Hemorrhagic disease of grass carp - Disease card[1]

by

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Pathogen Information

1. Causative Agent

1.1. Pathogen Type

Virus

1.2. Disease Name and Synonyms

Hemorrhagic disease of grass carp

1.3. Pathogen Common Name and Synonyms

Grass carp reovirus (GCRV)

Grass carp hemorrhagic virus (GCHV)

Fish reovirus (FRV)

1.4. Taxonomic Affiliation

1.4.1 Family: Reoviridae

1.4.2 Genus: Aquareovirus

1.5. Description of the Pathogen (Fig. 1-4)

Hemorrhagic disease was first discovered in a fish farm of Hubei Province, China, in 1972. It was considered to be a viral disease after etiological studies were reported in 1978. In 1980, virus particles were observed in kidney sections of infected grass carp, and the virus was named grass carp reovirus in 1984. The virus is icosahedral with a diameter of 65-70 nm with double capsids, no envelope, and contains 11 segments of double-stranded RNA. The virus is stable to chloroform treatment, acidic (pH 3) or alkaline (pH 10) conditions and heating at 56ºC for 30 min. Up to now, 2 serotypes have been found in China: GV-873 was found in Hunan province and GV-14 was found in Hubei province. They possess different patterns of 11 segments of dsRNA and different antigenicity.

1.7. Pathogen Environment

Freshwater

2. Modes and Routes of Transmission

Horizontal transmission (Water-borne or through ectoparasites). It is proven that the infection is transmitted horizontally from infected fish with clinical signs (such as sick fingerlings of grass carp and black carp) and/or carriers without clinical signs (such as silver

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carp, bighead carp, common carp and adult grass carp) through the water or through ectoparasites (e.g. *Argulus*)

3. Host Range

3.1. Host Type

Grass carp (*Ctenopharyngodon idella*)

Black carp (*Mylopharyngodon piceus*)

Topmouth gudgeon (*Pseudorasbora parva*)

3.2. Other Known or Suspected Hosts

Bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), golden carp (*Carassius auratus*) and commom carp (*Cyprinus Carpio*) can carry virus, but do not show clinical signs and mortalities.

3.3. Affected Life Stage

Fry and one-year-old fingerlings are usually infected. Occasionally, infections occur in older fish (2-3 years).

3.4 Additional comments

The disease is highly contagious, and cumulative mortality can approach more than 80% in affected fingerlings of grass carp. Disease usually occurs at high temperatures and most outbreaks occur when water temperature is between 25 and 28^{o}C

4. Geographic Distribution

In China, this disease was first discovered in 1972 and is now widely prevalent in central, southern and eastern China, especially in areas along Yangzhi River. It is possible that the actual geographical range is wider than the known geographical range. Susceptible species inhabiting waters of low temperature may be carriers and do not show clinical signs and mortality. The disease is also known to occur in Vietnam.

Disease Information

1. Clinical Signs and Case Description (Figure 5-8)

1.1. External observation

- Exophthalmia,
- Body color very dark,
- Hemorrhages at the base of fins, gill covers and mouth cavity,
- High mortalities at 25-28^{o}C.

1.2. Internal observation

- Hemorrhages throughout the musculature
- Enteritis (red intestine)
- Hemorrhagic gills or pale gills
- Internally, hemorrhages in organs such as liver, spleen, kidney and intestine.

1.3. Histopathological changes

- Degeneration and necrosis of liver cells
- Hyperemia and hemorrhages in vessels of liver and spleen.

1.4 Differential diagnosis

It is important to distinguish the disease from bacterial red spot disease in grass carp and other bacterial enteritis. Mixed infection with bacteria or secondary bacterial infection can often lead to similar clinical and pathological changes.

1.5 OIE Status

GCRV is not listed by the OIE

2. Social and Economic Significance

Grass carp is an important freshwater fish in China, which accounts for more than 20% of the total freshwater fishery production. Hemorrhagic disease causes serious losses of fingerlings, and only 30% of the fish survive to reach the market size

3. Zoonotic Importance

No data available

4. Diagnostic Methods

4.1. Screening Methods

4.1.1. Level I

There are no diagnostic signs exhibited by sub-clinical carriers.

4.1.2. Level II

Histopathological lesions in sub-clinical carriers are not detectable.

4.1.3. Level III

Virus isolation using CK cells and RT-PCR can be used for detection of carriers. It is easy to detect virus from carrier or sick fish in summer, specially, when water temperature is 25-28^{o}C. It is difficult to detect virus in winter.

4.2. Presumptive Methods

4.2.1. Level I (gross signs)

Increased number of fingerlings of grass carp and black carp show clinical signs and die in summer (22-30^{o}C). Sick fish exhibit some or all of above clinical signs described in section 1.1 and 1.2 under Disease Information.

4.2.2. Level II (light microscopy)

Degeneration and necrosis in liver cells may be observed in histological sections. Hyperemia and hemorrhages in vessels of liver and spleen is also commonly seen.

4.2.3. Level III

By electron microscopy, virus particles that are orderly arranged in a lattice form could be observed in kidney (head-kidney) sections of infected fish.

4.3. Confirmatory Methods

4.3.1. Level I

There are no pathognomonic signs of infection with GCRV.

4.3.2. Level II

There are no suitable methods.

4.3.3. Level III

Isolation of virus from organs of fish and/or detection of viral RNA directly from organs of fish using PCR.

4.3.3.1. Virus Isolation

For virus isolation, the preferred tissue is kidney, spleen and liver of infected or moribund fish. Isolation using CK cells at 25^{o}C for 7 days and blind passage for 2 times. CPE usually appears 3-5 days after inoculation.

4.3.3.2. Nucleic Acid Assay

Reverse-transcription polymerase chain reaction (RT-PCR) assays can be used for identification of virus in cell culture or for detection of viral RNA in organs of fish. Two pairs of specific primers are presently available.

One set amplifies 697 bp in S10 fragment of GCRV-873 strain:
GV873S10R: 5'-ccccg atcat cacca cgat-3'
GV873S10F: 5'-cgcgt tcgct gatgt aagg-3'

Another set amplifies 320 bp in M6 segment of GCRV-14 strain:
GV14S6R: 5'-agttc tcaaa gctga gacag-3'
GV14S6F: 5'-acgtg cgatt ggaag agctt-3'

4.3.3.3. Immunoassays

ELISA is available to identify whether the virus isolated from cell culture is GCRV.

5. Control Methods

5.1 Vaccination

To reduce the serious losses of grass carp fingerlings due to this disease, vaccination has been

successfully used. An inactivated (autogenous) vaccine prepared from organs of infected fish has been applied in China. The vaccine can be easily prepared and is effective. Tissue-culture based vaccine is also available in China. As a result of on farm vaccination programs, the mortality of grass carp fingerlings has been significantly reduced.

5.2 Restrictions on movements and transportation of fish

5.3 Destruction of pathogens

To eliminate contaminated pond water as a source of infection, treat water with chlorine prior to draining (OIE, 2003). Restrict release of water into environment from farms

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Figures

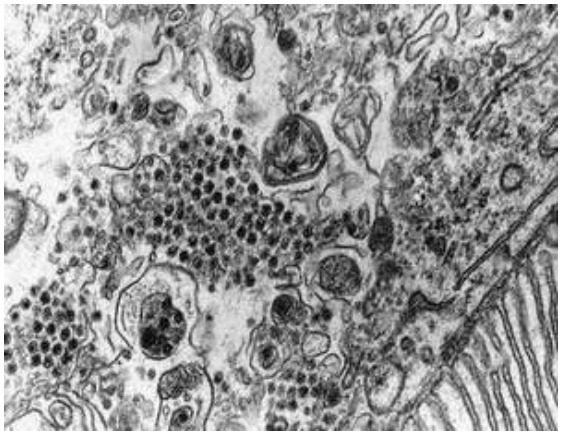

Fig.1 Electron micrograph of kidney section of grass carp with hemorrhagic disease. Note virus particles arranged in lattice form in cells

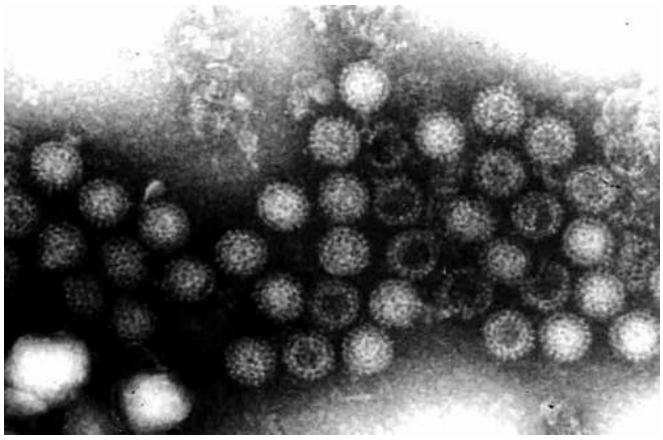

Fig.2 Electron micrograph of negatively stained grass carp Reovirus (GCRV).

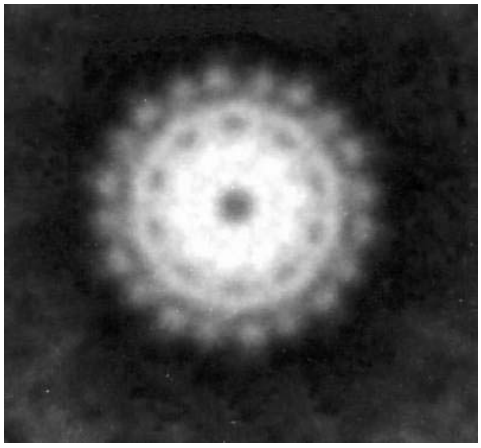

Fig.3 Electron micrograph of virus particles. 20 peripheral capsomers showed shape of sphericity with a diameter of 7-9 nm.

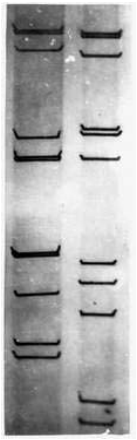

Fig.4 SDS-PAGE profile of viral RNA. left: GV-14, right: GV-873

Fig.5 Sick grass carp showing hemorrhage of fins.

Fig.6 Sick fingerlings. top: hemorrhage of musculature, bottom: hemorrhage of organs.

Fig.7 Sick fingerlings showing hemorrhage of musculature and organs.

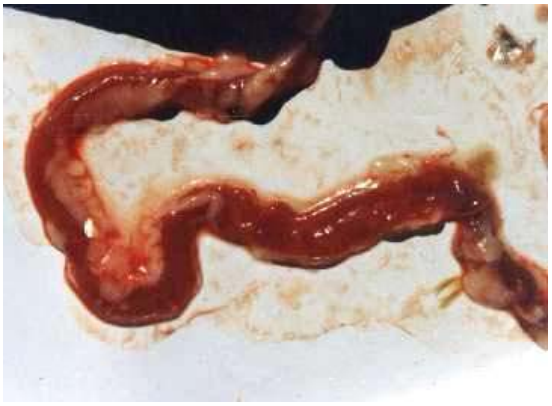

Fig.8 hemorrhage of intestine of grass carp.

Fig.9 Sick black carp showing hemorrhage of musculature and organs.

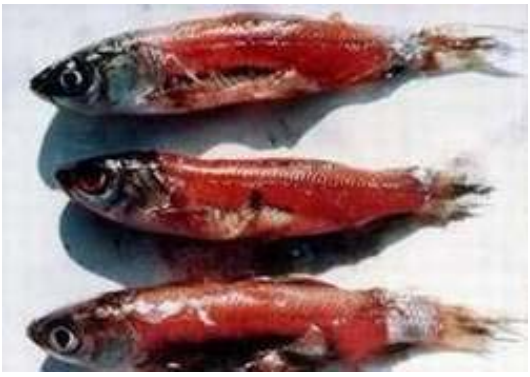

Fig.10 Sick fingerlings with mixed bacterial infection.

Fig.11 Inactivated vaccine from organs of sick fish.

Fig.12 immunization process in farms.