

# Feline Herpesvirus Infection of Snow Leopard

**Qiaoxing Wu**

Shaanxi Institute of Zoology

**Hongchao Wu**

College of Animal Science and Veterinary Medicine, Henan Agricultural University

**Shunfu He**

Qinghai-tibet Plateau Wild Zoo

**Yuxiu Liu**

National Research Center for Veterinary Medicine

**Yalei Chen**

National Research Center for Veterinary Medicine

**Xinzhang Qi**

Qinghai-tibet Plateau Wild Zoo

**Xiangyang Gu**

Huadong Medicine Co., Ltd

**Yifan Wen**

Chengdu Research Base of Giant Panda Breeding

**Xuelin Jin** (✉ [zhgpanda@163.com](mailto:zhgpanda@163.com))

Shaanxi Institute of Zoology

**Yipeng Jin**

College of Veterinary Medicine, China Agricultural University

**Kegong Tian**

National Research Center for Veterinary Medicine

---

## Research Article

**Keywords:** Feline herpesvirus Type 1, wildlife disease, Snow Leopard, pathology, epidemiological survey

**Posted Date:** November 30th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1097987/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

Feline herpesvirus type 1 (FHV-1) is a common causative agent of domestic cats rhinotracheitis and gradually threatens the wild felid worldwide. The endangered snow leopard belongs to the family Felidae and is also the top predator on the Tibetan Plateau. Herein, FHV-1 was identified and isolated from three dead snow leopards with symptom of sneezing and rhinorrhea. To explore the relationship between FHV-1 and their death, histopathology and molecular biology was performed. The organs and nasal swabs were collected for examinations of histopathology, the nucleic acid of the pathogen, viral isolation, and sequence analysis. The results reveal that all three snow leopards were infected with FHV-1. The first case primarily died of old cerebral infarction and secondary non-suppurative meningoencephalitis probably caused by FHV-1. The second case mainly died of renal failure accompanied by interstitial pneumonia caused by FHV-1. The third case was doubted to be related to the reactivation of latency of FHV-1. The gD and gE gene sequence alignment of the FHV-1 isolate strain revealed that the isolated strain originated from a domestic cat. Therefore, FHV-1 infection can cause different lesions of snow leopards and shows a high risk for the wild felid. We should focus more on protecting felid against threatening of FHV-1 infection originating from domestic cats.

## Introduction

Feline herpesvirus type 1 (order *Herpesvirales*, family *herpesviridae*, genus *Varicellovirus*, species *Felid herpesvirus 1*; FHV-1), known as the causative agent of feline rhinotracheitis, has been widespread in domestic cats<sup>1</sup>. The infection is often fatal to kittens; however, adult cats can generally survive and exhibit lifelong latency<sup>2</sup>. The initial clinical symptoms of FHV-1 in feline hosts are conjunctivitis, keratitis, and upper respiratory disease, in addition to occasional pneumonia<sup>3</sup>. Transmission of FHV-1 occurs mainly through direct contact between infected and susceptible animals, and vertical transmission has not yet been reported<sup>4</sup>. Although domestic cats are the main hosts for FHV-1, several cases of FHV-1 infections in wildlife have been reported recently<sup>5-7</sup>. FHV-1 infection has been found among European wildcats (*Felis silvestris silvestris*), sand cats (*Felis margarita*), leopard cats (*Felis bengalensis*), cheetahs (*Acinonyx jubatus*), mountain lions (*Puma concolor*), little spotted cat (*Leopardus tigrinus*), margay (*Leopardus wiedii*), ocelot (*Leopardus pardalis*), jaguarundi (*Herpailurus yaguarondi*), jaguar (*Panthera onca*), and south China tiger (*Panthera tigris amoyensis*) by serological or molecular methods<sup>8-12</sup>. In particular, the south China tiger, which died of infection with FHV-1, presented with clinical signs of sialorrhea, sneezing, and purulent rhinorrhea<sup>6</sup>.

The snow leopard (*Panthera uncial*, family *Felidae*) is a kind of rare and endangered animal and is known as the top predator on the Tibetan Plateau and its surrounding mountain ranges<sup>13</sup>. Its ranges these countries including Afghanistan, Bhutan, China, India, Kazakhstan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia, Tajikistan, and Uzbekistan. It is estimated that there are only approximately 4,000–7,000 individual snow leopards living in the wild now (Snow Leopard Network, 2014). Chinese Snow leopards are primarily distributed in Qinghai Province, Tibet, and Xinjiang autonomous regions. They are

also found in Gansu, Sichuan and Yunnan Provinces, and Inner Mongolia autonomous region<sup>4</sup>. In the past two decades, significant progress has been made in the conservation of snow leopards in China and poaching has been effectively curbed. With the development of tourism, pet infectious diseases have become a new threat for snow leopards, such as canine distemper<sup>14</sup>.

In December 2019, three snow leopards in the Qinghai-Tibet Plateau wild zoo had sneezing and rhinorrhea. Nasal swabs were collected and subjected to detection of potential pathogens such as canine distemper virus, FHV-1, feline calicivirus, mycoplasma, and chlamydia using real-time fluorescence quantification polymerase chain reaction (PCR). The results showed that FHV-1 was positive in three nasal swabs. Furthermore, virus isolation, immunohistochemistry, and other methods were used to analyze the characteristics of the snow leopard-origin FHV-1 strain. We firstly confirmed the infection and death cases with FHV-1 in the captive snow leopards in China.

## Results

### Pathological changes relative to FHV-1

In this study, the tissues fixed with formalin were histopathologically examined including the brain and tonsils of No. 1 and the lung, heart, liver, spleen, and kidney of No. 2.

In the case of No. 1, meningeal congestion and liquefactive necrosis region with a diameter of approximately 2 cm × 0.5 cm × 1 cm in the right cerebral hemisphere were observed (Fig. 1). Moreover, no obvious abnormality was observed in the other organs. The results of histopathology demonstrated meningeal hyperemia and hemorrhage, liquefactive necrosis of the cerebral cortex, a massive collection of foam cells, and hemosiderosis. Capillary hyperemia, bleeding foci, edema, vascular cuff reaction (lymphohistiocytic perivascular infiltrates), neuron necrosis, neurophagia, and demyelination reaction were visible in the brain parenchyma (Fig. 2). Therefore, the pathological diagnosis was old cerebral infarction and secondary non-suppurative meningoencephalitis.

In the case of No. 2, the bladder accumulated and dilated (20 cm × 10 cm in diameter), was full of 1050 mL dark red urine. The congestion presented on the surface of urinary bladder mucosa (Fig. 3A and 3B). The renal pelvis of the right kidney had effusion and dilation. The lung presented dull-red, swelling, and liquid leakage from the section (Fig. 3C). Moreover, there was no obvious abnormality in other organs. Microscopic examination revealed coagulation necrosis of massive glomerulus and tubules in the renal cortex, and bilirubin deposition in tubule epithelial cells (Fig. 4A). The bladder had severe autolysis and the mucosa structure was disordered and homogeneous. Pulmonary alveoli were expanded and filled with pink exudation. Many lymphocytes and exfoliative cells filled the bronchioli (Fig. 4B and 4C). Therefore, we can conclude that the pathological diagnosis was an acute postrenal renal failure owing to urinary retention and interstitial pneumonia.

### Detection of suspicious pathogens and FHV-1 distribution and location in tissues

The detection of suspicious pathogens in nasal swabs is shown in Table 1.

Table 1  
The clinical information and pathogen examination results of three snow leopards

Snow Leopards		No.1	No.2	No.3
Age		>10y	>10y	~10y
Gender		Male	Female	Male
Background disease		Cataract	The right leg fracture	ND
Clinical symptoms		sneezing, rhinorrhea and convulsion	sneezing, rhinorrhea and anuria	Sneezing and rhinorrhea
Outcome		Dead	Dead	Recovery but died 5 month later
Detection of suspicious pathogens	CDV	-	-	-
	FHV-1	+	+	+
	Feline calicivirus	-	-	-
	Mycoplasma	-	-	-
	Chalmydia	-	-	-
Detection of FHV-1	Nasal swab	+	+	+
	Tonsil	-	ND	ND
	Lung	+	+	ND
	liver	-	-	ND
	Spleen	-	-	ND
	Kidney	-	-	ND
	Brain	-	ND	ND
Note: +, positive; -, negative; ND, No data. The method of detection of suspicious pathogens is real-time qPCR. The method of detection of FHV-1 in tissues is PCR.				

The FHV-1 was found to be distributed in lungs and nasal swabs by PCR test. Liver, spleen, kidney, and tonsils were negative. The details are shown in Table 1.

The immunohistochemistry results showed that lung of No. 2 was positive and FHV-1 was primarily located in the cytoplasm of epithelial cells of a bronchial bronchiole and exfoliative cells (Fig. 4D).

### **Viral isolation and detection**

At 36 h post-inoculation with nasal swab supernatant of No. 2, FK81 cells showed an obvious cytopathic effect (CPE), characterized by round, pyknosis, fusion, aggregating like “Fleece-Pulling” (Fig. 5A). PCR revealed that culture inoculating with nasal swab was positive for FHV-1. Much positive signal (green) of FHV-1 was located in the cytoplasm of FK81 cells by indirect immunofluorescence (Fig. 5B).

### **Phylogenetic analysis based on gD and gE genes of FHV-1**

In this study, the three isolates shared 100% identity on gD (GenBank accession number: OK087390) and gE gene; therefore, the FHV-1 isolates from snow leopard were named SL/QH/2019. According to the alignment, SL/QH/2019 shared 99.9% identity with isolates of FHV-1 from cats and 99.7% identity with isolates of FHV-1 from tiger in China on gD gene. However, SL/QH/2019 shared a low identity (from 30.6–50.8%) with canine herpesvirus type 1 (CHV-1) on the gD gene (Fig. 6A).

Similarly, SL/QH/2019 shared 100% identity with isolates of FHV-1 from cats; however, low identity (from 27.6–61.7%) with CHV-1 on gE gene (Fig. 6B).

In particular, SL/QH/2019 is highly homologous with primarily epidemic isolates (Fig. 7B▲) in China.

The phylogenetic tree based on the gD and gE gene sequences showed that the isolate investigated in this study was closely related to the isolates of FHV-1 from cats (Fig. 7A and 7B), a result consistent with the alignment analysis.

## **Discussion**

FHV-1 existing in snow leopards has been found by next-generation sequencing using serum and rectal swab samples<sup>5</sup>; however, there is no clinical case report about snow leopards. Herein, three snow leopards infected with FHV-1 presented different lesions and clinical passages. FHV-1 often causes feline viral rhinotracheitis, ocular disease, ulcerative dermatitis, and pneumonia<sup>3; 7; 15</sup>. The pathogenesis of FHV-1 is based on two different mechanisms<sup>16</sup>. The first mechanism is that FHV-1 as a cytolytic virus can damage the epithelial cells of mucosae and cornea leading to ulceration. The second mechanism is immune-mediated reaction driven by antigenic stimulation<sup>16</sup>. In this study, all of the snow leopards with obvious sialorrhea and sneezing symptoms have been first confirmed to be infected with FHV-1 using real-time qPCR.

In the case of No. 1, foam cells and hemosiderosis showed that cerebral infarction was old<sup>17; 18</sup>. The meningoencephalitis was in the acute stage and an obvious demyelination reaction was visible in the white matter according to the clinical neural symptom of No. 1. It has been reported that one white-handed gibbon died of cerebral infarction and myocardial fibrosis with herpes simplex  $\alpha$  and Epstein-Barr

virus has been reported; however, the viral infection was not considered the main cause of death<sup>19</sup>. In cats and dogs, non-suppurative meningoencephalitis is frequently found and these pathogens include porcine herpesvirus 1, parvovirus, feline infectious peritonitis virus, feline leukemia virus, West Nile virus, and encephalomyocarditis virus are found in the central nervous system of dogs and cats with non-suppurative meningoencephalitis by immunohistochemistry. Therefore, the primary or virus-triggered secondary immune-mediated mechanisms cannot be ignored<sup>20</sup>. FHV-1 has also been reported as a causative agent of severe nonsuppurative meningoencephalitis in domestic cats<sup>21</sup>. In this study, though FHV-1 was not detected in organs other than the lung of No. 1 using PCR, the vascular cuff reactions and demyelinating lesions were generally suggestive of a viral etiology in the brain<sup>22</sup>. Additionally, herpesvirus is a common causative agent in humans and animals<sup>23-25</sup>. Thus, the FHV-1 infection may be relative to the non-suppurative meningoencephalitis of No. 1.

In the case of No. 2, lung, kidney, and urinary bladder presented the obvious pathological change. Histopathology examination showed renal failure owing to uroschisis and interstitial pneumonia. Similarly, only the lung was positive for FHV-1 using PCR and immunohistochemistry. Owing to the cataract of the right leg, the activity of No. 2 might be limited which would induce neurothlipsis of the urinary bladder<sup>26</sup>. This may lead to acute postrenal renal failure, according to the symptoms of anuria. To date, urinary system diseases with FHV-1 have not been reported. Using a pathological examination, immunohistochemistry, and PCR, pneumonia with FHV-1 infection became clear. Firstly, the lung was characterized by interstitial pneumonia, which was primarily caused by a viral infection in morphology, and immunohistochemistry demonstrated the antigen of FHV-1 located in the epithelial cells of a bronchiole<sup>22</sup>. Secondly, detection of the gD gene was also positive. According to the report, FHV-1 targets both respiratory epithelial cells and pneumocytes and enters the lung. Moreover, the FHV-1 can make infectious cells dead via apoptosis or inducing neutrophil infiltration<sup>3</sup>. In this study, FHV-1 was mainly located in the epithelial cells of the bronchiole with little neutrophil infiltration. However, massive necrotic cast-off cells are visible in the bronchiole. That also proves that the pathway of FHV-1 shedding is primarily the respiratory tract. Therefore, it is considered that No. 2 died of the combined effect of renal failure and FHV-1 pneumonia.

However, No. 3 recovered after 2 weeks of treatments and then died 5 months later. Since it was neither autopsied nor stored in any samples, the relationship between its death and FHV-1 infection was not clear. However, No. 3 was infected with FHV-1 and presented obvious clinical symptoms. Different from No. 1 and No. 2, No. 3 had no background disease clinically. No. 3 recovered gradually from infection underlying treatment before death. This may be relative to the reactivation of latency of FHV-1 that is a hallmark of alphaherpesvirus biology<sup>7</sup>. When animals suffer stress, viral reactivation easily occurs spontaneously<sup>27</sup>. In this case, FHV-1 poses a greater threat to sick snow leopards, similar to that FHV-1 primarily affects kittens and juvenile cats and persists lifelong of the host<sup>1</sup>. Therefore, the animal recovered from FHV-1 infection should be fed separately for further observation.

Furthermore, a nasal swab of No. 2 was used for the isolate of FHV-1 in F81 cells, and the obvious CPE was visible. Immunohistochemistry showed that FHV-1 isolate replicated in cells. The nucleic acid of FHV-1 was also detected in infected cells. The results further confirmed that snow leopards were infected with FHV-1.

Research shows that FHV-1 has only one serotype and is relatively homogenous genetically<sup>1</sup>. To explore the infectious origin, the sequence of gD and gE genes was analyzed and phylogenetic trees were constructed. The gD protein probably had host-selective and stimulated the host to produce high cellular immunity and anti-gD antibody<sup>28</sup>. While the gE protein is mainly related to the virulence of FHV-1<sup>29</sup>. The results showed SL/QH/2019 was highly homologous with the mainly epidemic isolates of cats in China. Thus, the origin of FHV-1 infecting snow leopards was probably from feral cats in the zoo. Additionally, the identity between these isolates and tiger isolate is lower compared to cat isolates. The genomic variation is not necessary for cross-species transmission of FHV-1 and FHV-1 from a domestic cat that can directly infect other wild felids.

The severity and symptoms of FHV-1 infection are not relative to the viral genome variants, and it is most likely due to other factors such as host response<sup>30</sup>. Therefore, the different pathological changes of No. 1 and No. 2 snow leopards may be the result of different background diseases.

## Conclusions

In this study, the first clinical cases of snow leopards dying of FHV-1 infection are described. The relationship between FHV-1 and the causes of death of three snow leopards has been explored using multiple methods. FHV-1 may induce massive death of wild felid with severe background disease. Therefore, administrators of the zoo and natural reserves should focus more on the threat of FHV-1 from domestic cats.

## Methods And Materials

### Case Descriptions

Three dead snow leopards in the Qinghai-Tibet Plateau Wild Zoo presenting with sneezing and rhinorrhea were numbered 1, 2, and 3. Their clinical information is shown in Table 1.

### Sample collection and pathological examination

After their deaths, an autopsy was performed for No. 1 and No. 2 snow leopards. The details of samples are shown in S1.

The frozen samples were sent for PCR examination. The samples fixed by 4% neutral formalin were processed to the paraffin section and were stained using hematoxylin-eosin for pathological examination. Additionally, the fixed tissues were immunohistochemically stained with murine monoclonal antibody 4G12 of FHV-1 antigen (ProtTech, China), and observed under an optical microscope and photographed.

## PCR Assays

According to the symptoms, we firstly detected the potential pathogens including canine distemper virus (CDV), FHV-1, feline calicivirus, mycoplasma, and chlamydia in nasal swabs using T8 real-time fluorescence quantitative PCR instrument and its commercial kit (Manufactured by Beijing Anheal Laboratories Co., Ltd. China).

Furthermore, viral genomic DNA was extracted from nasal swabs and tissue samples using a DNA Viral Genome Extraction Kit (D2400, Solarbio, China), subjected to PCR. The complete genome of the glycoprotein D (gD) gene and glycoprotein E (gE) gene of FHV-1 were amplified. The primer sequences and conditions of PCR are shown in S2.

## Virus isolation

The nasal swab from No. 2 was made into supernatant using phosphate buffer solution (0.1 mol/L, pH 7.4, PBS) and filtered using 0.22 µm filter membrane for sterilization. The viral culture refers to the method described by Zhang<sup>31</sup>. The F81 cells were selected for replication of FHV-1. F81 cells infected with FHV-1 were detected using PCR and indirect immunofluorescence using murine monoclonal antibody 5H8 of FHV-1 antigen (ProtTech, China).

## Sequence analysis and phylogenetic tree construction

The sequences of the gD and gE genes were analyzed and compared with other FHV-1 strains published in NCBI using MegAlign (7.1). The phylogenetic trees were constructed based on the gD and gE genes using MEGA-7 software (7.0).

## Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. All relevant guidelines for the use of animals in scientific studies were followed. The study did not include any experimentation on animals or humans, and samples were taken from natural dead animals that was approved by the owner of animal.

## Declarations

### Acknowledgments

We thank professor Zhenxuan Liu at Taiwan University for pathological observation.

### Author Contributions

X.L.J., Y.P.J., T.K.G. and Q.X.W. planned the assessments. Q.X.W., S.F.H., Y.X.L, Y.L.C., Q.X.Z. and Y.F.W collected samples and performed laboratory analysis. Q.X.W., X.Y.G., and X.L.J. coordinated logistics, Q.X.W and X.L.J. provided the funding. All authors have read, commented, and approved the manuscript

## Funding

This study was supported by the Youth Joint Innovation Project of Shaanxi Academy of Sciences (No.2020k-12) and High-level Talent Introduction Plan of Shaanxi Academy of Sciences (No.2019K-15)

## Conflicts of interest statement

The authors declare no conflict of interest

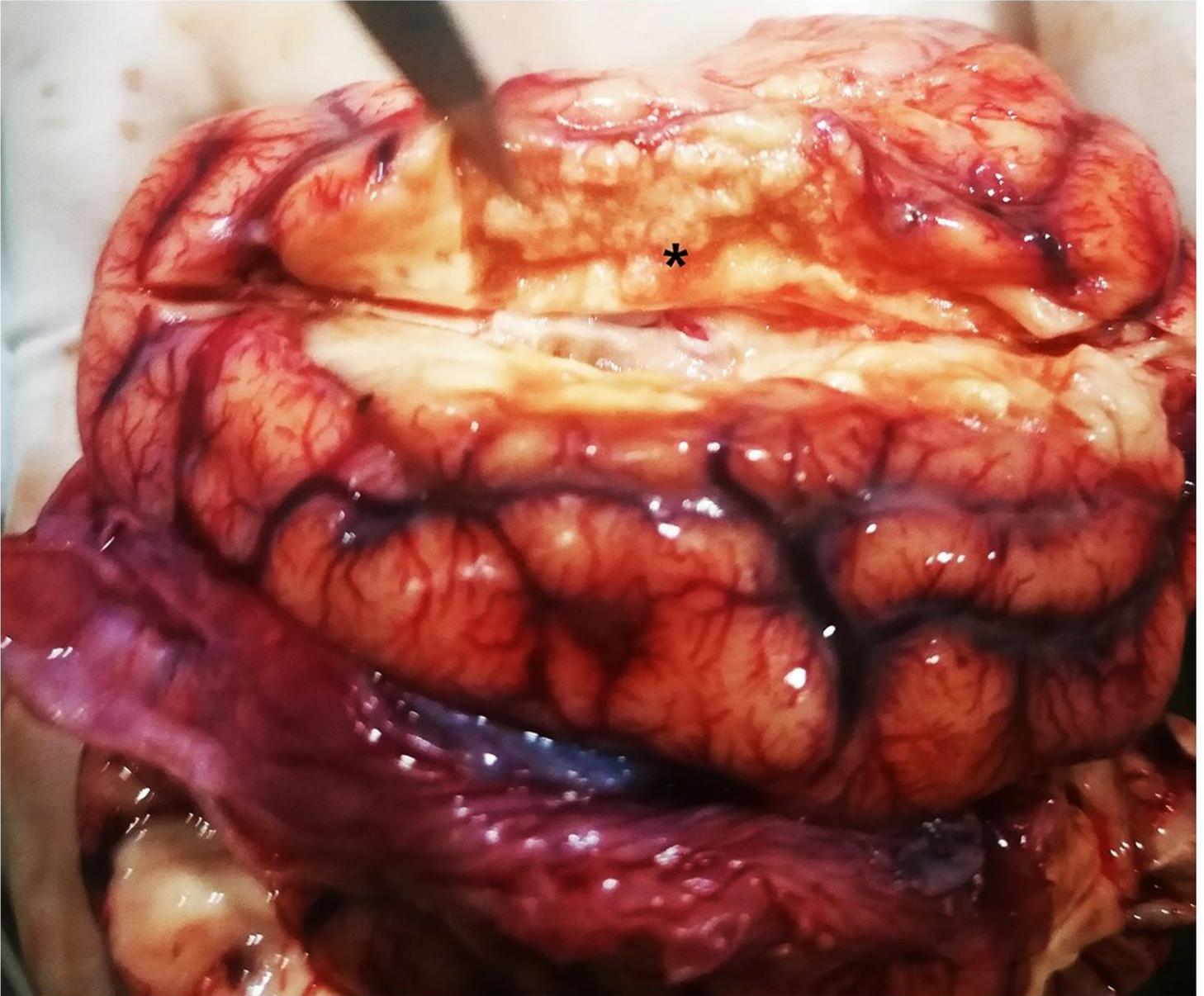
## References

1. Gaskell, R., Dawson, S., Radford, A. & Thiry, E. Feline Herpesvirus. *Vet. Res.* **38**, 337-354 (2007).
2. Maggs, D. J. Update On Pathogenesis, Diagnosis, and Treatment of Feline Herpesvirus Type 1. *Clinical Techniques in Small Animal Practice.* **20**, 94-101 (2005).
3. Monne Rodriguez, J. M., Leeming, G., Köhler, K. & Kipar, A. Feline Herpesvirus Pneumonia: Investigations Into the Pathogenesis. *Vet. Pathol.* **54**, 922-932 (2017).
4. Li, J., Xiao, L. & Lu, Z. Challenges of Snow Leopard Conservation in China. *Science China Life Sciences.* **59**, 637-639 (2016).
5. Johansson Ö., Ullman K., Lkhagvajav P., Wiseman M., Malmsten J., Leijon M.. Detection and Genetic Characterization of Viruses Present in Free-Ranging Snow Leopards Using Next-Generation Sequencing. *Frontiers in Veterinary Science.* 645 (2020).
6. Sun, H. et al. Isolation and Identification of Feline Herpesvirus Type 1 From a South China Tiger in China. *Viruses.* **6**, 1004-1014 (2014).
7. Maes, R. Felid Herpesvirus Type 1 Infection in Cats: A Natural Host Model for Alphaherpesvirus Pathogenesis. *Isrn Veterinary Science.* **2012**, 495830 (2012).
8. Ruthner Batista H. B. et al. Neutralizing Antibodies Against Feline Herpesvirus Type 1 in Captive Wild Felids of Brazil. *J. Zoo Wildlife Med.* **36**, 447-450 (2005).
9. Ostrowski, S., Van Vuuren, M., Lenain, D. M. & Durand, A. A Serologic Survey of Wild Felids From Central West Saudi Arabia. *J. Wildlife Dis.* **39**, 696-701 (2003).
10. Nakamura, K. et al. Comparison of Prevalence of Feline Herpesvirus Type 1, Calicivirus and Parvovirus Infections in Domestic and Leopard Cats in Vietnam. *J. Vet. Med. Sci.* **61**, 1313-1315 (1999).
11. Leutenegger, C. M. et al. Viral Infections in Free-Living Populations of the European Wildcat. *J. Wildlife Dis.* **35**, 678-686 (1999).
12. Scherba, G. et al. Comparison of a Cheetah Herpesvirus Isolate to Feline Herpesvirus Type 1. *Arch. Virol.* **100**, 89-97 (1988).
13. Ghoshal, A. Snow Leopard. *Resonance.* **22**, 677-690 (2017).
14. Ng, D., Carver, S., Gotame, M., Karmasharya, D. & Johnson, C. N. Canine Distemper in Nepal's Annapurna Conservation Area – Implications of Dog Husbandry and Human Behaviour for Wildlife

- Disease. *PLoS One*. **14**, e220874 (2019).
15. Argenta, F. F. et al. Ulcerative Dermatitis Caused by Feline Herpesvirus Type 1 in a Domestic Cat. *Semina: ciências agrárias*. **38**, 2857-2862 (2017).
  16. Nasisse, M. P., Guy, J. S., Davidson, M. G., Sussman, W. A. & Fairley, N. M. Experimental Ocular Herpesvirus Infection in the Cat. Sites of Virus Replication, Clinical Features and Effects of Corticosteroid Administration. *Invest. Ophthalm. Vis. Sci*. **30**, 1758-1768 (1989).
  17. Uitz, E., Bahadori, B., Mccarty, M. F. & Moghadasian, M. H. Practical Strategies for Modulating Foam Cell Formation and Behavior. *World Journal of Clinical Cases*. **2**, 497 (2014).
  18. Yanamoto, H., Murao, K. & Miyamoto, K. Etiology and Pathology of Cerebral Infarction]. *Nippon rinsho. Japanese journal of clinical medicine*. **64 Suppl 8**, 11-15 (2006).
  19. Borkowski, R., Taylor, T. G. & Rush, J. Cerebral Infarction and Myocardial Fibrosis in a White-Handed Gibbon (*Hylobates Lar*). *J. Zoo Wildlife Med*. **31**, 65-70 (2000).
  20. Schwab, S. et al. Non-Suppurative Meningoencephalitis of Unknown Origin in Cats and Dogs: An Immunohistochemical Study. *J. Comp. Pathol*. **136**, 96-110 (2007).
  21. Hora, A. S., Toniatti, P. O., Guerra, J. M., Leme, M. C. & Brandão, P. E. Felid Herpesvirus 1 as a Causative Agent of Severe Nonsuppurative Meningoencephalitis in a Domestic Cat. *J. Clin. Microbiol*. **51**, 676-679 (2013).
  22. Zachary, J. F. *Pathologic Basis of Veterinary Disease*. six edition edn, Elsevier (St. Louis, Missouri, 2017).
  23. Donovan, T. A., Schrenzel, M. D., Tucker, T., Pessier, A. P. & Nordhausen, R. W. Meningoencephalitis in a Polar Bear Caused by Equine Herpesvirus 9 (EHV-9). *Vet. Pathol*. **46**, 1138-1143 (2009).
  24. Claus, M. P., Alfieri, A. F. & Alfieri, A. A. Meningoencephalitis by Herpesvirus Type 5. *Semina Ciências Agrárias*. **23**, 131-141 (2002).
  25. Torre, D., Speranza, F., Martegani, R., Ferrante, P. & Fiori, G. P. Meningoencephalitis Caused by Human Herpesvirus-6 in an Immunocompetent Adult Patient: Case Report and Review of the Literature. *Infection*. **26**, 402 (1998).
  26. Fuselier, H. A. Etiology and Management of Acute Urinary Retention. *Comprehensive Therapy*. **19**, 31-36 (1993).
  27. Gaskell, R. M., Radford, A. D. & Dawson, S. *Feline Infectious Respiratory Disease*, Blackwell Publishing Ltd (Oxford, England, 2004).
  28. Maeda K. et al. Expression and Properties of Feline Herpesvirus Type 1 gD (Hemagglutinin) by a Recombinant Baculovirus. *Virus Res*. **46**, 75-80 (1996).
  29. Kruger, J. M., Sussman, M. D. & Maes, R. K. Glycoproteins G1 and gE of Feline Herpesvirus-1 are Virulence Genes: Safety and Efficacy of a g1-gE Deletion Mutant in the Natural Host. *Virology*. **220**, 299-308 (1996).
  30. Lewin, A. C., Coghill, L. M., McLellan, G. J., Bentley, E. & Kousoulas, K. G. Genomic Analysis for Virulence Determinants in Feline Herpesvirus Type-1 Isolates. *Virus Genes*. **56**, 49-57 (2020).

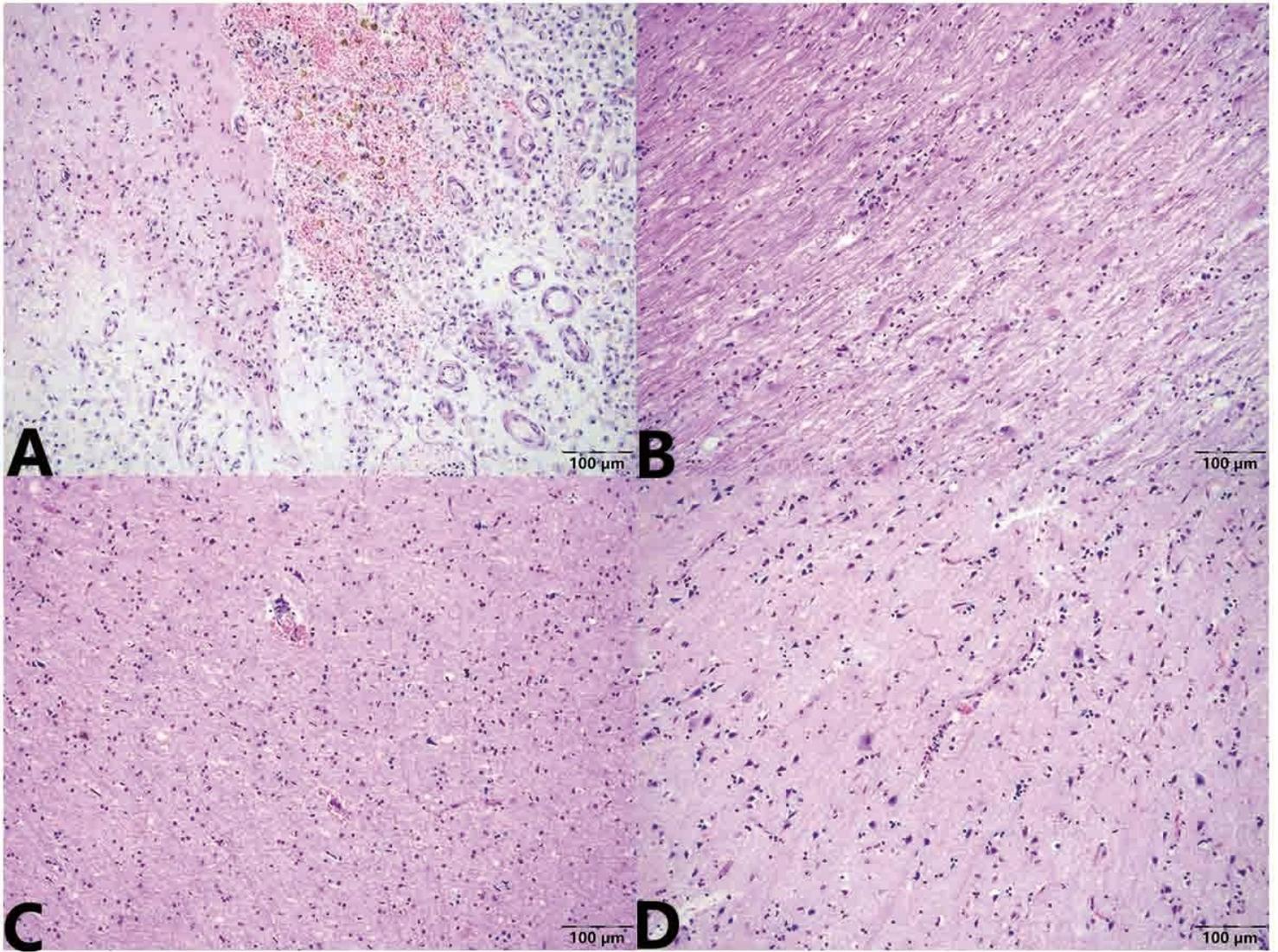
31. Zhang, S., Chun-Ling, L. I., Wang, B. Y., Liu, J. H. & Tian, K. G. Isolation and Identification of Feline Herpesvirus Type 1. *Laboratory Animal Science*. 1-5 (2010).

## Figures



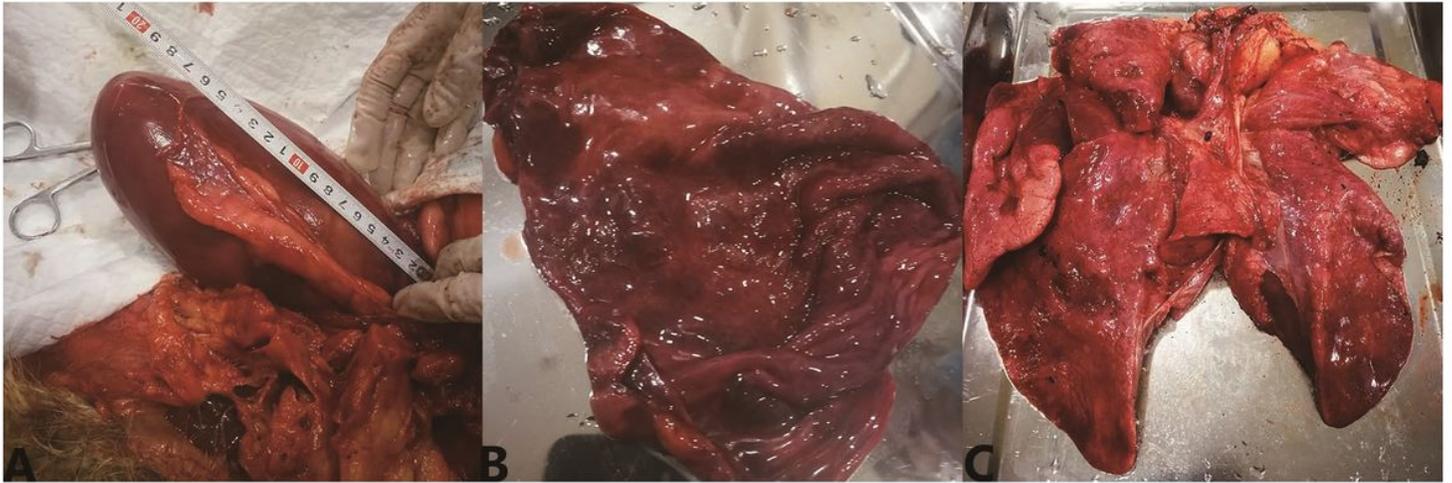
**Figure 1**

Gross observation of brain of No.1. Meningeal vascular is congested, and a liquefactive necrosis region approximately 2 cm × 0.5 cm × 1 cm in diameter is located on the right cerebral hemisphere (\*).



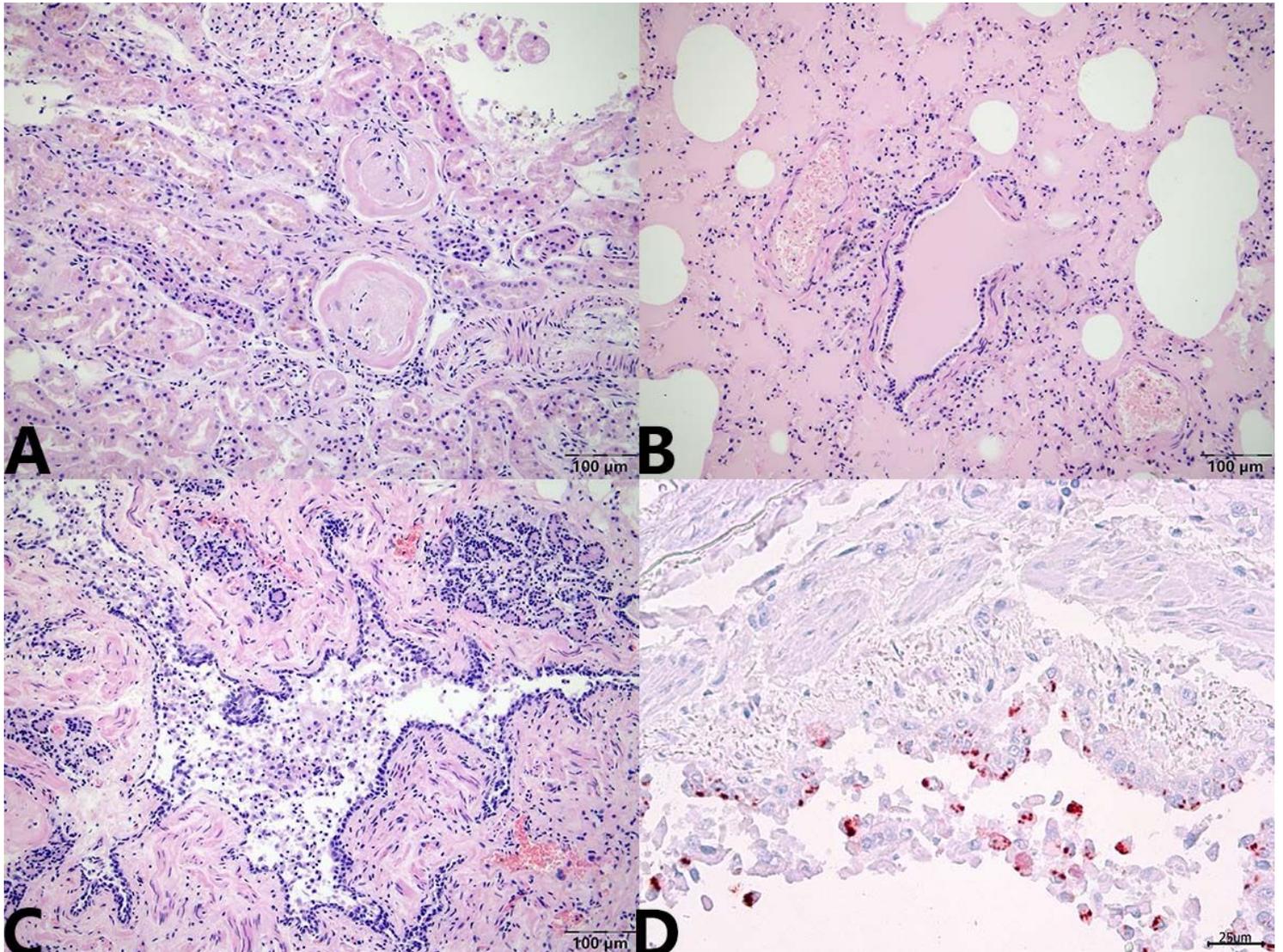
**Figure 2**

Microscopic change of brain of No.1. A. The lesions of hyperemia and hemorrhage, liquefactive necrosis, a massive collection of foam cells, and hemosiderosis are presented in the meninges and cerebral cortex. B. Demyelination reactions are visible in white matter. C. Bleeding foci scattered in cortex. D. Capillary hyperemia; edema; moderate vascular cuff reaction (lymphohistiocytic perivascular infiltrates); neuron necrosis; and neurophagia are also visible in the brain parenchyma. Stained using hematoxylin and eosin (H&E).



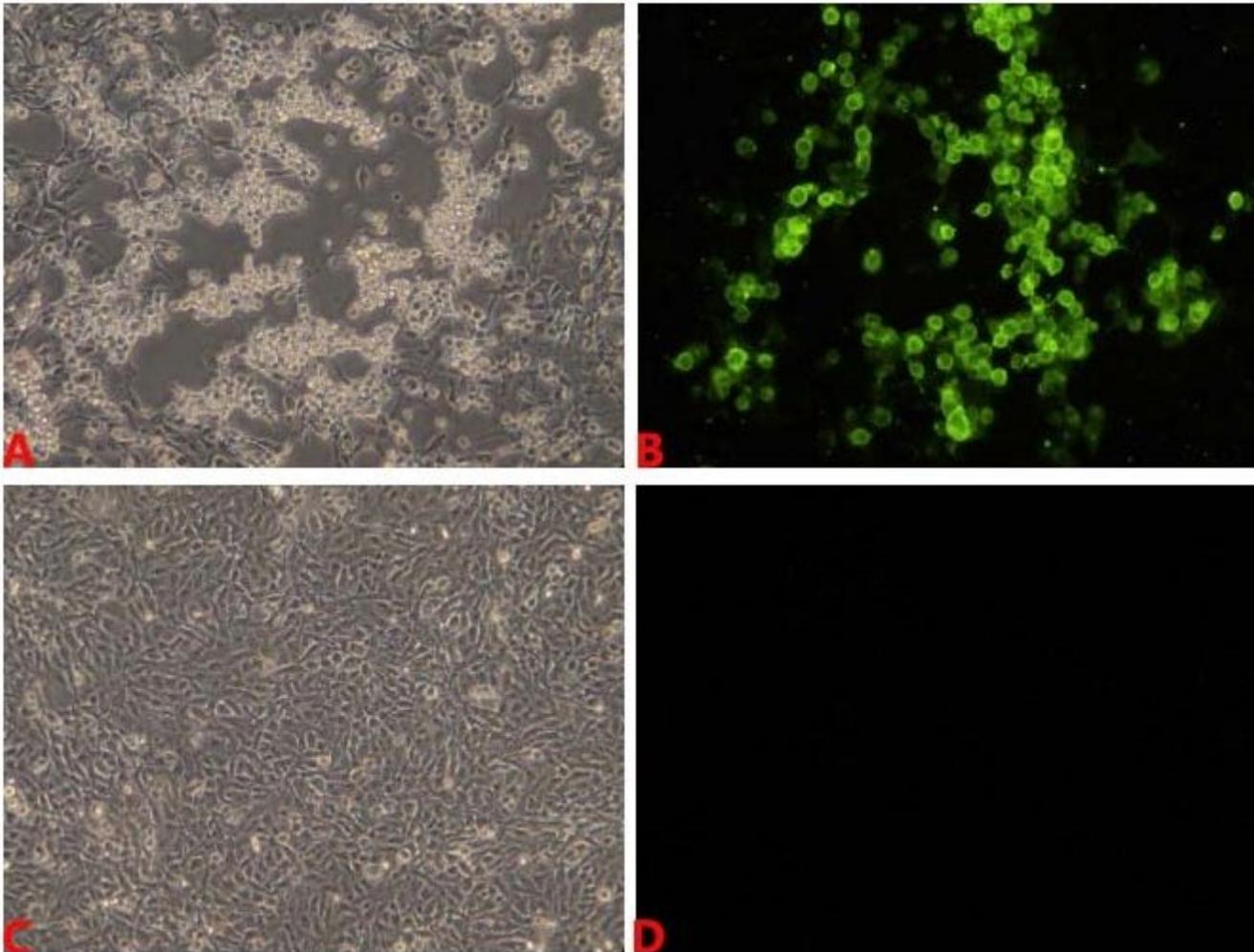
**Figure 3**

Gross observation of the urinary bladder and lung of No. 2. A. The bladder accumulated and dilated, 20 cm × 10 cm in diameter. B. The congestion present on the surface of the urinary bladder mucosa. C. The lung presented dull-red, swelling, and liquid leakage from the section.



## Figure 4

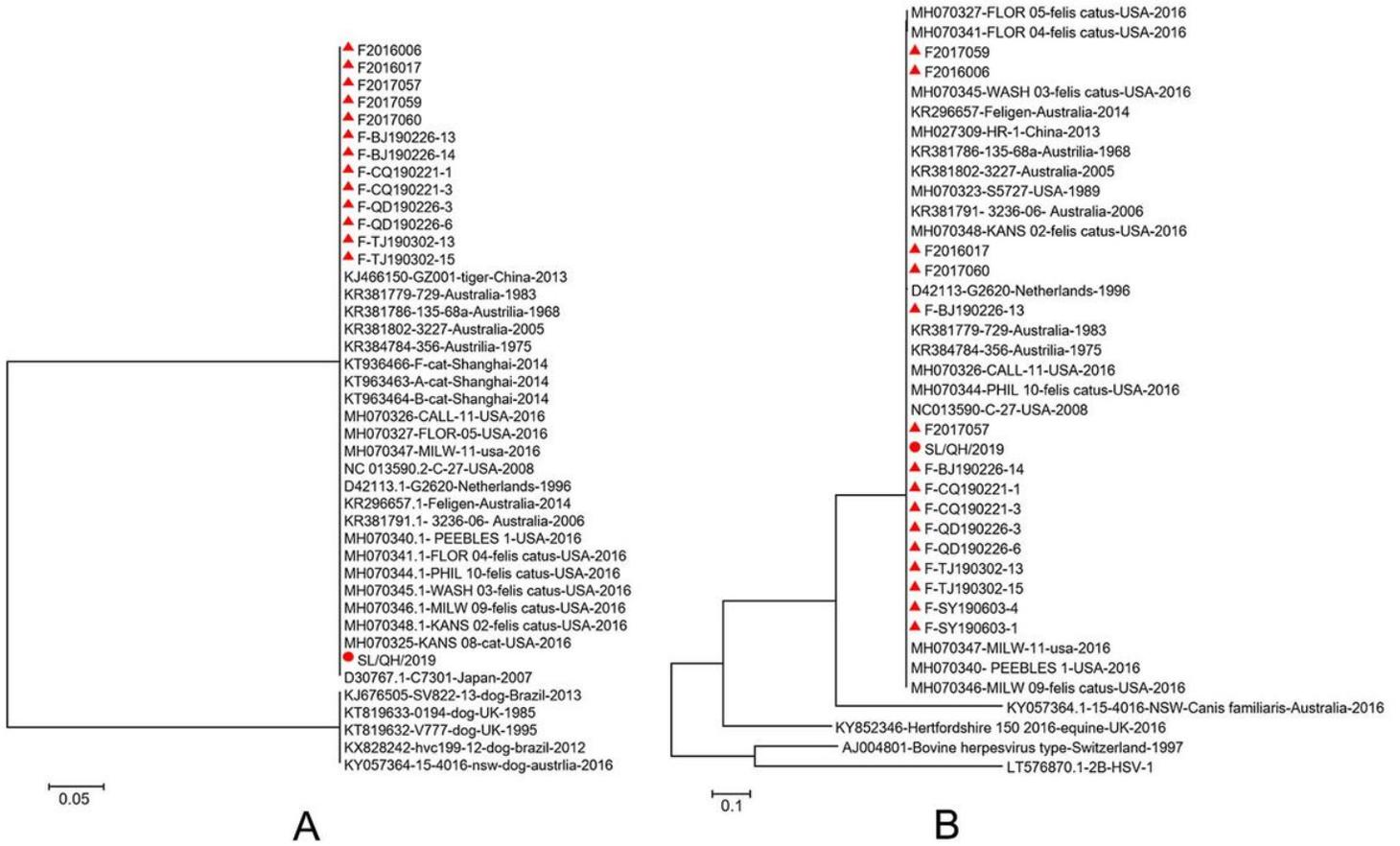
Microscopic change of kidney and lung of No. 2. A. Microscopic observation, coagulation necrosis of massive glomerulus and tubules in the renal cortex, and bilirubin deposition in tubule epithelial cells are visible. B. Pulmonary alveoli expanded and are filled with pink exudate. C. Many lymphocytes and exfoliative cells are filled with the bronchioli. D. Feline herpesvirus type-1 (FHV-1) primarily located in the cytoplasm of epithelial cells of a bronchial bronchiole and exfoliative cells by immunohistochemistry. A, B, and C stained using hematoxylin and eosin (H&E). D stained by the antibody for FHV-1.



## Figure 5

Isolation and identification of Feline herpesvirus type-1 (FHV-1) SL/QH/2019 strain. (A) FK81 cells inoculated with nasal swab supernatant of No. 2 for 36 h and show an obvious cytopathic effect (CPE), characterized by round, pyknosis, fusion, aggregating like "Fleece-Pulling." (B) Much positive signal (green) of FHV-1 located in the cytoplasm of FK81 cells by indirect immunofluorescence. C and D. Mock controls. FK81 cells from virus-free served as a negative control.





**Figure 7**

Phylogenetic tree based on the nucleotide sequences of the gD and gE gene. The tree is constructed with MEGA 7.0

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [S1.doc](#)
- [S2.doc](#)