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Research Article

Temperature-regulated type II grass carp reovirus establishes latent infection in *Ctenopharyngodon idella* brain

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ABSTRACT

Grass carp reovirus (GCRV) causes extensive infection and death in grass carp and black carp fingerlings, with a highly seasonal prevalence. Previous studies suggested that GCRV can become latent after primary infection. In this study, we investigated type II GCRV (GCRV-II) latency in asymptomatic grass carp with GCRV infection or exposure history. We found that during latent infection, GCRV-II was detectable only in the brain of grass carp, unlike the multi-tissue distribution observed in natural infection. GCRV-II only caused damage to the brain during latent infection, while in natural infection, brain, heart, and eye tissues had relatively higher viral loads. We also discovered viral inclusion bodies in infected fish brains. Additionally, GCRV-II distribution in grass carp was notably affected by ambient temperature, with the virus targeting the brain only during low temperatures and multi-tissue distribution during high temperatures. This study provides insights into the mechanisms of GCRV-II latent infection and reactivation and contributes to the prevention and control of GCRV pandemics.

1. Introduction

Grass carp reovirus (GCRV) is a highly contagious and pathogenic aquareovirus with high mortality (up to 80%) in grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) fingerlings (Ahne, 1994; Rangel et al., 1999; Liang et al., 2014). Temperature is a key factor in GCRV replication, with natural diseases prevalent at a 25–28 °C water temperature range (Jiang, 2009). GCRV can affect fish of various ages but causes higher mortality in fry. The clinical signs of GCRV infection include lumpy or punctate hyperemia, such as gill, visceral, intestinal wall, and mesenteric congestion. Additional studies have determined that the virus can exist in various fish tissues, including kidney, gill, brain, swimming bladder, spleen, liver, muscle, intestine, and blood (Liang et al., 2014, 2016).

GCRV is a nonenveloped icosahedral spherical virus comprised of 11 double-stranded RNA genome segments surrounded by multiple concentric protein capsids (Cheng et al., 2008). It is classified into three types, with GCRV-II being the most virulent strain (Fang et al., 2005) family. VP56 is a fiber protein of approximately 56 kDa encoded by GCRV-II S7 and is one of the signature proteins used to detect the presence of GCRV. GCRV infection activates caspases and increases inducible nitric oxide synthase (iNOS) expression, thereby promoting cell apoptosis (Jia et al., 2014; Liang and Su, 2019). Due to the resulting destruction of the blood system and loss of the original buffering function, kidney and intestine damages are exacerbated, the circulatory system is impaired, resulting in fish acidosis (Liang et al., 2014).

Viruses can infect the central nervous system (CNS) of animals, including herpes virus, adenovirus, influenza A, poliovirus, rabies virus, reovirus, coronavirus, and Ebola virus (Kennedy, 2004; Baig et al., 2020; Liu et al., 2022). In particular, the mammalian orthoreovirus can quickly multiply and infect many mammals, including humans. Neonatal mouse CNS infection is one of the most characteristic models for reovirus pathogenesis. Among these reoviruses, serotype 1 (T1) and serotype 3 (T3) exhibit markedly different infection patterns (Forrest and Dermody, 2003). Following oral infection in neonatal mice, reoviruses are taken up by intestinal M cells (intestinal epithelial cell subset) and undergo primary replication in Peyer's patches of lymphoid tissue (Wolf, 1981) before attacking the CNS virally. T1 and T3 strains use different transmission routes and exhibit different pathological effects. T1 reovirus is transported through the blood to the CNS and infects ependymal cells, resulting in hydrocephalus. In contrast, T3 reovirus is transported by nerves to the CNS and infects neurons, leading to fatal encephalitis (Weiner et al., 1977; Tardieu et al., 1983; Morrison et al., 1991).

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Reovirus is a cytolytic animal virus that can establish persistent infection (Ahmed et al., 1981; Wetzel et al., 1997; Miszczak et al., 2013), meaning the host does not exhibit any symptoms, but the virus is not cleared and remains in specific cells. This type of persistent infection is also known as latent infection. It is the most remarkable viral property as it ensures their genetic information remains in their hosts for an extended period in the absence of productive infection (Dermody et al., 1995).

A bluetongue virus type 17 infection can be regulated by incubation temperature, becoming persistent (producing low viral levels), lytic (producing a high titer of the released virus), or latent (producing no detectable virus) (Hallum et al., 1984). However, reactivation from latency may occur under stress conditions; carriers will produce this virus and infect other fish (Lin et al., 2017). Therefore, investigating GCRV latency is imperative for developing strategies against GCRV infection. Most viruses require specific conditions for latency; thus, they only establish this in particular types of host cells and microenvironments (Lieberman, 2016). For example, the herpes simplex virus often remains latent in the trigeminal nerve (Furuta et al., 1992); this is also observed for beta herpesviruses in bone marrow, lymphoid tissue, and kidneys (Compston et al., 2009), some gamma herpesviruses in splenic B cells (Pollock et al., 1997), human cytomegaloviruses in $CD34^+$ myeloid progenitor cells (Sindre et al., 1996), and human papillomaviruses in basal epithelial cells (Feller et al., 2009). However, some viruses establish latency in multiple cell types. Human cytomegalovirus latent infection has been found in endothelial cells, macrophages, neuronal progenitors, and myeloid precursor cells (Taylorwiedeman et al., 1994; Belzile et al., 2014).

This study investigated GCRV-II latency in asymptomatic grass carp with GCRV infection or exposure history. During natural disease, grass carp brain, heart, and eyes contained relatively higher viral loads, and demonstrated different pathological damage types and degrees. However, different from viral multi-tissue distribution in natural infection, GCRV-II can only be detectable in grass carp brain tissue in latent infection, thus causing damage. In addition, viral inclusion bodies (VIBs) in the brain tissue suggest that the virus can still exist during this stage. As mentioned, GCRV-II distribution in grass carp can be affected by ambient temperature. As temperature decreases, the virus shifts from multi-tissue distribution to only infecting the brain, with reducing viral load. However, with a rise in temperature, GCRV-II latency can reactivate. Research on GCRV latency and reactivation will not only introduce a cornerstone in viral detection and control strategies but also shed light on the understanding of reovirus evolution.

2. Materials and methods

2.1. Fish and sampling

To investigate GCRV latency, we purchased 200 grass carp individuals (weighing 25–30 g) associated with a suspected GCRV outbreak in 2019 from Hanchuan City, China. These fish didn't display any symptoms of disease or physical damage on their body surface and were temporarily raised in a recirculating freshwater system at 28 °C for more than two weeks. They were fed twice a day with a commercial pellet diet at a rate of 2% body weight. Five GCRV latent infections were identified using reverse transcription PCR (RT-PCR). Tissues including gill, trunk kidney, spleen, head kidney, brain, hepatopancreas, intestine, swim bladder, heart, eye, skeletal muscle, caudal fin, skin, and peripheral blood were collected and divided into four sections. The first section was soaked in TRIzol and placed in a −80 °C freezer for RNA extraction, the second section was prepared as tissue protein samples and placed at −20 °C for Western blotting (WB), the third section was fixed in 10% neutral formaldehyde solution at 4 °C, and the last section was fixed with glutaraldehyde solution for transmission electron microscopy (TEM). As summer approaches and water temperatures rise, some grass carp develop natural GCRV infection. We collected these diseased fish tissue samples in the same way.

For experimental infection, we purchased 150 grass carp individuals (weighing approximately 15 g) without any GCRV exposure history from another fish farm (Huanggang City, China). Before infection, these fish were temporarily raised under the same conditions. Random individuals were selected to detect whether these fish were GCRV carriers through RT-PCR.

2.2. Virus and infection

The GCRV-II strain GCRV-097 was used in this study (Liang and Su, 2019). Fish with acute infection were all selected from the naturally infected fish. For experimental infection, grass carp were acclimated in aquaculture tanks 14 days prior, then were intraperitoneally injected with GCRV-097 (1×10^6 $TCID_{50}$/mL, 4 μL/g). Each tank had a heating rod to maintain the water temperature at 28 °C.

2.3. Antibodies and western blotting (WB) analysis

The *anti*-VP56 mouse polyclonal antibody (Ab) was prepared and conserved by our lab. Anti-β-tubulin rabbit monoclonal antibody was purchased from Abcam (Cambridge, UK). For WB analysis, protein extracts were separated by 8% SDS-PAGE gels and transferred onto nitrocellulose membranes (Millipore). Membranes were blocked in fresh 2% bovine serum albumin dissolved in a TBST buffer (25 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.1% Tween 20 [pH 7.5]) at 4 °C overnight, then incubated with the appropriate indicated primary Ab for 2 h at 37 °C. Nitrocellulose membranes were washed three times with a TBST buffer and incubated with a secondary Ab for 1 h at 37 °C. After washing four times with a TBST buffer, nitrocellulose membranes were scanned and imaged by Image Quant (GE, Boston, America). Results were obtained from five independent experiments.

2.4. RT-PCR and real-time quantitative RT-PCR (qRT-PCR)

Total RNAs were extracted with TRIzol (Simgen) and converted to cDNA using the Reverse Transcription Kit HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme Biotech Co., Ltd., Nanjing, China). RT-PCR was performed using gene-specific primers (Supplementary Table S1) and PCR reaction conditions of 94 °C, 5 min, followed by 94 °C for 30 s, 58 °C for 45 s, and 68 °C for 2 min, a total of 35 cycles; with a final extension at 72 °C for 10 min. PCR products were subjected to gel electrophoresis, and β-actin was used as the internal control gene. qRT-PCR was established in a Roche LightCycler® 480 system using BioEasy Master Mix (SYBR Green) (Hangzhou Bioer Technology Co., Ltd. Hangzhou, China). mRNA expression levels were normalized to the 18S rRNA expression level, and data were analyzed using the $2^{-\Delta\Delta CT}$ method. RT-PCR and qRT-PCR primers were designed by Primer Premier 5 software based on GenBank gene sequence information, shown in Supplementary Table S1.

2.5. Histopathological examination

The brain, heart, and eye tissues were fixed immediately with a 10% neutral buffered formalin for 24 h, dehydrated, paraffin-embedded, sectioned, and mounted on aminopropyl triethoxysilane-coated slides. Next, samples were deparaffined with xylene, rehydrated, stained with hematoxylin and eosin. The entire tissues of brain, heart, and eye were photographed for an overall display.

2.6. Immunofluorescence microscopy

Brain tissue was sectioned and fixed with xylene for 15 min, soaked with ethanol for another 15 min, then washed three times with phosphate-buffered saline (PBS). Next, sections were denatured with 0.01 mol/L SSC (1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate) at 95 °C for 15 min. Then, sections were incubated with 5% bovine serum albumin at 37 °C for 1 h. Next, sections were incubated

with primary mouse *anti*-VP56 Abs (1:1000) and secondary Abs fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (1:200; ABclonal) at 37 °C for 1 h, respectively, and stained with 1 mg/mL 4′,6-diamidino-2-phenylindole (DAPI) (1:1000) at 37 °C for 10 min. After washing three times with PBS, sections were observed through the UltraVIEW VoX 3D Live Cell Imaging System (PerkinElmer).

2.7. Transmission electron microscopy

Grass carp brain tissues infected with GCRV-II were fixed with 2.5% glutaraldehyde, and ultrathin sections were prepared as previously described. Images were viewed on an HT-7700 TEM (Hitachi). GCRV-II non-infected grass carp brain tissue was used as a negative control.

2.8. GCRV-II latency establishment

Twenty grass carp individuals were subjected to a cooling program to determine whether GCRV–II–infected grass carp can establish latent infection when the water temperature is lowered. Previous studies established that GCRV proliferation peaked on day 7 post-infection (Compston et al., 2009). Therefore, we injected fish with the virus at 28 °C, maintained the temperature for seven days to allow virus proliferation, and collected our first sample on day 7. Then, the tank water temperature was lowered from 28 °C to 4 °C at a rate of 1 °C per day. During this period, when temperatures reached 24 °C, 18 °C, 14 °C, and 4 °C, it was held constantly for three days, and three grass carp were sampled. Each fish was fed twice daily and maintained with a natural photoperiod. Fish were necropsied, and tissue samples were collected on day 7, 13, 21, 27, 39.

2.9. GCRV reactivation through temperature stress

To determine whether GCRV latency can be reactivated, the water temperature was decreased from 28 °C to 14 °C at a rate of 1 °C per day seven days post-infection. The water temperature was held at 14 °C for ten days before being raised back to 28 °C at a rate of 1 °C per day. When the water temperature was lowered to 14 °C and maintained for ten days, virus detection in different tissues and organs was determined.

2.10. Statistical analysis

Statistical analysis and presentation graphics were crafted using the GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA). Data were presented as mean ± standard deviation (SD) for at least three independent experiments. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. GCRV-II establishes latent infection in grass carp brain

To investigate GCRV-II tissue tropism, 14 tissues (gill, trunk kidney, spleen, head kidney, brain, hepatopancreas, intestine, swim bladder, heart, eye, skeletal muscle, caudal fin, skin, and peripheral blood) were sampled from natural disease and latent infection grass carp. RT-PCR and Western blot results showed that all 14 tissues and organs from GCRV-II natural disease grass carp were GCRV-positive, while in latent-infection grass carp, only the brain was GCRV-positive (Fig. 1A and B). ImageJ was used to analyze gray band values in RT-PCR and WB results. Results

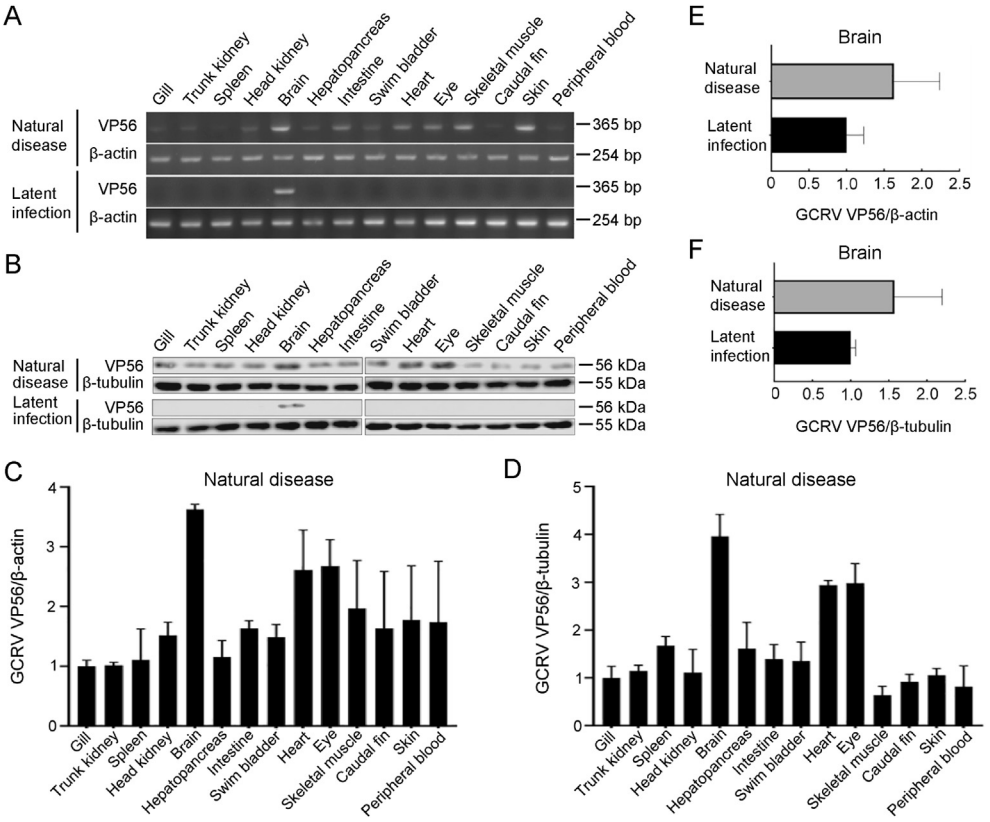

Fig. 1. Viral tissue tropism of natural and latent GCRV-II infection. Tissues were sampled from natural disease or latent infection grass carp. **A** VP56 mRNA expression levels in various tissues of grass carp were measured by RT-PCR. β-actin was used as an internal control (n = 3). **B** VP56 protein expression levels in various tissues of grass carp were analyzed by Western blot using VP56 polyclonal antiserum. β-tubulin was used as the internal control (n = 3). **C**, **D** Relative VP56 mRNA (**C**) and protein expression levels (**D**) in natural disease fish were analyzed by ImageJ software. **E**, **F** Relative VP56 mRNA (**A**) and protein expression levels (**B**) in brain tissues of natural disease and latent infection fish, analyzed by ImageJ software.

showed that the brain had the highest viral load, followed by the heart and eye (Fig. 1C and D). The viral load in the grass carp brain during natural GCRV-II infection was higher than that of latent-infection grass carp (Fig. 1E and F). These results suggest that the brain, heart, and eye have higher viral loads during natural GCRV-II infection, while GCRV-II becomes latent in the brain.

3.2. GCRV-II causes pathological damage to grass carp brain tissue during latency

The histopathological analysis was performed to explore GCRV-II latent infection effects on grass carp tissues (Fig. 2). Upon natural disease, grass carp brain tissue displays pathological changes like meningeal shedding, cytoplasmic enlargement, and loose brain matrix. Latently infected grass carp had milder pathological changes than natural disease, with a lesser extent and without cytoplasmic enlargement (Fig. 2A). Both the heart and eye showed clear pathological changes post-GCRV-II infection during natural infection, with severe damages observed to the heart and eye's basic structures. In contrast, latent-infected grass carp had no observable heart and eye pathological damage (Fig. 2B and C). These results indicate that upon latent GCRV-II infection, grass carp tissues suffer similar but milder pathological changes to those of natural disease.

3.3. Viral bodies found in natural disease and latent infection grass carp brain tissue

Indirect immunofluorescence experiments were performed to further determine if GCRV-II was responsible for pathological changes in grass carp brain tissues. Confocal microscopic results revealed that both natural disease and latent infection expressed the virus in brain tissue, and no green signal was observed in healthy grass carp brain tissue (Fig. 3A). Through TEM, we confirmed the presence of various virus particle forms and VIB in latently infection and naturally disease grass carp brain tissues (Fig. 3B). GCRV-II VIBs formed a distinct boundary in brain tissues. The scattered virus particles are round, 70–80 nm in size, have a uniform and clear structure, and are present in the brain cell cytoplasm (Fig. 3B). These results disclose that VIBs exist in brain tissues upon natural or latent GCRV-II infection.

3.4. Low temperature establishes GCRV-II latency

Epidemiological studies indicate that GCRV is primarily prevalent in summer when water temperatures are approximately 28 °C. In contrast, GCRV loses its infectivity when water temperatures are lower than 20 °C (Liang et al., 2016). To investigate GCRV-II viral tissue distribution under different temperature conditions, we manipulated environmental stress by artificially lowering water temperatures (Fig. 4A). Grass carp were infected intraperitoneally at 28 °C, and the cooling procedure was initiated. GCRV-II tissue distribution was detected through qRT-PCR under the five temperature gradients 28 °C, 24 °C, 18 °C, 12 °C, and 4 °C (Fig. 4B–F). At 28 °C, the virus had distributed to all grass carp tissues with the highest viral load in the brain, heart, and eye. As temperature decreased, viral distribution in different tissues diminished and decreased. When temperatures reached 4 °C, the virus was only detectable in the brain, indicating that low temperatures help GCRV-II establish latent infection in grass carp brain tissue.

3.5. GCRV-II can reactivate under temperature stress

To investigate whether GCRV-II can reactivate under temperature stress, we injected the virus into eight healthy grass carp at 28 °C and initiated the reactivation procedure (Fig. 5A). Viral mRNA was detected at day 50 post temperature variation in experimental fish. Two grass carp individuals died in the process, and four of the six remaining individuals were determined as GCRV-II positive through RT-PCR and clinical symptoms were reminiscent of natural disease and mainly manifested as muscle congestion (Fig. 5B). Virus distribution in the brain, heart, and eye of six grass carp individuals were detected with RT-PCR. Results conveyed that four of six grass carp individuals were detected GCRV-II positive. Only the brain tissue of three (3/4) tested GCRV-II positive, indicating that the virus may not successfully be activated and remain latent. Only one grass carp tested GCRV-II positive in all tissue, indicating that the virus had been reactivated (Fig. 5C). These results suggest that latent GCRV-II in low temperatures can reactivate through temperature stress.

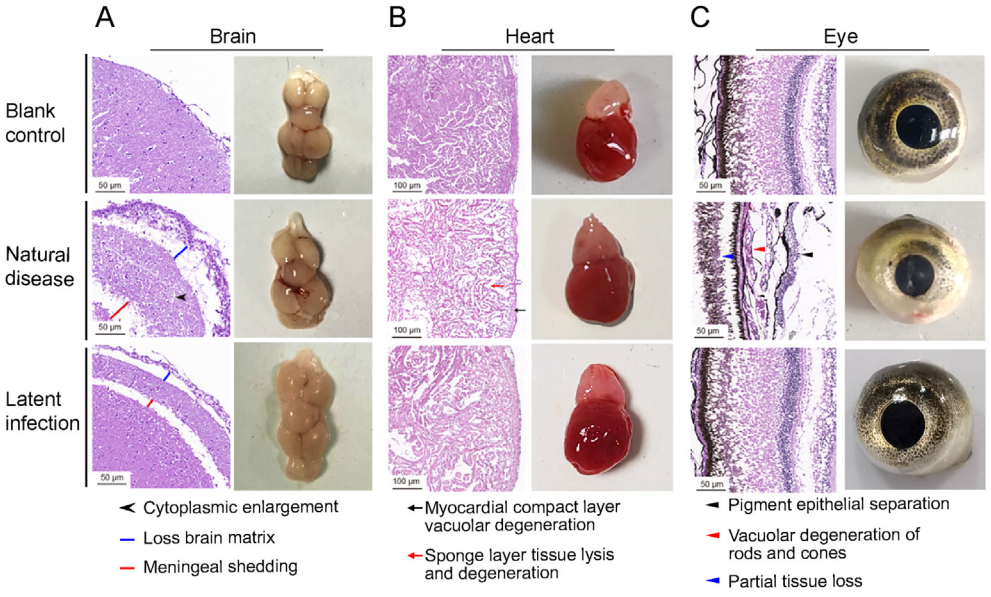

Fig. 2. Pathological damage of grass carp brain, heart, and eye caused by natural and latent GCRV-II infection. Histopathological analysis of brain (**A**), heart (**B**), and eye (**C**) with tissues from control, natural disease, and latent infection grass carp. Different symbols in the picture indicate different pathological symptoms.

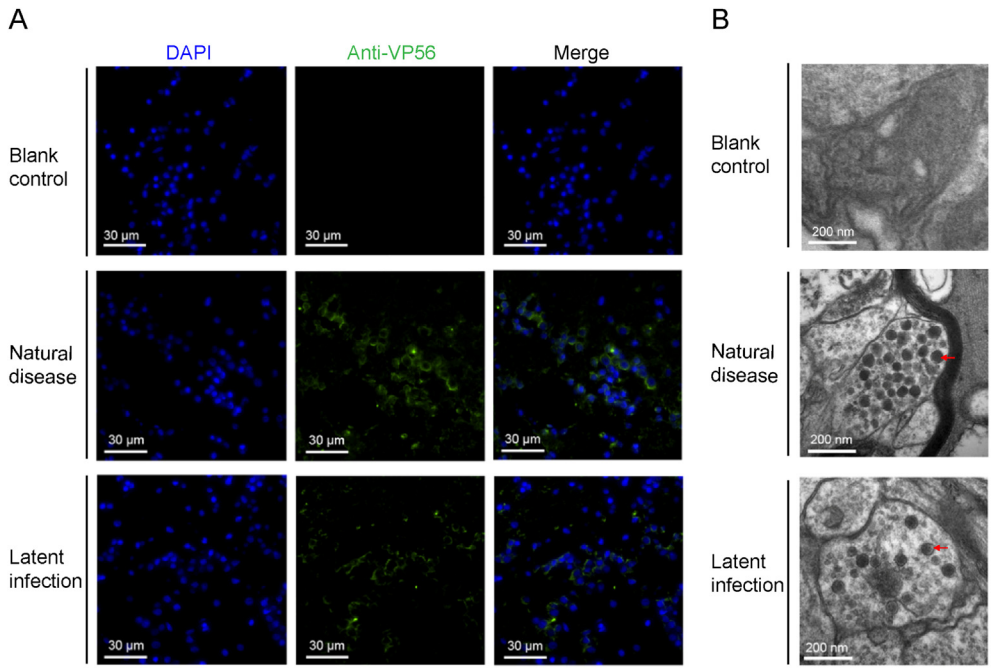

Fig. 3. Natural and latent GCRV-II infection immunofluorescence and TEM observations in grass carp brain tissues. **A** Immunofluorescence analysis of GCRV-II VP56 protein expression in brain tissues of natural disease, latent infection, or no infection. Green represents FITC-labeled fluorescent secondary Ab to detect GCRV-II's VP56 proteins, and blue represents DAPI-stained nuclei (scale bar: 30 μm). **B** TEM images of brain tissues from natural disease or latent infection grass carp. The red arrows represent virus particles.

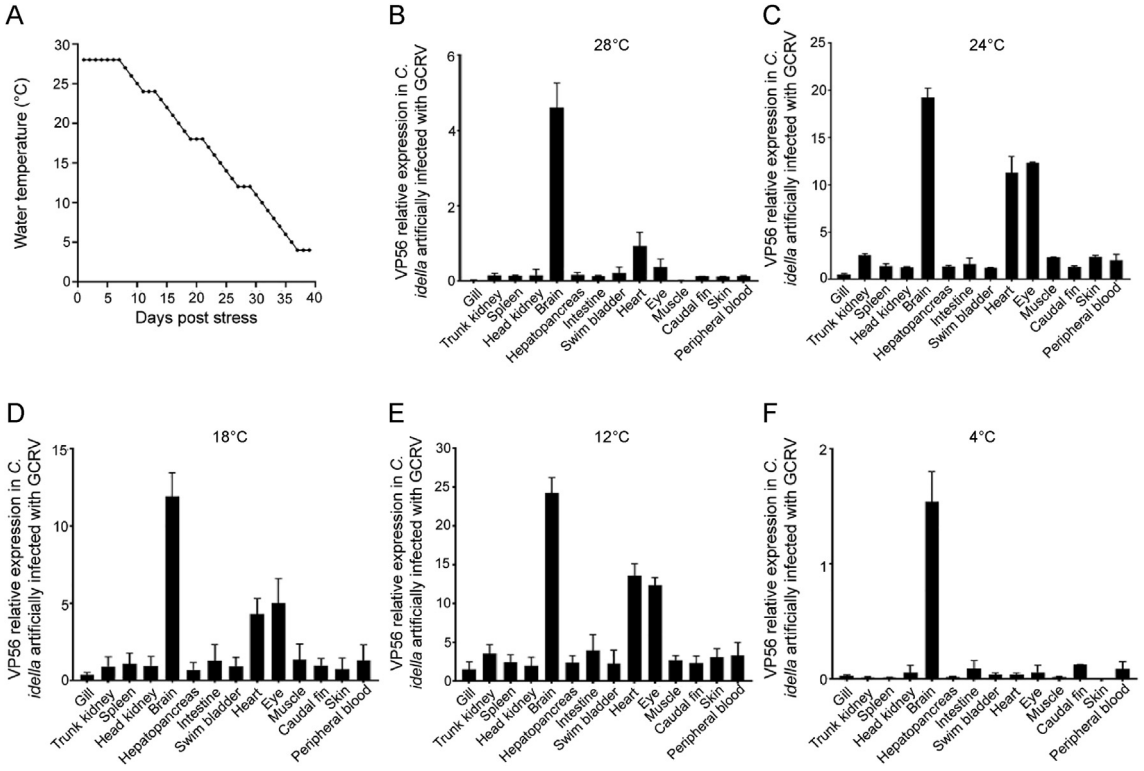

Fig. 4. Virus distribution under various temperature gradients post-GCRV-II experimental infection. **A** Experimental design of the temperature gradient. Grass carps were injected with the virus and then cultured at 28 °C for seven days. The water temperature was then lowered from 28 °C to 4 °C at a rate of 1 °C per day and stabilized at 24 °C, 18 °C, 12 °C, and 4 °C for three days. **B**–**F** VP56 mRNA expression levels in various tissues of grass carp infected with GCRV-II and subjected to different temperatures, measured by qRT-PCR (n = 3).

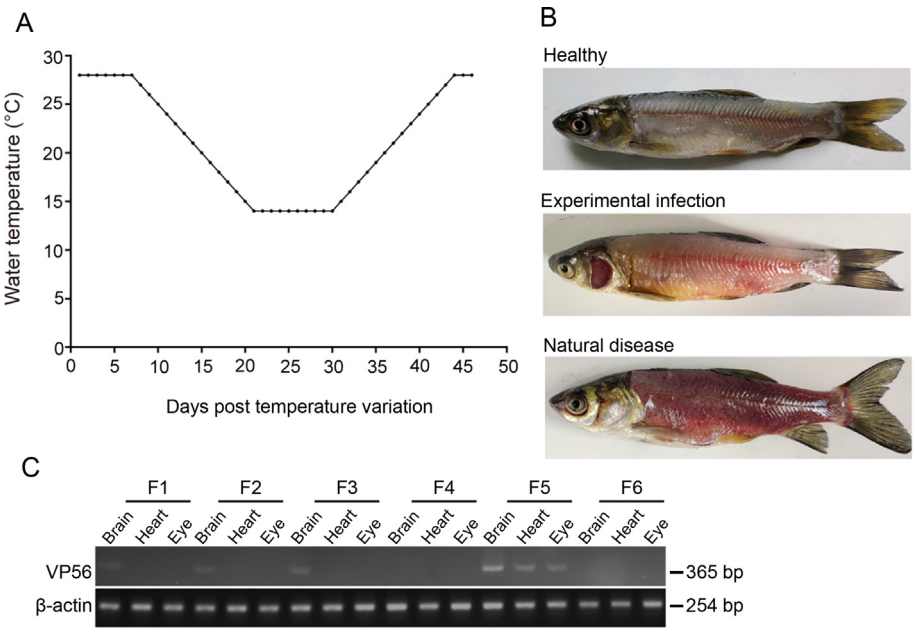

Fig. 5. GCRV-II latency was reactivated through a temperature regime. **A** Temperature reactivation model. After being injected with the virus, fish were cultured at 28 °C for seven days. The water temperature was then gradually lowered from 28 °C to 14 °C at a rate of 1 °C per day, and stabilized at 14 °C for nine days. Next, the water temperature was increased from 14 °C to 28 °C by 1 °C per day and then stabilized at 28 °C for three days. **B** Hemorrhage symptoms of grass carp at 50 day of the temperature reactivation experiments. **C** VP56 mRNA expression levels in brain, heart, and eye tissues of six grass carp individuals (F1–F6) at 50 day following GCRV-II experimental infection and temperature reactivation, measured by RT-PCR.

4. Discussion

In this study, we found that GCRV-II infection has a strong association with brain tissue compared with other organs in fish. The brain is an important neuroendocrine, sensory, and motor center, regulating various complex physiological functions and behaviors, and the highest viral load was observed during natural disease. Our findings suggest that the brain may serve as a "virus reservoir" for GCRV-II infection. Essentially, this means that when subjected to environmental stress or changes in the body's autoimmune conditions, the virus will spread to other tissues and cause clinicopathological symptoms at its entry site, as demonstrated by the herpes virus (Eide et al., 2011). This type of infection is highly similar to the pattern of latent infection, which is a type of persistent infection. Persistent infection is characterized by the fact that when a virus infects the body, the infected cells carry the virus for a long time and often expel the virus. The difference is that the virus does not replicate in the state of latent infection. When affected by the external environment or the immune state of the body is weak, the virus is activated and replicated, resulting in pathological symptoms. Virus latency is characterized by an infectious virus' absence everywhere except in the latent infection tissue (Eide et al., 2011). For instance, GCRV-II latency is only reported by the high viral load of GCRV-II in the brain. GCRV-II's ability to invade the brain is similar to mammalian orthoreovirus' neurophilic characteristics.

In addition to the brain, the heart and eye are two other sites with higher viral loads under natural disease conditions. Proven through experimental infection, the heart is the first tissue where the viral load increases as it is the center of blood circulation (Liang et al., 2014). Therefore, it is considered GCRV-II's "initial cycle point" in grass carp (Liang et al., 2016). As an organ connected to the brain through the optic nerve, the eye also has a high viral load and may provide a channel for viral entry and/or escape (Miner et al., 2016).

Histopathological examination was performed on the brain, heart, and eye of grass carp exposed to natural disease, distinguishing different pathological change types and degrees closely related to their high viral load. We also noticed that this was the first instance where pathological changes were noticeable in the grass carp heart's appearance under natural disease. This discovery can be used as an essential basis to diagnose grass carp hemorrhagic disease in the future. Viral latent infection is usually asymptomatic but can cause pathological damage in the host's latent site, resulting in chronic inflammation (Valyi-Nagy et al., 2000). Unlike natural infection, GCRV-II only appears in the brain during latent infection, and causes histopathological changes.

Environmental pressure exerted on the host can reactivate the latently infected virus (Chai et al., 2020). Previous studies have proven that GCRV prevalence is highly seasonal, with a significant incidence in summer when water temperatures are 25–30 °C (Liang et al., 2014). However, GCRV has been detected in grass carp cultured at 7.8–35 °C temperatures from February to November (Feng et al., 2022). This observation strongly suggests a GCRV latent infection. In our study, lowering the temperature modified the virus from multi-tissue to single-tissue distribution.

Moreover, increasing the water temperature can successfully reactivate the virus. Environmental stress and autoimmune condition changes are two decisive factors for the mutual transformation between natural and latent viral infection. We artificially created environmental stress by controlling water temperature and successfully established a latent GCRV-II infection model. In this model, the brain was determined as the primary target for latent GCRV-II infection in grass carp. The brain is vital in the host immune response and is also known as the "immune-privileged organ". Even the slightest inflammatory reaction will damage this organ's functional integrity. Immune responses either do not proceed in this area or proceed differently (Ferguson et al., 1997). For example, the blood-brain barrier prevents humoral immune factors from entering the brain (Miller, 1999). These results indicate that GCRV-II chooses the brain as a latent site to avoid the host immune system.

The initiation of viral replication includes adsorption, endocytosis, uncoating, and VIB formation (Brookes et al., 1993). VIBs are sites of viral synthesis and assembly (Rivas et al., 1998). The virus shape is approximately circular, with a double-layer capsid and a size of 70–80 nm (Su et al., 2021). We observed scattered GCRV-II virions and VIBs in the cytoplasm of grass carp brain cells for the first time. This finding further corroborates the previous results revealing viral presence in the

brain under latent infection. In addition, we observed that GCRV-II induces cytopathic changes in brain tissue (including cytoplasmic vacuoles), disordered endoplasmic reticulum arrangement, and nuclear membrane dissolution.

5. Conclusions

In this study, we found that the brain, heart, and eye exhibited relatively higher viral loads during GCRV-II infection, and GCRV-II can establish latent infection in brain tissue. Pathological results showed that these organs suffer different pathological damage types and degrees when exposed to natural disease, whereas the virus only causes damage to brain tissue during latent infection. In addition, ambient temperature can influence GCRV-II distribution in grass carp. When temperatures decrease, GCRV-II shifts from a multi-tissue distribution to appearing only in brain tissue. Furthermore, manipulating temperature stress can reactivate GCRV-II. This study provides significant insight into the mechanisms underlying GCRV-II infection in fish and identifies potential targets for viral detection and control.

Data availability

The datasets supporting the conclusions of this article are included within the article.

Ethics statement

All fish used in this study were cultured and cared at HZAU in accordance with Institutional Animal Care and Use Committee guidelines that was approved by the Scientific Ethic Committee of Huazhong Agricultural University.

Authors contributions

Rui Jiang: writing-original draft, data curation, formal analysis, visualization. Jie Zhang: investigation, data curation, formal analysis, visualization. Zhiwei Liao: investigation, formal analysis, validation. Wentao Zhu: investigation, methodology, validation. Hang Su: software, validation. Yongan Zhang: supervision, resources. Jianguo Su: conceptualization, funding acquisition, project administration, supervision, resources, writing-review & editing.

Conflict of interest

The authors have no financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.04.006.

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