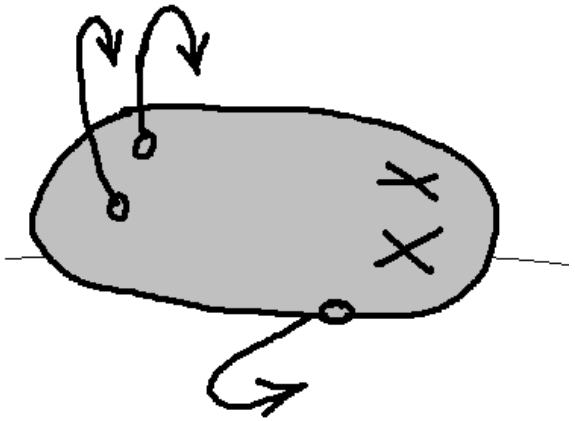
What does such a finding mean/not mean for fish health?

OLECULAR TESTS vs "LIVE BUG" ?



SO, ARE YOU A *REAL* PATHOGEN, OR JUST TALK AND SEQUENCES ?

Let's get MODERN and QUICKER !!!



PCR

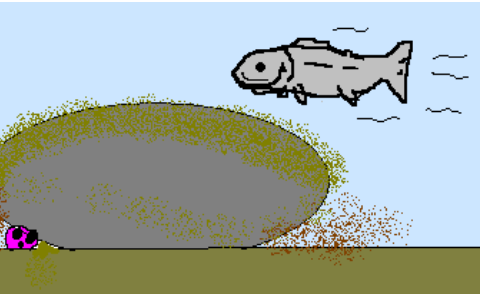
One cell,
Dead or Alive !

One cell – in what size of sample ?

The initial sample needs to have fairly high numbers of the pathogen present in a tissue in order to be detected in a small sample volume.

But PCR methods and sequences sure speed up positive identifications !

What evidence is needed to show that something is NOT there ?



e.g. **WHIRLING DISEASE**
Myxobolus cerebralis

Ontario: "not found"

- disease signs observed in susceptible fish
- positive diagnostic tests in sick fish
- detection of sub-clinical infections in screening tests of "at risk" fish populations

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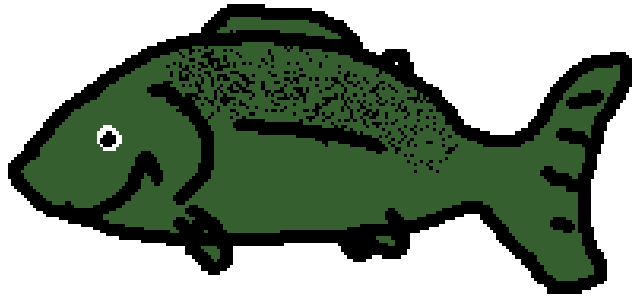
AND THE NEXT STEP IS ……?

More fish? More places? Better tests ?

ow many different tests is it
~~ossible~~ to run for routine screening ?

$$ FEASIBLE $$?

USEFUL?



"MOSTLY HEALTHY"

CELL LINES FOR VIROLOGY
TISSUES SAMPLED
ENRICHMENTS ? BLIND PASSES?
BACTERIOLOGICAL CULTURE MEDIA
MICROSCOPIC EXAMINATION
POLYMERASE CHAIN REACTION PRIMERS

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