Abstract

Background: Suid herpesvirus type 1 (SHV1) is a type of neurotropic virus able to infect various species. However, the clinical cases of human SHV1 encephalitis are still rarely reported, and the clinical characteristics, treatment, and prognosis of human SHV1 encephalitis are still unclear.

Methods: In this study, we reported 2 cases of human encephalitis associate with SHV1 infection, and reviewed the other 18 cases from the literatures. A total of 20 cases with human SHV1 encephalitis were summarized and re-analyzed.

Results: Nineteen of 20 patients had a history of swine-related occupational exposure before illness onset. All patients initially presented with influenza-like symptoms, and then quickly developed seizures, disturbed consciousness, and respiratory failure. The results of cerebrospinal fluid (CSF) indicated aseptic or viral infection. MRI findings of SHV1 encephalitis were prone to distribute in temporal-frontal and insular cortex, which was similar to the pattern of herpes simplex virus encephalitis. Some cases with disease progression and poor prognosis might expand to thalamus, basal ganglia and brain stem. Metagenomic next-generation sequencing (mNGS) revealed that all patients had unique SHV1 sequences with variable reads in the CSF.

Conclusions: SHV1 is a zoonotic pathogen that can cause a new type of human viral encephalitis, characterized by acute, fulminating and catastrophic central nervous system infection. Currently, there are no available approved treatments for the encephalitis, but it is possible to diagnose encephalitis quickly by mNGS.

1. Background

Suid herpesvirus type 1 (SHV1), also called pseudorabies virus (PRV), is a neurotropic virus that belongs to the alpha-herpesvirinae subfamily and genus varicellovirus in the family herpesviridae[1]. Swine is the primary natural host for SHV1[2]. The classic viruses, first discovered in swine in 1902, have been associated with fatal encephalitis in piglets, boars sterility, reproductive disorders in sows, and respiratory diseases in growing and fattening pigs, and have brought about a great number of economic loss to the swine industry since its global spreading since 1980s[3,4].

SHV1 can infect not only swine, but also a wide range of vertebrates including cattle, sheep, cats, dogs, minks, bears, skunks and mice, indicating that the virus has a strong ability of transmission cross species[5]. Mravak et al. in 1987 reported 3 cases of suspected human SHV1 infection diagnosed by SHV1 positive antibody without subtype identification[6]. Nevertheless, the diagnosis of human SHV1 infection had been debatable until Ai et al. in 2018 found some unique SHV1 sequences in the vitreous humor from an endophthalmitis patient through metagenomic next-generation sequencing (mNGS), and the possibility of SHV1 infecting humans across the species barrier was identified at the first time[7]. In the same year, Zhao et al. reported 4 cases of suspected SHV1 encephalitis for the first time based on clinical features and pathogen analysis of cerebrospinal fluid (CSF) by mNGS[8]. However, the cases of human SHV1 encephalitis are still rarely reported, and the clinical characteristics, treatment and prognosis of encephalitis associated with SHV1 remain unclear. Therefore, we reported 2 cases of human SHV1 encephalitis, and reviewed the other 18 cases reported in the literatures. We retrospectively analyzed the clinical processes of SHV1 encephalitis in order to improve awareness about this new type of human viral encephalitis.

2. Materials And Methods

2.1 Subjects

The patients were retrospectively collected from neurological intensive care unit in the first affiliated hospital of Nanchang university. Initial symptoms, age of onset, occupation, clinical manifestations, treatments and prognosis were collected from their relatives and medical records.

2.2 Patient Consent Statement

All samples and medical data were obtained after a written consent signed by each family in compliance with the bioethical laws of China as well as the Declaration of Helsinki. The research was also approved by the ethic committee of the first affiliated hospital of Nanchang university.

2.3 Brain MRI

All magnetic resonance image (MRI) examinations were performed as routine clinical care with 3.0 T MR scanner. Conventional T1-weighted spin echo (T1WI) sequences, T2-weighted spin echo (T2WI) sequences, fluid attenuated inversion recovery (FLAIR) sequences, and diffused weight image (DWI) sequences were conducted according to their own routine procedures. MRI with contrast was conducted at least one time in the 2 patients.

2.4 Cerebrospinal fluid NGS

Five milliliters CSF sample were collected in a sterilized RNase free container for mNGS, then stored and delivered at dry ice. DNA was extracted with QIAamp DNA Microbiome Kit (at#51704, Qiagen, Germany). After enzymatic fragmentation of DNA to a size of 200–300 bp, the sequencing libraries were constructed through end repair, adapter ligation and amplification. Sequencing templates were prepared by using the OneTouch2 System (Life Technologies, CA, USA) and then sequenced on the BioElectronSeq 4000 sequencer (CapitalBio Corporation, Beijing, China). The original sequencing data is first subjected to data quality control, and the sequences of less than 50bp and low complexity were removed. The qualified reads were mapped to the human reference genome (GRCh38) using the Burrows-Wheeler Aligner to remove the human sequence. The remaining data were aligned to the NCBI microbial genome database (ftp://ftp.ncbi.nlm.nih.gov/genomes/) for the detection of pathogens. The final pathogen results included a list of suspected pathogens, hitreads and statistics on the coverage of the genome. All pathogen genome sequences contained 13,992 species of bacteria, 1,659 species of fungi, 13,000 species of viruses, and 287 species of parasites. The reference values, hitreads and coverage of microbial pathogen were calculated according to the samples of 100 control people. The 5%, 75%, 95% quartile, median and maximum values were obtained, respectively, and then the intervals (5%-95%) were used as the
3. Results

3.1 Clinical features of two patients with SHV1 encephalitis

3.1.1 Patient 1

The patient was a 51-year-old woman without specific medical history, but she is a housewife who frequently handles raw pork when cooking. She was transferred to our unit for a critical care with fever for 1 week, delirium and seizures for 3 days. Initially, the patient had a fever accompanied by headache and drowsiness, but she did not visit doctor since the symptoms were considered as a common cold. After 4 days, the patient had abnormal behaviors such as ramblings and tearing the sleeve, then immediately presented with generalized tonic-clonic seizure. The recurrent seizures lasted for 2–3 minutes, and caused the patient in a comatose state. She had respiratory failure due to status epilepticus, and endotracheal intubation was conducted. The patient was clinically diagnosed as encephalitis with status epilepticus, since no obvious pathological findings were identified in cranial computed tomography (CT). She was transferred to our neurological critical care unit immediately when her vital signs were stable. On admission, physical examination revealed a sedative state with mechanical ventilator supporting, and the Glasgow coma scale (GCS) was 6/15 (E1; V1; M4). The Kernig’s, Brudzinski’s and Babinski’s signs were all negative, but JOLT test was not performed due to the sedative state.

A routine blood test revealed a total white blood cell (WBC) count of 12.47 x10^9/L (normal 4–10 x10^9/L), and the percentage of neutrophils was 85% (normal 40–75%). Biochemical examination revealed a mild elevation of liver enzymes. The serological assays for hepatitis B antibodies and antigens, hepatitis C antibodies, syphilis antibodies, and HIV antibodies were all negative. The serological assays for rubella virus and Epstein-Barr virus IgGs were positive, but the IgMs for all three viruses were negative. The serological assays showed that the IgG and IgM of herpes simplex virus type 1/2 and cytomegalovirus were all negative. CSF was clear with an opening pressure of 220 mmH_2O (normal 80–180 mmH_2O). CSF analysis showed WBC count of 1x10^5/L (normal 0–5 x10^5/L), glucose of 3.1 mmol/L (normal 2.8–4.5 mmol/L), chloride of 122.3 mmol/L (normal 120–132 mmol/L), and protein of 340 mg/L (normal 150–450 mg/L). CSF cytology revealed the total count of cells was 80 with lymphocyte 60% (48 cells), neutrophils 30% (24 cells) and monocytes 10% (8 cells). The acid-fast staining, India ink staining, as well as bacterial smear and culture were all negative. CSF and serum were negative to antibodies of autoimmune encephalitis including NMDAR IgG, AMPAR1 IgG, AMPAR2 LgG, LG1 IgG, CASPR2 IgG, GABAR IgG, DPXY IgG, IgLON5 IgG, Glya1 IgG, GABAAR IgG, GABAAR3 IgG, mGlur5 IgG, D2R IgG, Neurexin3 IgG and GAD65 IgG. Blood cultures for bacteria, tuberculosis and fungus were all negative. Brain MRI on admission revealed high signals on FLAIR and DWI distributing in bilateral temporal lobes and the right insular cortex (Fig. 1A–E). Brain MRI at one month after admission showed diffused lesions involving bilateral temporal-frontal-parietal lobes, right occipital lobe, insular cortex, thalamus, basal ganglia and brain stem with variable enhancements on bilateral temporal lobes, as well as hemorrhagic lesion at the occipital lobe (Fig. 1F–J). Electroencephalogram (EEG) showed generalized slow wave (1-2Hz delta frequency) on the background (Fig. 2A).

Since the clinical features and MRI findings indicated acute diffused cortical inflammatory involvement, and the CSF tests showed normal pattern and mild lymphocytic pleocytosis, the patient was clinically suspected as viral encephalitis, and immediately was initiated treatment with antiviral drugs (acyclovir, 10mg/kg, q8h), antibiotic (linezolid, 600mg, q12h), antiepileptic (midazolam, 0.05mg/kg/h and sodium valproate, 500mg, q12h), and decreasing intracranial pressure therapy (mannitol, 25g q6h) based on empirical cares. At one week post-treatment, lumbar puncture had been rechecked, the opening pressure of CSF dropped to 190 mmH_2O, protein rose significantly to 0.95 mmol/L, glucose was 3.9 mmol/L, chloride was 127.5 mmol/L, and WBC count was 5x10^5/L. CSF cytology revealed the total count of cells was 180 with lymphocyte 83% (150 cells), monocytes 10% (18 cells), neutrophils 5% (9 cells) and basophils 2.5% (3 cells). To identify the causative virus, CSF sample was sent to mNGS screening, and revealed 34 unique reads of suid herpesvirus type 1 with 2.43% coverage (Fig. 3A). Additionally, Sanger sequencing of a gB segment in SHV1 further supported the mNGS result (Fig. 3B). Therefore, the encephalitis was probably associated with SHV1 infection. After 6 weeks of treatment, she weaned off ventilator but remained metal tracheostomy tube and still was in unconsciousness. Her modified Rankin scale (mRS) score was 5 and GCS was 7 (E2, V1, M4) when discharged to a local hospital.

3.1.2 Patient 2

A 68-year-old man, a swineherd, was transferred to our clinic due to fever for 4 days and recurrent seizures for 1 day. Initially, the patient had a persistent fever with apathy, and was treated as influenza without improvement at the local clinic. Three days later, a chest CT scan was conducted, but no abnormalities were identified. The patient was transferred to our intensive care unit due to frequent seizures, rapidly developed status epilepticus and respiratory failure, and then was immediately intubated and treated with sedatives. Physical examination showed medical sedative condition with a GCS score of 3/15 (E1; V1; M1). Kernig’s and Brudzinski’s signs were negative, while the left Babinski’s sign was positive. Routine blood test revealed a high white blood cell count of 16.33...
x10⁶/L. Brain MRI on admission showed abnormal signals in the bilateral temporal-parietal lobes and the left insular cortex (Fig. 4A-D), indicating viral encephalitis. Lumber punctuation revealed an increased opening pressure of 190 mmH₂O. CSF analysis revealed WBC count of 20x10⁶/L, glucose of 3.3 mmol/L, chloride of 119 mmol/L, and protein of 1290 mg/L. CSF cytology showed the total count of cells were 150 with lymphocyte 60% (90 cells), neutrophils 20 % (30 cells), monocytes 16% (24 cells) and basophils 4% (6 cells). India ink stain, acid-fast stain, bacterial smear, and antibodies of autoimmune encephalitis were all negative. EEG showed paroxysmal spike wave discharge with diffused slow wave background (Fig. 2B). Based on empirical therapy, the patient was administered acyclovir (10mg/kg, q8h), meropenem (1.0g, q8h), midazolam (0.05mg/kg/h) and sodium valproate (500mg, q12h). One week later, mNGS screening of CSF sample revealed 29 unique reads of SHV1 with 2.55% coverage (Fig. 3C). Additionally, Sanger sequencing of a gB segment in SHV1 further supported the mNGS result (Fig. 3B). Nevertheless, brain MRI at 10 days after admission showed that the lesions significantly expanded along the temporal-frontal-parietal lobes and thalamus (Fig. 4E-H). Subsequently, intravenous immunoglobulin (IVIG) of 0.4g/kg/d for 5 days were attempted to prevent the disease progression. However, after hospitalization for three weeks, the patient still was unconscious and ventilator-dependent, and then was palliative care at home. At the end, the patient died one week after discharge from the hospital.

3.2 Summarization of cases with human SHV1 encephalitis reported

3.2.1 Clinical features and treatment outcome

The patient enrollment was depicted in Supplemental Fig. 1. In total, 25 records were identified by the keywords in the multiple databases. After removing the duplication records, 14 of 25 records were screened for cases with SHV1 encephalitis, and then 3 of 14 records were removed due to not clinical reports. Three of 11 records were excluded due to diagnosis ineligibility with evidence insufficiency (n = 2) and repeated report (n = 1). Finally, 8 of 11 records including 18 cases were qualified to summarize the clinical data of human SHV1 encephalitis.

Besides our study, 18 other cases have been reported as human SHV1 encephalitis with pathogenic evidence[1,2,5,7−12]. All patients came from Mainland China, including 8 cases from Shandong province, 2 cases from Inner Mongolia, 2 cases from Henan province, 2 cases from Hebei province, one case from Hubei province, one case from Guangdong province, one case from Anhui province, and one case from Beijing. Therefore, a total of 20 cases (male: 17; female: 3) of human SHV1 encephalitis were included in the summarization (Table 1 and Supplemental Table 1). The median age was 50 years (interquartile range [IQR] 43–52 years; range 25–63 years). Nineteen of 20 patients worked in industries related to swine, and most of them had a history of occupational exposure before illness onset, such as hand injury or needle stick at work. All patients initially presented with influenza-like symptoms such as fever, headache, and drowsiness, and then developed seizures, disturbed consciousness, and respiratory failure in the first week. The clinical manifestations mainly included fever (20/20), seizures (20/20), disturbed consciousness (20/20), respiratory failure (16/20), headache (10/20), eye involvement (10/20), and psychiatric symptoms (4/20). Pneumonia was the most common complication for SHV1 encephalitis patients, which probably was associated with long-term bedridden and airway dysfunction. Hepatic dysfunction was observed in 3 patients.
### Table 1
The summarization of clinical features from reported patients with human SHV1 encephalitis.

| Case | Reference | Age(y)/sex | Occupation       | Symptoms                                                                 | Stiff-neck | HSV1 reads/raw reads | HSV1 antibody | Treatments                                           | Outcome |
|------|-----------|------------|-----------------|--------------------------------------------------------------------------|------------|----------------------|---------------|------------------------------------------------------|---------|
| 1    | Current study | 51/F       | Housewife       | fever, headache, seizures, disturbed consciousness, psychosis, respiratory failure | positive  | 34/17,712,312       | ND            | full-dose acyclovir, antiepileptic, antibiotics      | com trcl statr |
| 2    | Current study | 63/M       | Swineherd       | fever, seizures, disturbed consciousness, respiratory failure            | negative  | 29/22,998,995       | ND            | full-dose acyclovir, antiepileptic, IVIG antibiotics | died    |
| 3    | Fan et al. [1] | mid-aged/M | Pig butcher     | fever, seizures, disturbed consciousness, respiratory failure, eye involvement | ND        | 141/48,512,319      | positive      | full-dose acyclovir, antiepileptic, IVIG             | died    |
| 4    | Fan et al. [1] | mid-aged/M | Swineherd       | fever, seizures, disturbed consciousness, respiratory failure, eye involvement | ND        | 30/20,833,434       | positive      | acyclovir, foscarnet, antiepileptic, IVIG            | men hani mR§ |
| 5    | Fan et al. [1] | young /M   | Pig driver      | fever, seizures, disturbed consciousness, respiratory failure            | ND        | 52/24,761,904       | positive      | full-dose acyclovir, antiepileptic, IVIG             | mR§     |
| 6    | Fan et al. [1] | young /F   | Pork dealer     | fever, seizures, disturbed consciousness, respiratory failure, eye involvement | ND        | 64/20,062,695       | negative      | acyclovir, foscarnet, antiepileptic                  | died    |
| 7    | Liu et al. [2] | 25/M       | Veterinarian    | fever, headache, seizures, disturbed consciousness, eye involvement     | positive  | 8/ND                 | positive      | ganciclovir, foscarnet, antiepileptic               | pen bllx mR§ |
| 8    | Liu et al. [2] | 35/M       | Pig butcher     | fever, headache, seizures, disturbed consciousness, respiratory failure  | negative  | 7/ND                 | positive      | acyclovir, foscarnet, antiepileptic                | mild impi mR§ |
| 9    | Liu et al. [2] | 49/M       | Pig butcher     | fever, headache, seizures, disturbed consciousness, respiratory failure, eye involvement | negative  | 43/ND                | positive      | ganciclovir, foscarnet, antiepileptic              | mini cons mR§ |
| 10   | Yang et al. [6] | 43/M       | Veterinarian    | fever, headache, disturbed consciousness, seizures, respiratory failure  | positive  | 6198/ND             | positive      | full-dose acyclovir, antiepileptic, antibiotics, IVIG, GC | tracl and gast tube |
| 11   | Zhao et al. [6] | 55/M       | Pig butcher     | fever, headache, seizures, disturbed consciousness, respiratory failure, eye involvement | negative | 21/ND                | positive      | full-dose acyclovir, antiepileptic, antibiotics, IVIG, GC | com wee mR§ |
| 12   | Zhao et al. [6] | 51/M       | Pork cutter     | fever, headache, seizures, disturbed consciousness, psychosis, respiratory failure | positive | 373/ND              | positive      | full-dose acyclovir, antiepileptic, antibiotics, IVIG, GC | died    |
| 13   | Zheng et al. [8] | 59/M       | Swineherd       | fever, seizures, disturbed consciousness, respiratory failure, eye involvement | positive | 8/ND                 | positive      | ganciclovir and foscarnet, antiepileptic, antibiotics | loss mR§ |
| 14   | Wang et al. [10] | 44/M       | Pork vendor     | fever, seizures                                                              | negative  | 74/ND                | positive      | full-dose acyclovir, antiepileptic, antibiotics, GC | follic instr mR§ |
| 15   | Yang et al. [11] | 50/M       | Pig butcher     | fever, headache, seizures, disturbed consciousness                         | positive  | 37/ND                | ND            | full-dose acyclovir, antiepileptic, antivirals, IVIG, GC | slow resp occi seiz mR§ |

CSF: cerebrospinal fluid; AE: autoimmune encephalitis; F: female; GC: glucocorticoids; IVIG: intravenous human immunoglobulin; M: male; mRS: modified Rankin Scale; ND: no data; full-dose acyclovir means 10mg/kg, q8h, for variable days dependent on different disease course.
classical SHV1 strains from the USA and Europe are categorized as SHV1 genotype I, and variant SHV1 strains from China are categorized as SHV1 genotype II.

Suid herpesvirus type 1 is a double-stranded DNA virus with icosahedral symmetric structure.

4. Discussion

Identified in 11 patients.

Most patients showed poor response to acyclovir and immunotherapy with the prognosis of 4 died patients and the median mRS score of 4. Most of them left severe disability and dysfunction of living capability. Conversely, antiviral treatments were taken as ganciclovir plus foscarnet in patient 7, acyclovir plus IVIG, GC and glucocorticoids in patient 8, and penciclovir (0.5 g/day q12h, for 17 days) and foscarnet (6 g/day q12h, for 17 days) in patient 13, and the 3 patients showed good response to antiviral treatments with the mRS 3.0.

A total of 33 times of CSF examinations were conducted in 20 patients (Supplemental Table 2), which showed median opening pressure of 200 mmH₂O (IQR 167–273 mmH₂O), median WBC count of 23 x10⁶/L (IQR 6–44 x10⁶/L), and median protein level of 0.5 g/L (IQR 0.39–0.67g/L). CSF cytology revealed that neutrophils were predominant in the initial stages of the disease in some patients, and lymphocytic pleocytosis with the development of the disease. The level of glucose and chloride in CSF were within normal limits. The findings of CSF indicated the possibility of aseptic encephalitis or viral encephalitis. Five patients underwent long-term EEG monitoring. All of them had generalized front-temporal slow activity, and three showed epileptiform discharges. These abnormal changes of EEG presumably were associated with impairment of brain function or sedative medicine for seizures.

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All patients received antiviral treatment after initial diagnosis with viral encephalitis including acyclovir (10mg/kg, q8h) in 17 patients, foscarnet in 6 patients, ganciclovir in 2 patients, and penciclovir in one patient. Fourteen patients also received immunotherapy including IVIG in 2 patient, glucocorticoids in 3 patients, and both of them in 9 patients. However, the actual dosage of foscarnet, ganciclovir, IVIG and glucocorticoids were unavailable in most of patients. All patients received antiviral treatment after initial diagnosis with viral encephalitis including acyclovir (10mg/kg, q8h) in 17 patients, foscarnet in 6 patients, ganciclovir in 2 patients, and penciclovir in one patient. Fourteen patients also received immunotherapy including IVIG in 2 patient, glucocorticoids in 3 patients, and both of them in 9 patients. However, the actual dosage of foscarnet, ganciclovir, IVIG and glucocorticoids were unavailable in most of patients.

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MRI changes

Nineteen of 20 patients underwent brain MRI, and the remaining one performed a cranial CT scan with no obvious abnormalities. Generally, brain MRI showed high intensity on FLAIR, T2WI and DWI, as well as low intensity on T1WI. The lesions distributed at insular lobe in 19 cases, temporal lobe in 16 cases, basal ganglia in 10 cases, frontal lobe in 8 cases, and brainstem in 3 cases (Supplemental Tables 1 and 3). One of our patients had hemorrhagic lesion in the occipital lobe. There were 3 patients with contrast MRI; and 2 patients had linear enhancement along the surface of cortex.

3.2.3 Microbial analysis

Microbiological analysis was performed on serum or CSF samples in all patients to identify causative pathogen. India ink stain, acid-fast stain, and bacterial smear test of CSF in all patients showed no abnormalities. mNGS test of CSF were performed on all patients who had unique sequence reads of SHV1. Most patients had reads number between 2 and 373, and low coverage between 0.1% and 16%, while one patient had unique sequence of 6198 reads with 80.58% of coverage (Table 1). All reads were uniformly distributed in the whole genome region of SHV1. In addition, serum or CSF positive SHV1 IgG antibody was identified in 11 patients.

4. Discussion

Suid herpesvirus type 1 is a double-stranded DNA virus with icosahedral symmetric structure[13], phylogenetic analysis for the genome sequences shows that classical SHV1 strains from the USA and Europe are categorized as SHV1 genotype I, and variant SHV1 strains from China are categorized as SHV1 genotype II.
II, which might have caused several outbreaks of SHV1 infection among swineherds in Jiangxi province of China since 2011\textsuperscript{[2,14]}. The symptoms of pseudorabies were assumed to be associated with classical SHV1, while the variant SHV1 was considered to be the pathogen of viral endophthalmitis or encephalitis described in this study.

The first descriptions about pseudorabies came from two technicians exposed to contaminated materials in 1914, and showed weakness, restlessness, sore throat and itching\textsuperscript{[15]}. Additionally in 1987, three patients with seropositive SHV1 antibodies initially developed fever, sweating, weakness and tiredness, and then progressed to dysphagia, paraesthesia and tinnitus\textsuperscript{[6]}. The clinical symptoms of encephalitis associated with classical SHV1 were very similar to those of rabies that is one of the most lethal diseases known to human, while the clinical courses showed slow but complete recovery. Therefore, SHV1 used to be named pseudorabies virus. However, the clinical features associated with variant SHV1 showed quiet differences from pseudorabies\textsuperscript{[1,24]}. According to our summarization, the affected patients initially presented with influenza-like symptoms such as fever, headache and drowsiness, then quickly developed seizures, disturbed consciousness, endophthalmitis and respiratory failure. Consequently, all patients required ICU supporting for a long time, and had an unfavorable prognosis. Therefore, the encephalitis associated with variant SHV1 presented with severe disable or lethal viral encephalitis.

Viral glycoprotein gD of SHV1 mediates the entry process into host cell through binding the host cell by the nectin-1 receptor, similar to herpes simplex viruses (HSV)\textsuperscript{[16]}. Therefore, the MRI lesions of variant SHV1 encephalitis are prone to distribute in temporal-frontal lobes and insular cortex similar to those in HSV encephalitis\textsuperscript{[10]}, but some severe cases with disease progression have lesion expansions to parietal-occipital lobes, thalamus, basal ganglia, and brain stem, which are rarely observed in the HSV encephalitis. Additionally, the hemorrhagic lesions used to be considered as a differentiated point between SHV1 and HSV encephalitis\textsuperscript{[11]}, but our SHV1 encephalitis patient also showed hemorrhagic lesion in the advanced stage of disease.

mNGS is a revolutionary method in the identification of potential pathogens including rare and novel viruses\textsuperscript{[17]}. Currently, mNGS tests of CSF were conducted in all 20 patients, then unique sequence reads uniformly distributing in the whole genome region of SHV1 were identified, consequently a diagnosis of SHV1 encephalitis was made. The first human case of SHV1 infection with mNGS result was a pig farmer who was diagnosed as viral endophthalmitis with fever, headaches and visual impairment\textsuperscript{[7]}. The mNGS showed that the most likely pathogen was variant SHV1, which belonged to genotype II according to the phylogenetic analysis of the SHV1 genome sequence. Next, a patient of SHV1 encephalitis had 6198 unique sequence reads covering 80.58% of the SHV1 genome region, also indicating the genotype II of SHV1\textsuperscript{[2,5]}. The other 19 patient with lower coverage of SHV1 genome presented with a very similar phenotype to the patient with high coverage. Therefore, we speculated that all 20 patients with SHV1 encephalitis were associated with the SHV1 genotype II, though available mNGS data were not enough to identify the SHV1 genotype II.

At present, there is no available treatment guideline for SHV1 encephalitis treatment. According to the Clinical Practice Guidelines of Encephalitis by the Infectious Diseases Society of America (IDSA), acyclovir is recommended (I level, A evidence) for herpes virus infection\textsuperscript{[18]}. Therefore, 17 of 20 patients were given full-dose acyclovir, but the patients showed poor response to the conventional antiviral therapy. Studies about piscidin 1 and resveratrol have provided some alternative measures for SHV1 infection, but clinical trials need to be carried out in the future\textsuperscript{[19,20]}. Intriguingly, some SHV1 encephalitis patient showed an improvement of consciousness after immediate administration of both penciclovir or ganciclovir plus foscarnet sodium treatment\textsuperscript{[2,9]}. The prompt antiviral treatment might attenuate severe SHV1 encephalitis and facilitate the clinical recovery. Additionally, antiepileptic, respiratory support, nutritional support and pneumonia treatment are also important to improve the prognosis in patients with SHV1 encephalitis\textsuperscript{[12]}. Future studies to establish the optimal treatment regimen for SHV1 encephalitis are urgently needed.

According to the guidelines of viral encephalitis\textsuperscript{[21,22]} and clinical features of the 20 patients with SHV1 encephalitis, we made a proposal for diagnostic workflow of human SHV1 encephalitis (Table 2). (1) Patient should have at least an epidemiological history of occupations related to swine, or history of direct contact with raw pork, or location in SHV1 epidemic area. (2) Patient should have a fever and one symptom of acute brain parenchyma dysfunctions that manifested recurrent seizures/status epilepticus, or severe disturbance of consciousness including coma, delirium, decorticate state, minimally conscious state and vegetative state, or mental disorder. Additionally, acute vision loss due to endophthalmitis with fundus hemorrhage and papilledema could be considered as one of clinical features. (3) Patient had abnormal EEG, aseptic/viral encephalitis in CSF, and abnormal multiple cerebral MRI lesions similar to HSV encephalitis. (4) Patient should be identified with specific sequences of SHV1 in CSF or blood by mNGS or PCR, and positive SHV1 antibody in serum or CSF. The definite diagnosis of SHV1 encephalitis should include an epidemiological history, typical clinical features, abnormal auxiliary examinations, and definite SHV1 DNA evidence. The detailed workflow can find in the Table 2.
Table 2
Criteria for clinical diagnosis of human SHV1 encephalitis.

| Variables       | Descriptions                                                                 |
|-----------------|-----------------------------------------------------------------------------|
| A: Epidemiology | 1. History of direct contact with raw pork                                    |
| one or more 1–3 | 2. Occupations related to swine                                              |
|                 | 3. Location in SHV1 epidemic area                                            |
| B: Cardinal symptoms | 1. Fever (> 37.5°C in mouth)                                               |
| 1 + one or more 2–5 | 2. Recurrent seizures or status epilepticus                                 |
|                 | 3. Severe disturbance of consciousness (coma, delirium, decorticate state, | |
|                 | minimally conscious state and vegetative state)                            |
|                 | 4. Acute vision loss due to endophthalmitis with fundus hemorrhage and      |
|                 | papilledema                                                                 |
|                 | 5. Psychiatric symptoms                                                     |
| C: Auxiliary examinations | 1. EEG: generalized or predominantly front-temporal slow activity; periodic |
|                 | lateralized epileptiform discharges                                          |
|                 | 2. CSF: consistent with the features of CSF of aseptic encephalitis or      |
|                 | viral encephalitis                                                          |
|                 | 3. Brain MRI: high signals in front-temporal cortex, insular lobe, basal    |
|                 | ganglia and brain stem on FLAIR                                              |
| D: Virological tests | 1. Specific sequences of SHV1 identified in CSF or blood by mNGS or PCR    |
|                 | 2. Positive SHV1 antibody in serum or CSF                                    |

Definite diagnosis: A + B + C + D 1
Probable diagnosis: A + B + C1 + C2 + D 1
Possible diagnosis: A + B + C + D 2

SHV1: Suid herpesvirus type 1; EEG: electroencephalogram; CSF: cerebrospinal fluid; FLAIR: fluid attenuated inversion recovery; mNGS: metagenomic next generation sequencing.

This study had some limitations that need to be explicitly acknowledged. First, it was a retrospective study, thus some clinical data in our cases were incomplete that would lower the clinical significance, for example, the SHV1 antibodies were not measured due to lack of serum sample, therefore it might weaken the diagnosis of SHV1 encephalitis. Second, the descriptions about reported cases were incomplete, so some important clinical features and laboratory results might have bias in the summarization. Third, the diagnostic workflow of human SHV1 encephalitis was just formulated on literature review and our own experiences, so the reliability of diagnostic protocols was undermined and need to be validated in a perspective observation including more cases.

5. Conclusion
The variant SHV1 is a zoonotic pathogen that is associated with a new type of human viral encephalitis characterized by acute, fulminating and catastrophic central nervous system infection. Since the underlying pathological pathway for SHV1 in humans is limited, there is no available proven treatment for the encephalitis, though a rapid diagnosis is becoming possible through mNGS.

Abbreviations
SHV1: Suid herpesvirus type 1; CSF: cerebrospinal fluid; mNGS: Metagenomic next-generation sequencing; PRV: pseudorabies virus; MRI: magnetic resonance image; T1WI: T1-weighted spin echo; T2WI: T2-weighted spin echo; FLAIR: fluid attenuated inversion recovery; DWI: diffused weight image; PCR: polymerase chain reaction; GCS: Glasgow coma scale; HSV: herpes simplex viruses

Declarations
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Authors’ contributions:
ZY draft manuscript and analysis of data. WH, NC and LY contributed to the acquisition and analysis of data. ZM and XZ contributed to critical revision of the manuscript. HD contributed the study design and drafting the manuscript.

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Availability of data and material:
All relevant data are described within the paper. Deidentified data can be requested. Data can be requested by all interested researchers, who can be contacted via the corresponding author.

Ethics approval:
The research was approved by ethics committee of the first affiliated hospital of Nanchang University.

Consent for Publication:
Not Applicable.

Conflicts of interest/Competing interests:
The authors have declared no conflicts of interest.

References
1. Fan S, Yuan H, Liu L, et al. Pseudorabies virus encephalitis in humans: a case series study. J Neurovirol. 2020. 26(4): 556-564.
2. Liu Q, Wang X, Xie C, et al. A novel human acute encephalitis caused by pseudorabies virus variant strain. Clin Infect Dis. 2020. Epub ahead of print.
3. Hanson RP. The history of pseudorabies in the United States. J Am Vet Med Assoc. 1954. 124(925): 259-261.
4. Pomerantz LE, Reynolds AE, Hengartner CJ. Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. 2005. 69(3): 462.
5. Yang H, Han H, Wang H, Cui Y, Liu H, Ding S. A Case of Human Viral Encephalitis Caused by Pseudorabies Virus Infection in China. Front Neurol. 2019. 10: 534.
6. Mravak S, Bienzle U, Feldmeier H, Hampl H, Habermehl KO. Pseudorabies in man. Lancet. 1987. 1(8531): 501-502.
7. Al JW, Weng SS, Cheng Q, et al. Human Endophthalmitis Caused By Pseudorabies Virus Infection, China, 2017. Emerg Infect Dis. 2018. 24(6): 1087-1090.
8. Zhao WL, Wu YH, Li HF, et al. [Clinical experience and next-generation sequencing analysis of encephalitis caused by pseudorabies virus]. Zhonghua Yi Xue Za Zhi. 2018. 98(15): 1152-1157.
9. Zheng L, Liu X, Yuan D, et al. Dynamic cerebrospinal fluid analyses of severe pseudorabies encephalitis. Transbound Emerg Dis. 2019. 66(6): 2562-2565.
10. Wang D, Tao X, Fei M, et al. Human encephalitis caused by pseudorabies virus infection: a case report. J Neurovirol. 2020. 26(3): 442-448.
11. Yang X, Guan H, Li C, et al. Characteristics of human encephalitis caused by pseudorabies virus: A case series study. Int J Infect Dis. 2019. 87: 92-99.
12. Wang Y, Nian H, Li Z, Wang W, Wang X, Cui Y. Human encephalitis complicated with bilateral acute retinal necrosis associated with pseudorabies virus infection: A case report. Int J Infect Dis. 2019. 89: 51-54.
13. Guan X, Liu J, Jiang H, Wu CX, Chen HC, Liu ZF. Expression of pseudorabies virus-encoded long noncoding RNAs in epithelial cells and neurons. J Neurovirol. 2018. 24(5): 597-605.
14. Wong G, Lu J, Zhang W, Gao GF. Pseudorabies virus: a neglected zoonotic pathogen in humans. Emerg Microbes Infect. 2019. 8(1): 150-154.
15. Von Ratz ST. Die empfänglichkeit der tier fur paralysis bulbaris infectiosa. Zeitschrift fur Infektionskrankheiten. 1914. 15:2.
16. Li A, Lu G, Qi J, et al. Structural basis of nectin-1 recognition by pseudorabies virus glycoprotein D. PLoS Pathog. 2017. 13(5): e1006314.
17. Guan H, Shen A, Lv X, et al. Detection of virus in CSF from the cases with meningoencephalitis by next-generation sequencing. J Neurovirol. 2016. 22(2): 240-245.
18. Tunkel AR, Glaser CA, Bloch KC, et al. The Management of Encephalitis: Clinical Practice Guidelines by the Infectious Diseases Society of America. Clinical Infectious Diseases An Official Publication of the Infectious Diseases Society of America. 2008. (3): 3.
19. Zhao X, Cui Q, Fu Q, et al. Antiviral properties of resveratrol against pseudorabies virus are associated with the inhibition of IκB kinase activation. Sci Rep. 2017. 7(1): 8782.
20. Hu H, Guo N, Chen S, et al. Antiviral activity of Piscidin 1 against pseudorabies virus both in vitro and in vivo. Virol J. 2019. 16(1): 95.
21. Solomon T, Michael BD, Smith PE, et al. Management of suspected viral encephalitis in adults–Association of British Neurologists and British Infection Association National Guidelines. J Infect. 2012. 64(4): 347-473.
22. Venkatesan A, Geocadin RG. Diagnosis and management of acute encephalitis: A practical approach. Neurol Clin Pract. 2014. 4(3): 206-215.