KOI HERPESVIRUS: USING BIOSECURITY AND SURVEILLANCE TO PRODUCE DISEASE FREE FISH

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Introduction

- Koi herpesvirus is a recently emerged viral disease of carp (*Cyprinus carpio*) in all of its varieties
- First officially identified in 1998.
- Examination of archive material indicated KHV presence as early as 1996

Poorly regulated international trade of ornamental carp (Koi) and goldfish

Worldwide distribution

molecular comparison of diverse geographic isolates indicated that these represented a largely homogenous group with only slight differences between Western and Asian isolates

Major impact on commercial food carp production

- Japan
- South East Asia
- North America
- United Kingdom
- Poland
- KHV recently reported in confiscated illegal koi imports in the Philippines

History

- In the past international movement of carp was governed by concerns over the spread of spring viraemia of carp (SVC)
- Health certification requirements applied to carp but not to koi.
- Spread of SVC in recent years has also been blamed on movement of koi

Spring viraemia of carp (SVC)

- Rhabdovirus
- Can survive outside of the host in fresh water for up to 5 weeks at 10°C
- Disease of cyprinids (carp, goldfish and koi)
- Spread from the European continent
- 1988 – Brazil 2002 – USA
- 2004 – China 2006 - Canada

Introduction of exotic diseases threatens:-

- natural aquatic species diversity
- livelyhood of subsistence and commercial fishermen
- aquaculture development and investment
- employment opportunities
- local economies

Family Herpesviridae

Cyprinid herpes virus 1 - carp pox herpes virus

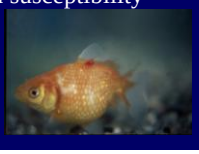
Cyprinid herpes virus 2 – herpes viral haematopoietic necrosis virus of goldfish

Cyprinid herpes virus 3 - Koi herpes virus

CyHV-1, CyHV-2 and CyHV-3 have been shown to be closely related to each other and distantly related to channel catfish virus (IcHV-1)

KHV and other species

- Recent evidence indicates that gold fish (*Carassius auratus)* may act as asymptomatic carriers of the koi herpes virus
- No information available on susceptibility of African cyprinids



Manifestation of disease

- Temperature dependant- permissive temperature for infection lies between 16 and 25ºC
- Disease follows introduction of stressed infected fish into naïve populations or after contact with strange fish
- Virus enters host through the skin. Frequent netting and high stocking density contribute to rapid spread
- susceptibility and mortality are greatest between 22 and 25ºC

History of a typical outbreak

- Clinical signs are not specific.
- Incubation period 7 days or less
- Rapid onset of mortality. Within 24 to 48 hours of onset of clinical signs. (82 % or more of exposed fish at 22^0C can die within 15 days)
- 80 to 100 % mortality
- All ages affected, but fingerlings more susceptible than older fish

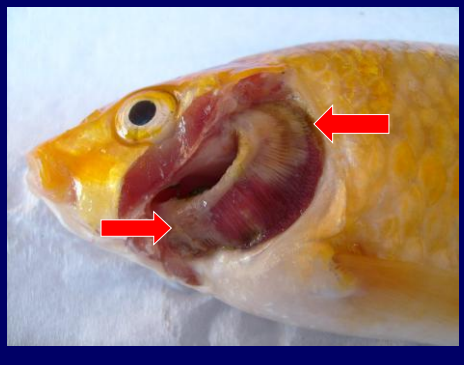
Symptoms

- Affected fish remain near the surface
- Swim lethargically
- Respiratory distress
- Aberrant swimming behaviour
- Sick fish may still be attracted to feed

External signs of disease

- External signs in infected fish are non-specific but include
- sunken eyes,
- hyperaemia and discolouration of the skin,
- fin erosion,
- thickening and loss of surface mucus.
- gills may take on a characteristic mottled appearance, with visible white or brown streaks of dead gill tissue.
- Changes in the internal organs are inconsistent and non-specific

Gill necrosis (KHV)



An increase in opportunistic parasites in KHV infected fish can be diagnostically misleading

- *Ichthyobodo necatrix*
- monogenean flukes
- bacterial infections with *Flavobacterium columnaris*

KHV - need for certified pathogen free fish

- Difficulty of identifying carrier fish
- Unquantifiable risk associated with:-
  - naturally resistant fish
  - survivor fish
  - 'vaccinated' fish
- Access to export markets

Diagnosis

- Opportunistic infections may mislead a diagnosis of KHV
- PCR testing of gill and kidney swabs will reliably confirm active infection

Direct diagnostic tests

1. PCR (polymerase chain reaction) detection of viral DNA.
2. histopathology
3. virus isolation on koi tissue culture. The virus is difficult to isolate on conventional fish tissue cell cultures and culture based tests are not available in South Africa

Current diagnostic limitations

- As with other herpes viruses, an asymptomatic carrier state appears to exist and recrudescence may occur. PCR techniques in use in South Africa are unable to identify carrier fish and a negative PCR result from a healthy fish does not rule out KHV infection.
- Reliable tests for virus isolation and tests to identify antibodies to the disease have been developed elsewhere but are not yet available in South Africa.

Diagnostic confirmation

- Indirect diagnostic test

  ELISA to detect antibody to KHV.

  Limitations:

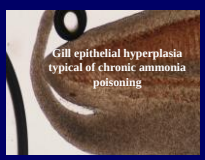
- although a positive test indicates previous exposure, it gives no indication of whether the fish is still infected.
- unknown how long antibodies persist

Effect of temperature

- Temperature is the predetermining factor that controls whether KHV develops into lethal infection.
- Peak mortality at permissive temperature of 22 – 25^{0}C.
- KHV outbreak can result in up to 98 % mortality

Other factors

- host susceptibility
- transport stress
- stocking density
- water quality – ammonia gas super-saturation



Diagnostic limitations

- Where KHV is causing mortality the current diagnostic techniques are highly accurate in identifying the virus.
- Once fish have recovered or if they have been exposed but are held outside of the permissive temperature it may be difficult to detect the presence of the virus

How can we limit the risk of spreading KHV?

- No single or group of measures will give absolute certainty that a single fish or a group of fish are free from Koi herpesvirus unless the fish originate from a certifiable KHV free population

Proposed strategies to limit KHV losses

- avoidance of exposure particularly at shows,
- development of vaccines,
- use of thermal temperature regimes
- selective breeding for increased resistance and
- use of resistant hybrids in fish production

KHV Regulations

- KHV has been included in the OIE Aquatic Animal Health Code as a notifiable disease
- EU import certification requirements have been amended to include guarantees for freedom from KHV by some EU member countries

Virus free hatchery protocol was developed based on:

- the 95% confidence level for detecting a disease with a 2% prevalence in a population of fish exceeding 1000 individuals with 6 monthly testing over a 2 year period and
- on the assumption that vertical transmission would not occur
- no other cyprinid species would be allowed on the farm

Prerequisites for a disease free status

- Closed population of fish
- Closed water supply
- Any newly introduced fish must originate from a source with the identical or higher standard of disease free certification
- Disease free status based on EU directives and on principles laid down by the International Aquatic Animal Health Code (OIE)

Prevent transmission from brood fish to ova

- Only healthy brood fish were selected
- Hormonally induced artificial spawning
- Artificial fertilization of ova
- Disinfection of fertilized ova
- Incubation of ova in isolation hatchery
- Raising fry and fingerlings in isolation ponds

Hormonal induction - GnRH analogue

Collecting milt

Stripping ripe eggs

Fertilization

Fertilized eggs placed on brushes or in Zoug jars

Fertilized eggs water hardening prior to disinfection

Eggs moved to disinfectant

Egg disinfection - iodophore disinfectant at 50 ppm for 10 minutes

Disinfected eggs being moved out of the brood fish facility

Grow out

- Fry were kept in hatchery tanks and initially fed *Artemia* spp. nauplii before being released into recently filled and fertilized earthen grow-out ponds.
- The grow-out ponds were used on an "all-in all-out" basis with each pond being emptied, disinfected and dried between use.

Principles of good biosecurity

- Access control
- Brood fish separated from hatchery and grow out facility
- Separate staff designated to each facility
- Closed water supply
- Fenced and net covered ponds
- Strategic disinfection
- All in all out stocking

6 monthly disease free testing procedure

1. After harvesting of earth ponds, fish were placed into holding ponds for observation for at least 2 weeks
2. 400 - 800 fish were packed into bags with oxygen and subjected to normal transport stress
3. Further quarantine in recirculated facility for minimum of 2 weeks at or near permissible temperature
4. Ammonia levels allowed to build up during quarantine

Sample submission from a minimum of 150 fish destroyed humanely for sampling

- Spleen, liver and kidney in phosphate buffered saline on ice for *Rhabdovirus carpio* virus isolation on tissue culture
- Gill swabs for PCR detection of Koi herpesviral DNA

Replacement of original broodstock

Over the following 2 years the original broodstock was replaced with offspring from the tested population

Disease free certification

- Following the initial two-year period with six monthly tests the fishery was able to meet EU import requirements for cyprinids, providing the necessary guarantees for freedom from *Rhabdovirus carpio* as well as from KHV.