Siniperca chuatsi rhabdovirus derived from Mandarin fish was pathogenic to Largemouth Bass

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Abstract

Background

Siniperca chuatsi rhabdovirus (SCRV) is one of the most important causativea gents of fish viral diseases, especially the virulent strains. SCRVcould cause significant loss and damages in the fish breeding industry. However, the pathogenesis of SCRV is unclear.

Methods

In the present study, two SCRV strains (SCRV-QY strain, SCRV-GM strain) derived from Mandarin fish was pathogenic to Largemouth Bass and the pathogenicity was revealed. The viral RNA copy numbers and innate immune activity detected by real-time qRT-PCRThe tissue sections were stained with hematoxylin-eosin (HE). And the sections of tissue were then examined by light microscope.

Results

Both of two strains were lethal to Largemouth Bass, which showed obvious clinical symptoms/pathology. The SCRV-GM strain was more virulent than the SCRV-QY strain and had higher mortality. Largemouth Bass infected with the SCRV-GM strain had higher viral RNA copy numbers than those of fishes infected with the SCRV-QY strain in all tissues. The viral RNA copy numbers were higher in the kidney, brain and spleen, which may be the target organ of virus. Additionally, the expression levels of innate immune activity-related genes, including Viperin, IRF-7, IRAK1 and Mx, were slightly up-regulated in the brain on the 7^{th} and 21^{st} day. Moerover, the Mx was slightly up-regulated in the intestine and spleen on the 7^{th} and 21^{st} day.

Conclusions

In the study, the two SCRV strains derived from Mandarin fish was lethal to Largemouth Bass and were characterized by systematic elucidation, which can help to understand the virus and diagnose the associated disease.

Introduction

Rhabdoviruses are causative agents of virulent and serious aquatic viral diseases in farmed fish (Fu et al., 2017). Fish rhabdoviruses have been well documented since the 1950s(Gadd, 2013). In the past decades, more than 20 rhabdoviruses have been identified in fish. Siniperca chuatsi rhabdovirus (SCRV) is a member of the Perhabdovirus that belongs to Rhabdoviridae(Zhou Guang-zhou, 2012).

SCRV has a bullet-like shape, which is a typical characteristic of the rhabdoviruses and was first reported in 1999(Zhang and Li, 1999). SCRV can infect different kinds of fishes and no validated therapy is available, which could result in mortality rates of up to 90%. Currently, the study of SCRV is mainly focused on the isolation and structural biology of the virus. However, the study of the tissue distribution

and dynamic changes of SCRV in tissues and organs is lacking. Furthermore, the pathogenicity and pathology of SCRV are not fully understood. In particular, the characteristics of highly virulent Siniperca chuatsi rhabdovirus strains are unclear.

The aim of the present study evaluate that two SCRV strains (SCRV-QY strain, SCRV-GM strain) derived from Mandarin fish was pathogenic to Largemouth Bass. The study clarified the pathogenicity, pathological changes, and dynamic changes after fish infected by SCRV-QY strain or SCRV-GM strain.

Materials And Methods

Cells and viruses

The Chinese perch brain cell line (CPB)was used for SCRV replication and was grown in Leibovitz's L-15medium (Gibco, USA) supplemented with 10% fetal calf serum (FBS;HyClone,USA)at 28°C. The SCRV-QY and SCRV-GM strains were obtained from the Pearl River Fishery Research Institute (Guangzhou, China). Largemouth Bass, with a body length of 7±1.0 cm and an average weight of 12.0±0.9g, were obtained from an SCRV-free zone in FOSHAN (Guangdong, China).

Fish pathogenicity experiments

Largemouth Basses (70 in each group) were intraperitoneally (i.p.) injected with 0.1 mL of 50TCID50 SCRV-QY or SCRV-GM; the control group was i.p. injected with 0.1 mL of PBS. The Largemouth Basses were monitored over 50 days for clinical signs.

Detection of viral RNA copy numbers by real-time qRT-PCR

Three largemouth Basses from each group were sampled from the first day to the fourteenth day, then on the twenty-first and twenty-eighth days. The different tissues were collected and were stored at -80°C until use.

The viral RNA copy numbers were detected by quantitative real-time RT-PCR (qRT-PCR).First, RNA was extracted using TRIzol reagent (Invitrogen, NY, USA) according to the manufacturer's protocol. Then, the isolated RNA was reverse transcribed using the First Strand cDNA Synthesis Kit (Takara, Japan). The cDNA was used as a template for qRT-PCR, which was performed using PremixExTaq^{TM} (PerfectRealTime) (Takara, Japan) to assay samples with the following SCRV N gene-specific primers: F, 5'-GACATGTTCTTCTACAGATTCAAC-3' and R, 5'-CAATCCAGCACTCCACTG-3'. The probe was 5'-AGGTTCAAAGACTGTGCAGCTCTGT-3', which was labeled on its 5'endwithFAMandonits3'endwithEclipse. To estimate virus replication, virus-specific mRNA expression was measured using qRT-PCR and was expressed as the number of RNA copies per mg of tissue. The results were analyzed using Applied Biosystems 7500software.

Detection of innate immune activity by real-time qRT-PCR

Three largemouth Basses from each group were sampled on the first, seventh, fourteenth, twenty-first, twenty-eighth and forty-second days. The different tissues were collected and stored at -80℃ until use.

First, total RNA was extracted using TRIzol reagent (Invitrogen, NY, USA) according to the manufacturer's protocol. The isolated RNA was then reverse transcribed using the TransScript first-Strand cDNA Synthesis SuperMix (Trans, Guangzhou, China). The cDNA was used as a templatefor qRT-PCR performed using TransStart Top Green qPCR SuperMix (Trans, Guangzhou, China) to assay samples with the following primers: GAPDHgene-specific primers: F, 5'-ATCAAGGAAGCGGTGAAGAAGG-3' and R, 5'-CGAAGATGGAGGAGTGGGTGTC-3'; IRF-7gene-specific primers, F, 5'-AGTGAAGGTGGTCCCTCTGA-3' and R, 5'-CGGCAGACCAAAGACAGAGT-3'; IRAK1 gene-specific primers, F, 5'-CGCTGTTAGCCGTTAGCCTG-3' and R, 5'-CGTAGAGAAACCGTCCCCTC-3'; Mxgene-specific primers, F, 5'-GGATTCTGACATCGGGAGCAA-3'; gene-specific primers, R,GTGCAGTAGACTCATGCTGTand Viperin gene-specific primers, F, 5'-CCAAGAGGGGCCTCAAACTT-3' and R, 5'-CTGACACTTGGGAGCTGGAG-3'.GAPDH was used as an internal control. To detect the innate immune activity, the mRNA expression levels of four specific innate immune-related genes were measured using qRT-PCR. The results were analyzed using Applied Biosystems 7500software.

Histopathological examination of liver, heart, spleen, kidney, brain and intestine

Three largemouth Basses from each group were sampled on the first, seventh and fourteenth days. The pieces of liver, heart, spleen, kidney, brain and intestine were fixed with paraformaldehyde and embedded with paraffin. The tissue sections were stained with hematoxylin-eosin (HE). The sections of tissue were then examined by light microscope.

Statistical analysis

All experimental measurements are expressed as the mean ± SE. The data were analyzed by one-way ANOVA and Student's t test using SPSS 13.0 statistical software.

Results

Fish challenge experiments

Both the SCRV-QY and SCRV-GM strains were virulent to Largemouth Bass. The cumulative mortality rate of Largemouth Bass infected with 50 TCID50 SCRV-GM was 48.6%, and that of SCRV-QY was 15.7% (Figure 1B). Most of the infected fishes died from the fourth day to the tenth day. Therefore, the SCRV-GM strain was more virulent and higher cumulative mortality than the SCRV-QY strain. The clinical signs of

fish infected with SCRV-GM or SCRV-QY were including muscle hemorrhage, swelling or hemorrhage of the liver and spleen and some skull deformation (Figure 1A).

Histopathological examination of the tissues

The spleens of Largemouth Bass infected with SCRV-GM and SCRV-QY showed that parenchymal cells were often loosely arranged in the tissue (Figure2 B, C) and the nuclei were deeply stained or dissolved; the heart section of Largemouth Bass infected with SCRV-GM and SCRV-QY showed a moderate degree of myocardial fiber atrophy and myocardial edema (Figure2 F, G); the intestines section of Largemouth Bass infected with SCRV-GM and SCRV-QY showed intestinal epithelium and necrosis(Figure2 I, J).

Viral RNA copy numbers in tissues of infected fish was determined by quantitative reverse transcription PCR (qRT-PCR)

The viral RNA copy numbers in tissues of infected fish were evaluated via qRT-PCR. The spleen, brain, kidney of infected fish showed significantly higher viral loads than that of heart, liver and intestine, suggesting that spleen, brain and kidney were primary target organs of the virus. The viral loads reached a peak during fourth to the sixth day post-infection in organs of infected fish. However, fish infected with the SCRV-GM strain had higher viral loads than that of SCRV-QY strain in all tissues, suggesting that SCRV-GM strain were more virulent than SCRV-QY strain (Figure 3).

The innate immune response detected by qRT-PCR

The expression levels of innate immune-related genes (Viperin, IRF-7, IRAK1 and Mx) were evaluated in infected fish by qRT-PCR. The expression levels of innate immune activity-related genes, including Viperin, IRF-7, IRAK1 and Mx, were slightly up-regulated in the brain on the 7th and 21st day. Moerover, the Mx was slightly up-regulated in the intestine and spleen on the 7th and 21st day, which was more significant in the fish infected with the SCRV-QY strain (Figure 4-5).

Discussion

SCRV is a fatal pathogen to Siniperca chuats, Largemouth Bass, and can cause a high mortality rate [5]. SCRV is expected to be transmitted to and to cause disease in other species of fish by the transportation of infected fish[6].To date, there is no effective antiviral treatment against SCRV infection. A better understanding of the viral pathogenicity and pathology of the high-virulence Siniperca chuatsi rhabdovirus strains will be helpful to control the disease.

In the present study, two virulent Siniperca chuatsi rhabdovirus strains were used: SCRV-QY strain and SCRV-GM strain. SCRV-QY strain and SCRV-GM strain derived from Mandarin fish was pathogenic to Largemouth Bass. The SCRV-GM strain may be more virulent than the SCRV-QY strain, as there were more deaths in the group infected with the SCRV-GM strain. The clinical signs of disease among the fish included muscle hemorrhage, liver and spleen swelling or hemorrhage and even some skull deformation after infection with the SCRV-QY or SCRV-GM strains.

There were morphologic changes in the spleen, heart and intestine after infection [12]. Fishes infected with the SCRV-GM strain had more significant pathological changes than fishes infected with the SCRV-QY strain, possibly because the SCRV-GM strain was more virulent than the SCRV-QY strain. Moreover, the pathological changes were more serious on the seventh day after infection than on the fourteenth day after infection.

Attenuated virus strains replicate quickly and express large amounts of antigen protein, thereby inducing strong adaptive immune responses that result in rapid virus clearance. In contrast, pathogenic virus strains replicate at a lower rate than attenuated strains and can reach their target tissues [7]. In this study, the SCRV-GM strain may be more virulent than the SCRV-QY strain; thus, it could be detectable for a longer time, with more copies detected in the tissues by qRT-PCR. The brain, spleen, kidney may be the target organs, as they had higher viral RNA copy numbers. By the way, the brain had higher viral loads than other organs, which maybe suggest that the blood-brain barrier of fish is very different from that of other animals.

During the pathogen-host coevolution, many viruses have developed ways to evade the host innate immune responses, particularly the IFN pathways [8]. For example, the oldest and most famous rhabdovirus-rabies virus has selected mechanisms to hinder the host interferon response to sustain infection[9]. The P protein, one of the five rabies virus proteins, has been identified as a crucial factor for the inhibition of the IFN system[10].Moreover, attenuated rabies virus activate the innate immune responses, including the IFN pathway and inflammatory reactions, but the pathogenic rabies virus strain SHBRV induces very little or no inflammation and little or no up regulation of gene expression in the IFN and inflammatory pathways[11].In the present study, innate immune factors, such as interferon signaling genes (IRF-7), interferon effector genes (Mx), interleukin-1 receptor-associated kinase1 (IRAK-1) or antiviral responses, such as antiviral protein (viperin), were analyzed. However, none of them induce dupregulation of gene expression in the IFN and inflammatory pathways. It maybe that SCRV-QY and SCRV-GM strains were pathogenic and could inhibit the IFN and inflammatory pathways.

Conclusion

In the study, the two SCRV strains derived from Mandarin fish was lethal to Largemouth Bass and were characterized by systematic elucidation. SCRV is a threat to fishes, especially the virulent strains. In the present study, the characteristics of the highly virulent Siniperca chuatsi rhabdovirus were revealed and showed that the pathogenicity and pathology were different when fish were infected with strains with

different levels of virulence. This study can help to understand the virus and the diagnosis of SCRV disease.

Declarations

Acknowledgments

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Author Contributions

Methodology, H.-R.L.and Z.-Y. F.; validation, X.-Z.F. and X,-T, Z., and X.L.; writing—original draft preparations, Q.L.and Y.-J.N.; writing—review and editing: L.-H.L., N.-Q.L. and X. L.., All authors have read and agreed to the published version of the manuscript.

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Ethics declariations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

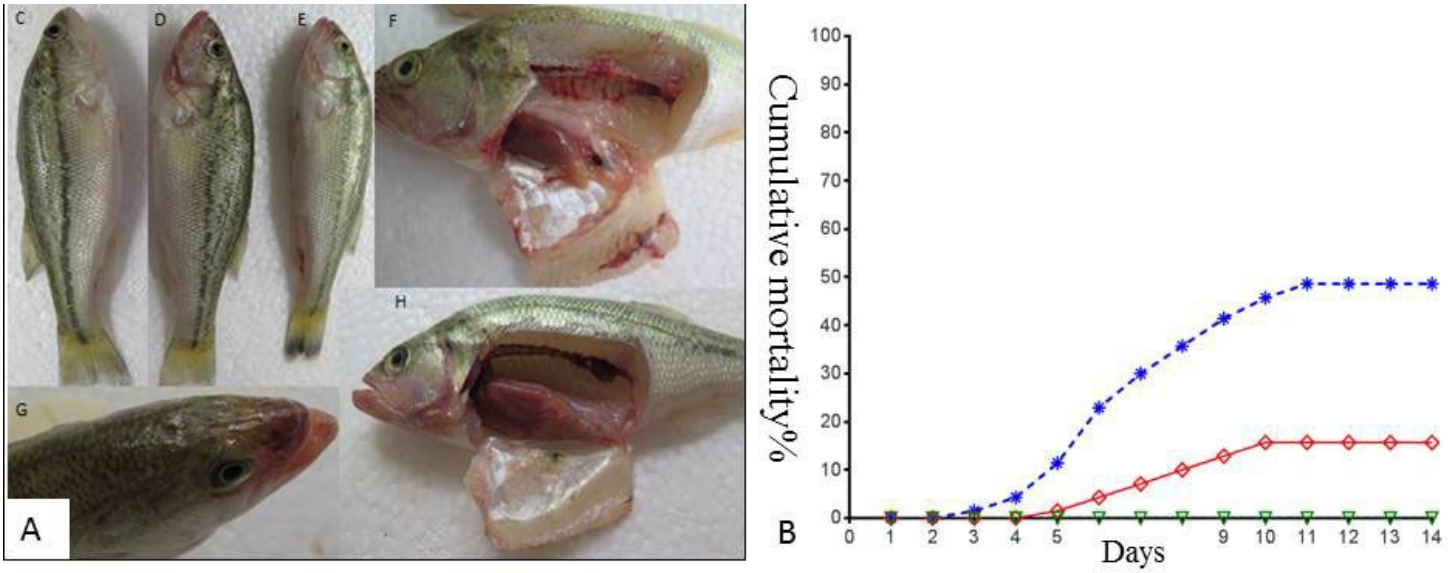

Figure 1

Figure 1

Largemouth Basses were infected intraperitoneally.

1A. Clinical signs of fish infected with SCRV. C. Largemouth Basses injected with PBS. D. Largemouth Basses infected with SCRV-GM; E. Largemouth Basses infected with SCRV-QY. F. Largemouth Basses infected with SCRV-GM showed swelling in the liver and spleaen. G. Largemouth Basses infected with SCRV-GM showed skull deformation. H. Largemouth Basses infected with SCRV-QY showed swelling in the liver and spleen.

1B.Cumulative mortality after fishes was infected i.p.

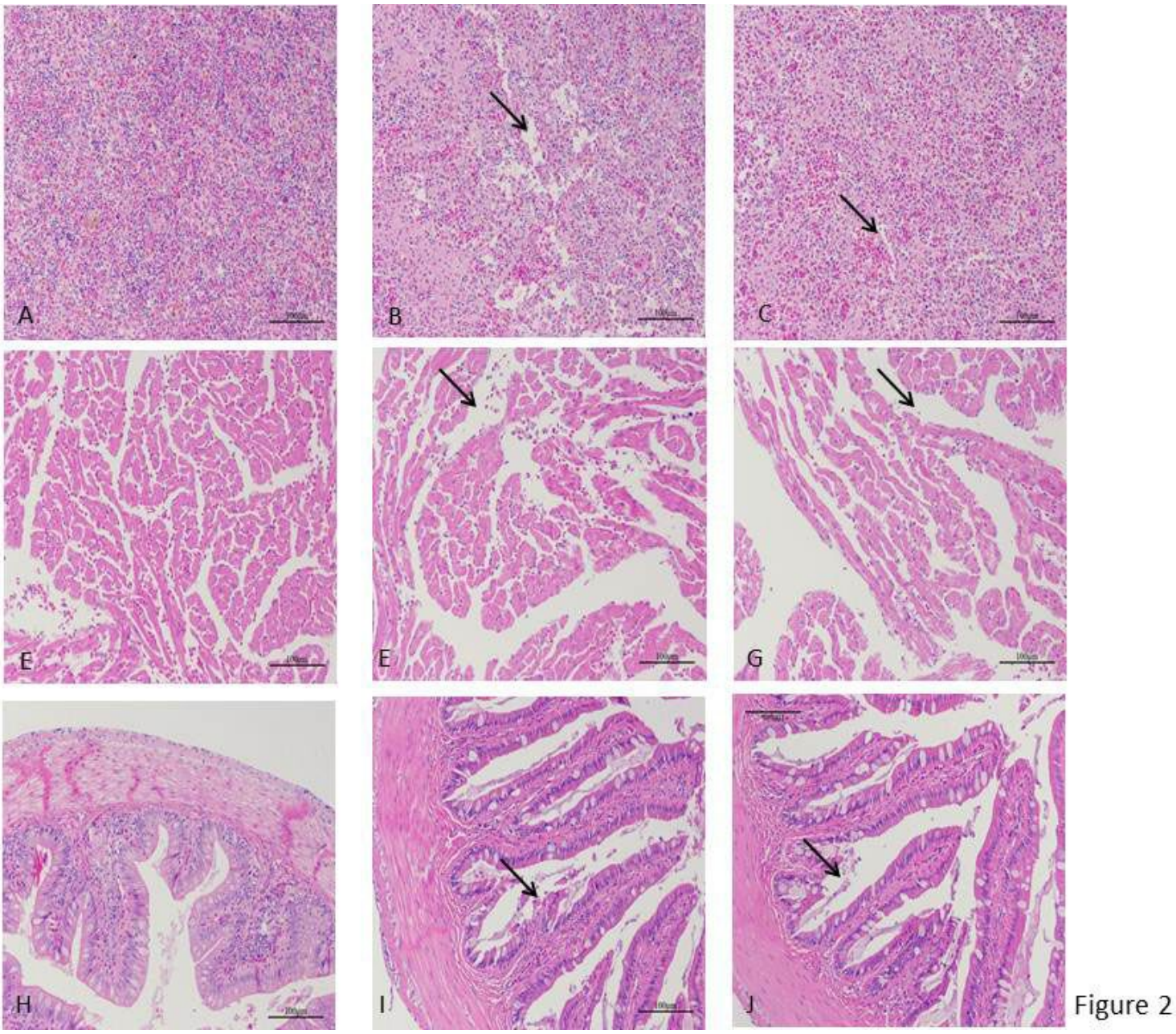

Figure 2

Figure 2

Histopathological examination showed pathological changes

A. A spleen section of a Largemouth Bass injected with PBS; B. A spleen section of a Largemouth Bass infected with SCRV-GM, The black arrow shows parenchymal cells loosely arranged in the tissue; C. A spleen section of a Largemouth Bass infected with SCRV-QY, The black arrow shows parenchymal cells loosely arranged in the tissue; E. A heart section of a Largemouth Bass injected with PBS; F.A heart section of a Largemouth Bass infected with SCRV-GM, the black arrow shows loosely arranged myocardial fibers; G. A heart section of a Largemouth Bass infected with SCRV-QY, The black arrow shows loosely arranged myocardial fibers; H An intestine section of a Largemouth Bass injected with PBS; I. An intestine section of a Largemouth Bass infected with SCRV-GM, the black arrow shows

intestinal epithelium and necrosis; J. An intestine section of a Largemouth Bass infected with SCRV-QY, the black arrow shows intestinal epithelium;

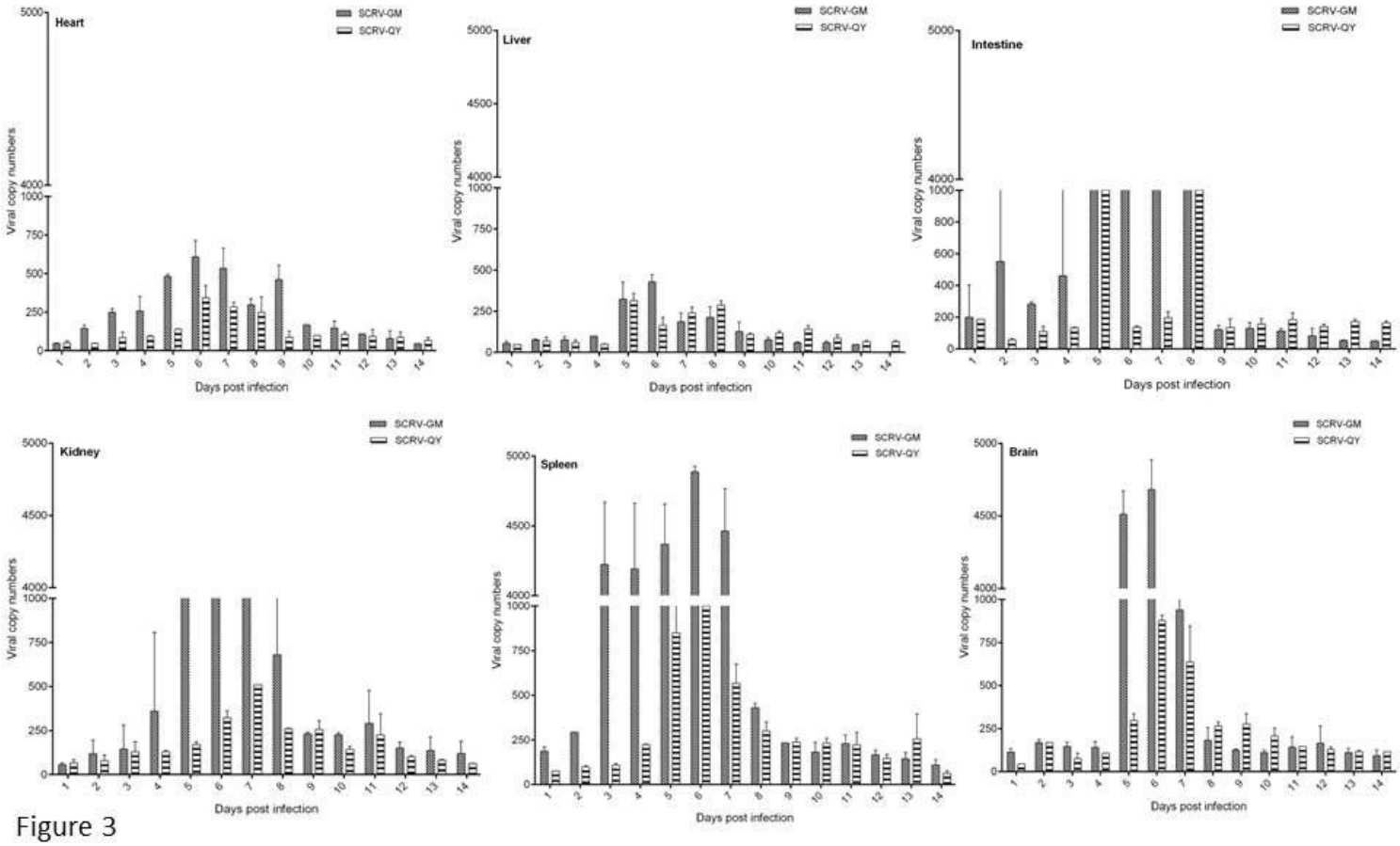

Figure 3

Figure 3

The viral RNA copy numbers were detected by real-time qPCR. The viral copy numbers in tissues were detected by real-time qPCR, including the intestine, liver, brain, spleen, kidney and heart.

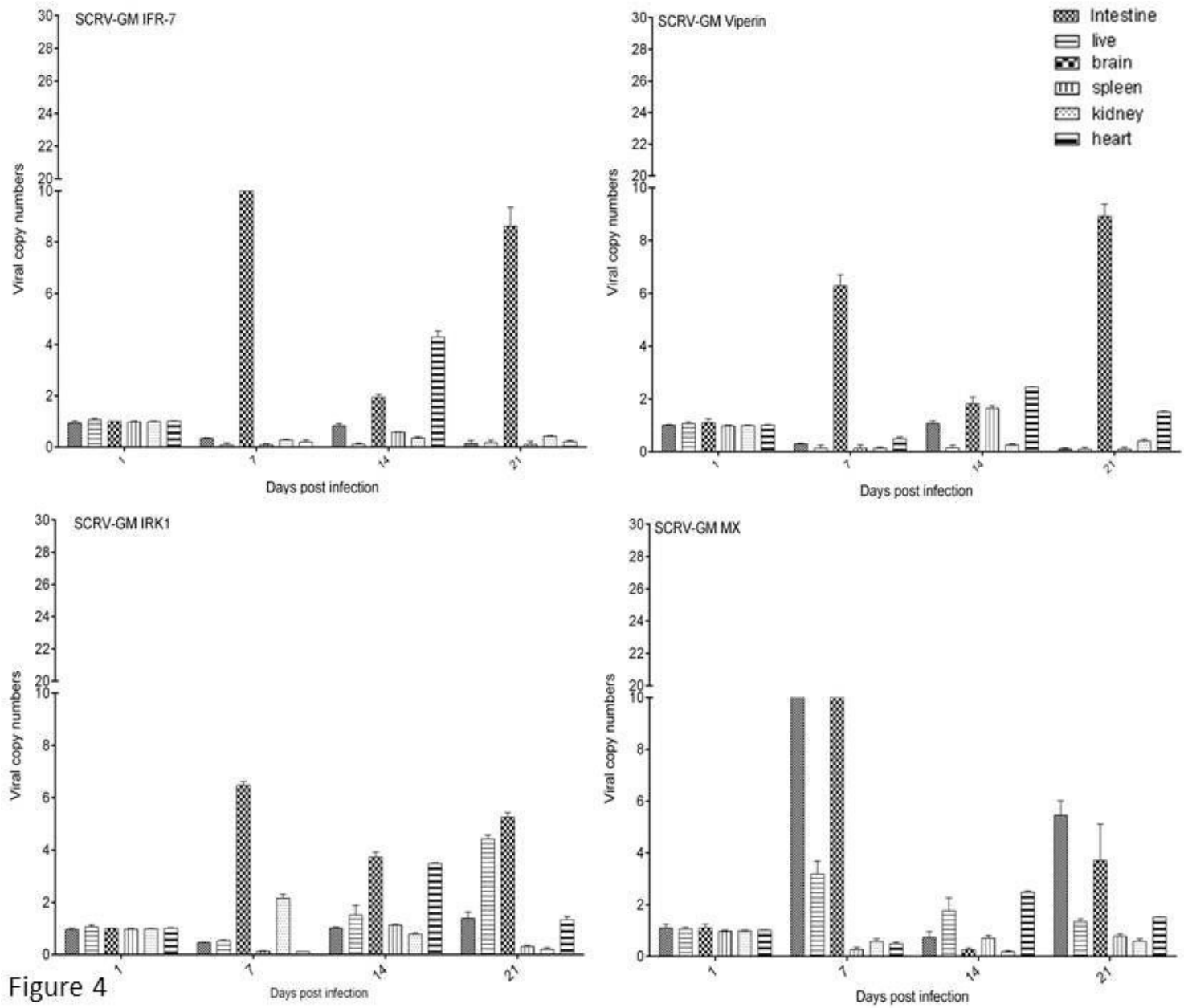

Figure 4

Innate immune activity of SCRV-GM was detected by real-time qPCR. The innate immune activity levels of tissues, such as intestine, liver, brain, spleen, kidney and heart, were evaluated via qRT-PCR. The expression levels of innate immune activity-related genes were detected, including Viperin, IRF-7, IRAK1 and Mx.

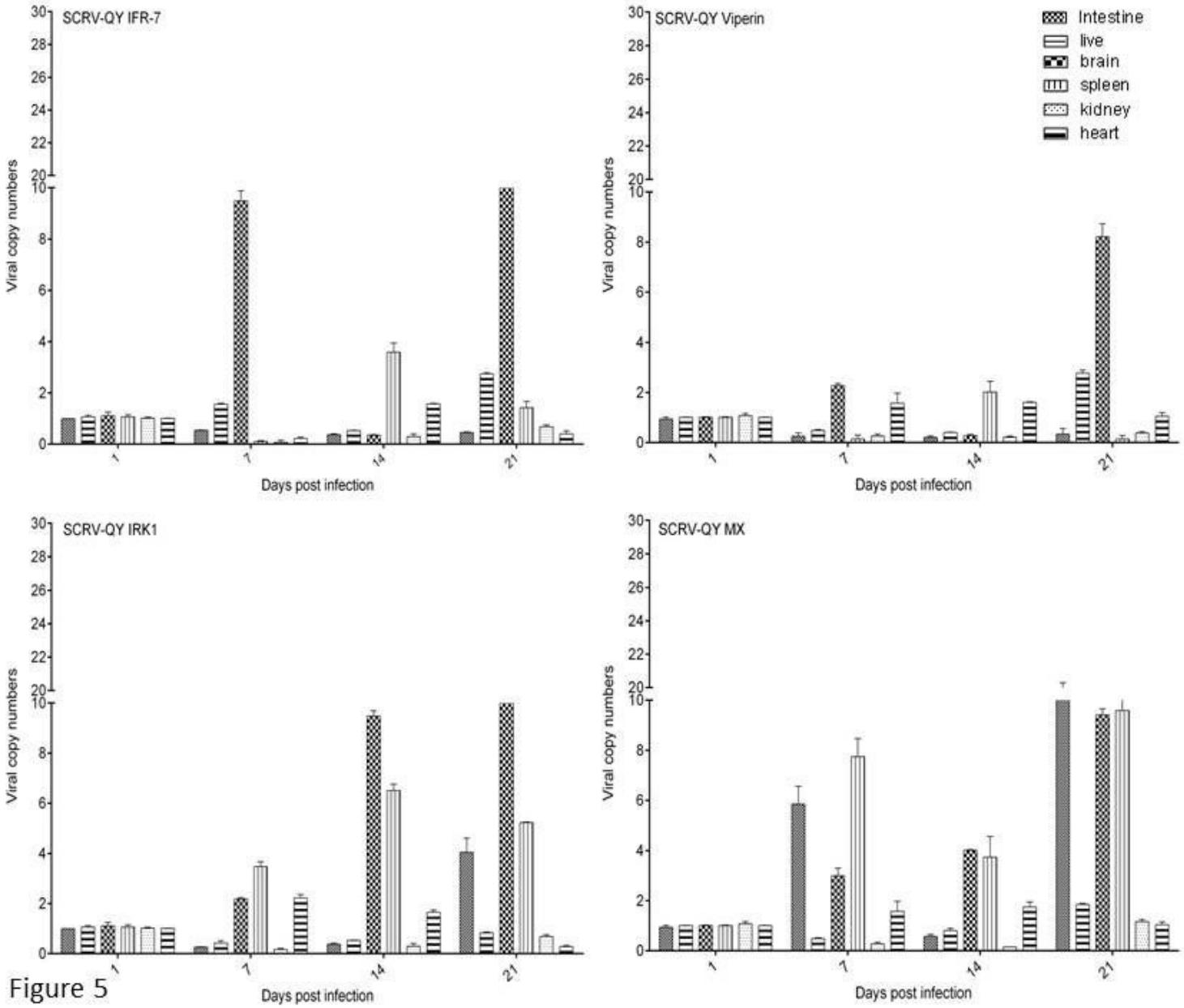

Figure 5

Innate immune activity of SCRV-QY was detected by real-time qPCR. The innate immune activity levels of tissues, such as intestine, liver, brain, spleen, kidney and heart, were evaluated via qRT-PCR. The expression levels of innate immune activity-related genes were detected, including Viperin, IRF-7, IRAK1 and Mx.