

Human Endophthalmitis Caused By Pseudorabies Virus Infection, China, 2017

Jing-Wen Ai,¹ Shan-Shan Weng,¹ Qi Cheng,
Peng Cui, Yong-Jun Li, Hong-Long Wu,
Yi-Min Zhu, Bin Xu, Wen-Hong Zhang

We report human endophthalmitis caused by pseudorabies virus infection after exposure to sewage on a hog farm in China. High-throughput sequencing and real-time PCR of vitreous humor showed pseudorabies virus sequences. This case showed that pseudorabies virus might infect humans after direct contact with contaminants.

Pseudorabies virus (PRV) primarily infects swine and has several secondary hosts, including cattle, dogs, and cats. PRV, also called Aujeszky disease virus or Suid herpesvirus 1, is a member of the *Alphaherpesvirinae* subfamily within the family *Herpesviridae*. PRV infection has not been confirmed in humans (1), but previous reports have suggested the possible presence of PRV infection in 3 immunocompetent humans in whom fever, sweating, and neurologic complaints developed; virus neutralization and immune precipitation tests were positive for PRV antibody (2,3). We report a human case of human infectious endophthalmitis caused by PRV in a woman from Jiangxi Province, China, in July 2017.

The Study

On June 14, 2017, sewage from a hog farm spilled onto a 46-year-old woman from Jiangxi Province, China, who worked as a swineherder; her daily work was to feed swine and clean hoggerly sewage. The next day, she had a headache and fever of 39.5°C. Three days later, she became visually impaired and was admitted to a local hospital, where she was treated empirically with meropenem, vancomycin, and acyclovir. On June 29, after no significant improvement, she was transferred to Huashan Hospital of Fudan University (Shanghai, China), to treat unresponsive fever, headaches, and visual impairment.

On examination, she had palpebral conjunctival congestion, and visual acuity to light perception of both eyes

had worsened. Slit-lamp examination showed keratic precipitates and Tyndall effect flare. Funduscopic examination revealed vitreous opacity (Figure 1, panel A) and a pale white lesion on the posterior pole of the right eye (Figure 1, panel B), which suggested acute retinal necrosis and occlusive vasculitis. Results of routine laboratory testing were normal, including serology tests for HIV and hepatitis B and C, T-SPOT.TB test (Oxford Immunotec Ltd., Oxford, UK), blood culture, cryptococcal latex agglutination test, autoantibodies, and cerebrospinal fluid test. Test results for plasma Epstein-Barr virus and cytomegalovirus (CMV) IgG were positive; IgM was negative for both pathogens.

The on-staff ophthalmologist diagnosed endophthalmitis in the patient, considering viral infection as most likely. The patient was transferred to the ophthalmology department for vitrectomy surgery on the right eye on June 30. During the operation, ≈2 mL of vitreous humor was taken for culture and next-generation sequencing (NGS) (online Technical Appendix, <https://wwwnc.cdc.gov/EID/article/24/6/17-1612-Techapp1.pdf>)

On July 2, NGS results showed 4,832 unique sequence reads of PRV in vitreous humor, covering 84% of the nucleotide sequences (Table; Figure 2, panel A); NGS results for cerebrospinal fluid (CSF) were negative for PRV. Other detected sequences were within laboratory reference ranges. On the basis of NGS results, the physicians, who suspected that the patient may have acquired PRV infection, immediately initiated valacyclovir therapy. Sanger sequencing (online Technical Appendix Figure 1) and PCR analysis (online Technical Appendix Figures 2, 3) confirmed identification of PRV in vitreous humor (4–6). Phylogenetic analysis disclosed a close connection between the isolated strain and 3 emergent and highly pathogenic PRV variants in China (Figure 2, panel B; online Technical Appendix) (7,8).

To further validate our results, we constructed a plasmid and a standard curve for real-time PCR and obtained quantitative results of viral DNA load as 2.7×10^6 copies/mL (online Technical Appendix Figure 3). To rule out contamination, we performed PCR of the PRV on a control group of 7 persons; all were negative (online Technical Appendix Figure 2). Other possible viral causes of infective endophthalmitis, such as varicella-zoster virus, herpes simplex virus, and cytomegalovirus, were excluded through

Author affiliations: Huashan Hospital of Fudan University, Shanghai, China (J.-W. Ai, S.-S. Weng, Q. Cheng, P. Cui, Y.-M. Zhu, B. Xu, W.-H. Zhang); BGI genomics, BGI-Shenzhen, Shenzhen, China (Y.-J. Li); Binhai Genomics Institute, Translational Genomics Center, Tianjin, China (H.-L. Wu)

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¹These authors contributed equally to this article.

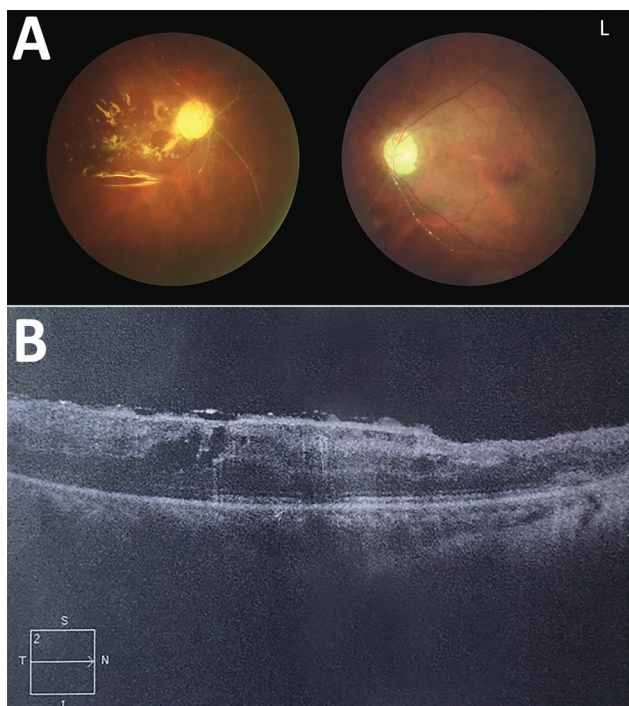


Figure 1. Ocular examination conducted 2 months after eye surgery in patient with human endophthalmitis caused by pseudorabies virus, China, 2017. A) Fundus photography of both eyes showing retinal necrosis and occlusive vasculitis and postoperative change after pars plana vitrectomy with silicone oil injection in the right eye. B) Optical coherence tomography of the patient's right eye showed postoperative change after pars plana vitrectomy. 2, scan depth (2 mm); I, inferior; L, left eye; N, nasal side; S, superior; T, temporal side.

PCR. One week after surgery, culture result for vitreous humor was negative; the patient's fever and headache had resolved, and visual acuity had improved slightly. The patient was discharged on July 11; during the last clinical check-up (December 6, 2017), the visual acuity of her left eye had improved to 0.2, and the right eye remained at slight light perception.

During the follow-up period, we obtained the patient's plasma and CSF samples and ordered PRV antibody testing. PRV antibody was detected in all plasma

samples at 4 and 5 months after disease onset and in CSF samples at 2 weeks to 2 months after disease onset, indicating the patient's previous contact with PRV (online Technical Appendix Figure 4). Epstein-Barr virus, CMV, and PRV serologic tests were also conducted on control samples and ruled out the possibility of cross-reaction (online Technical Appendix Table 2) (9,10). Although the patient experienced headache and fever during disease progression and CSF PRV antibody test was positive, routine tests, NGS, and PCR of the CSF all failed to disclose abnormality. Therefore, we do not have enough evidence to suggest possible PRV central nervous system infection. Considering epidemiologic history, clinical symptoms, and serologic and molecular testing results, we diagnosed PRV endophthalmitis.

Conclusions

The first 2 suspected cases of human PRV infection were reported in 1914, but detection of antibodies or cultivation of the virus had failed. In 1987, Mravak reported 3 suspected cases of human PRV infection with positive serum antibodies (2). All 3 patients were immunocompetent and their clinical manifestation occurred 1–3 weeks after possible animal contact. Initial symptoms included fever, sweating, and weakness; later, central nervous system symptoms developed. Some symptoms persisted for months, and serologic PRV antibodies were positive 5–15 months after the onset of clinical symptoms. In our study, the patient had similar symptoms, plus visual impairment and a unique infection route of PRV through direct exposure to contaminants.

Since 2011, prevalence of PRV infection among swineherds in China has risen several times (11). Among swineherds in Jiangxi Province, studies have shown varying positive rates for PRV DNA: from 5.5% to 26.5% during 2014, (12), and of positive PRV antibodies, from 84.4% to 89.9% during September 2013–September 2015 (13). PRV vaccine is still provided for swineherds on a voluntary basis rather than as a requirement in China, and swine were not vaccinated in the hogery in which this patient worked at that time. On the day of disease onset,

Table. Pathogens detected by using next-generation sequencing in vitreous humor from a patient with human endophthalmitis caused by pseudorabies virus, China, 2017

Pathogen	Coverage, %	Depth, bp	Unique reads
Virus			
<i>Suid herpesvirus 1</i>	84	6.8	4,832
<i>Bovine herpesvirus 5</i>	8.9	1	1
Bacteria			
<i>Thermoanaerobacter wiegelsii</i>	0.52	5.3	9
<i>Corynebacterium urealyticum</i>	0.56	1	0
<i>Haloquadratum walsbyi</i>	0.47	2.8	0
<i>Brachyspira pilosicoli</i>	0.41	1	1
<i>Candidatus Nitrososphaera</i>	0.46	1	0
Fungus			
<i>Cryptococcus gattii</i>	0.46	1	1

the patient's eyes were directly contaminated with sewage containing pig excrement when cleaning pig sties. Although no previous study had reported a confirmed case of PRV-caused infectious endophthalmitis, the atypical infectious route in this case made hoggerly the most probable infectious source in this study.

NGS is marked by its rapid diagnostic ability to precisely identify certain pathogens in peripheral blood,

respiratory, and CSF samples. In this case, NGS testing in facilitating the diagnosis of infective endophthalmitis is supported. The fact that real-time PCR and Sanger sequencing results were consistent with the NGS results further validated the credibility of this technique. Furthermore, a serologic test was conducted during the follow-up period; results showed that, even 5 months after symptom onset, the antibodies to PRV in plasma were

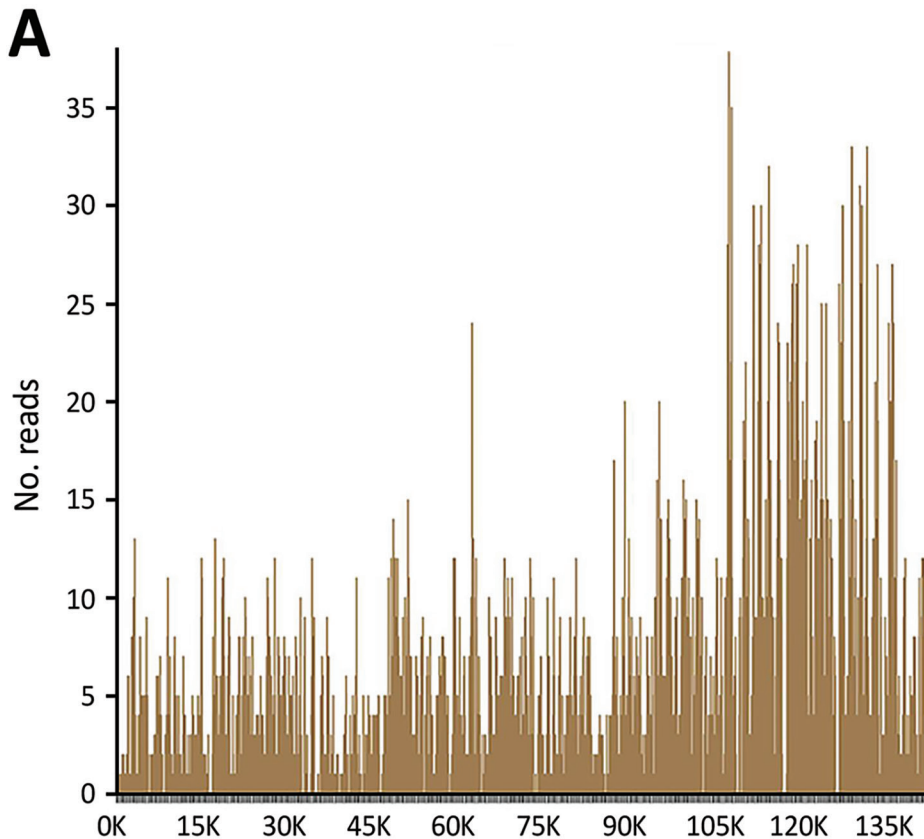
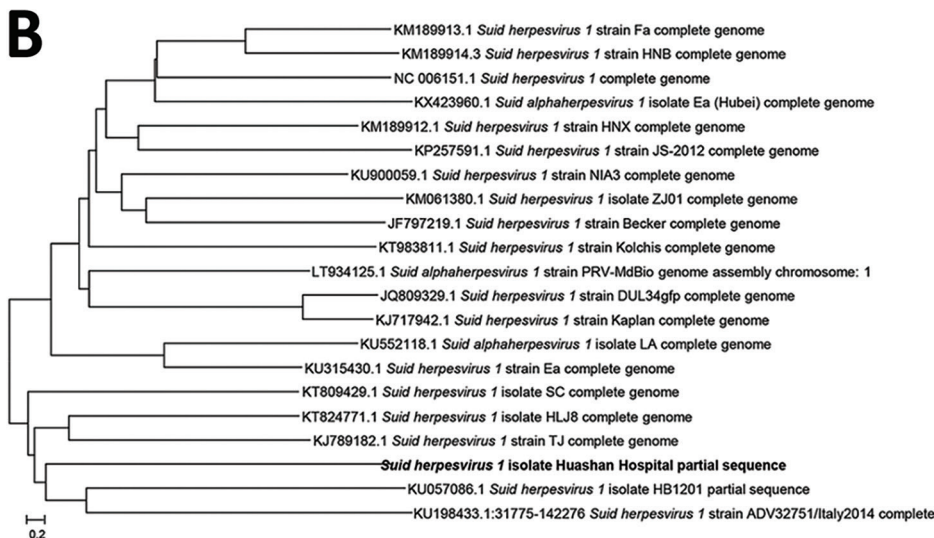


Figure 2. Results of gene sequencing, serologic testing, and phylogenetic analysis of pseudorabies virus from patient with human endophthalmitis, China, 2017. A) Sequencing of Suid herpesvirus 1 (pseudorabies virus) yielded a total coverage of 84%. B) Evolutionary relationships of taxa. Phylogenetic analysis disclosed a close connection between the isolate from the patient (boldface) and 4 other Suid herpesvirus 1 strains. Scale bar indicates amino acid substitutions per site.



still active, and the antibodies in CSF persisted during the entire follow-up period (online Technical Appendix Figure 4). This result is similar to that reported in 1987, indicating that PRV antibodies may persist long after the initial infections.

In summary, this case of PRV-caused human infectious endophthalmitis indicates that PRV could affect humans through direct contact with pig contaminants. NGS of the vitreous humor provided a strong technical support for rapid diagnosis of PRV infection in this patient. However, the pathogenesis of PRV infection remains to be explored. This case stresses the importance of mandatory PRV vaccine among swineherds and the necessity for workers in the breeding industry to increase awareness of self-protection when handling animal containments.

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About the Author

Dr. Ai is a clinical resident at Huashan Hospital affiliated to Fudan University. Her research interests include the precision medicine of infectious diseases and the use of high throughput sequencing in assisting the rapid diagnosis of causative pathogens.

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Address for correspondence: Bin Xu, Department of Infectious Diseases, Huashan Hospital, Fudan University, Rm 504, Bldg 5, Huashan Hospital, No. 12, Middle Wulumuqi Road, Jing'an District, Shanghai, China; email: xbin2007@aliyun.com; Wen-Hong Zhang, Department of Infectious Diseases, Huashan Hospital, Fudan University, Rm 501, Bldg 5, No. 12, Middle Wulumuqi Road, Jing'an District, Shanghai, China; email: wenhongzhang_hs@126.com

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Technical Appendix

Next Generation Sequencing

The ophthalmologist collected 2 mL of the vitreous humor from the patient according to standard procedures, and we processed it accordingly. A 1.5ml microcentrifuge tube with 0.5mL sample and a 1g, 0.5mm glass bead were attached to a horizontal platform on a vortex mixer and agitated vigorously at 2,800–3,200 rpm for 30 minutes. A 0.3mL sample was separated into a new 1.5 mL microcentrifuge tube and DNA was extracted by using the TIANamp Micro DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's recommendation. We constructed DNA libraries through DNA-fragmentation, end-repair, add A-tailing, adaptor-ligation and PCR amplification. We used Agilent 2100 for quality control of the DNA libraries. Quality qualified libraries were sequenced by BGISEQ-100 platform.

Polymerase Chain Reaction, Sanger Sequencing and Real-time PCR

DNA was extracted by using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) for extraction according to the manufacturer's recommendations and finally eluted in 100µL of buffer. Three pairs of specific primers were designed according to related articles (1,2,3) and the sequences of primers are summarized in Technical Appendix Table 1. All oligonucleotides were synthesized by BGI.

All PCRs were completed by using the Takara RR902A Kit in a total reaction volume of 25 µL. For a single reaction, 8.5 µL RNase-free water, 12.5 µL Premix Taq and 2 µL primers were merged as a master mix. Finally, we added the 2 µL DNA template and PCR was completed by using a ProFlex PCR System. The following thermal profile was used: PCR initial activation step at 95°C for 5 min; 35 cycles of 3-step cycling consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s; finally extension at 72°C for

5 min. After obtaining positive results by electrophoresis, Sanger sequencing was performed by BGI as of which result were shown in Technical Appendix Figure 1.

PCRs were conducted among vitreous humor, plasma and cerebrospinal fluid obtained during follow-ups and only in vitreous humor was the PCR result positive (Technical Appendix Figure 2A). Besides, we also conducted PCR of pseudorabies virus in specimens of other patients during the same period, and the results were all negative, ruling out the possibility of contamination (Technical Appendix Figure 2B).

Real-time PCR were used to measure the viral load. Primer PRV -gE-Sybr were used since it works best. We ligated the PCR product to the Peasy-T3 plasmid and constructed standard curves with eight different concentration gradients from 10^2 to 10^9 . The same pair of primers and the same profile of cycling stage were used and the melt curve stage was 95°C for 15 s, 60°C for 60 s, followed by 95°C for 30 s and 60°C for 15 s at last. The quantitative result of the viral DNA load is 2.7×10^3 copies/uL (File 3, Figure S3).

Serologic Tests

IDEXX PRV gB Antibody Test Kit (IDEXX Laboratories, Westbrook, ME, USA) was used to test the antibodies of the patient's plasma, vitreous humor and cerebrospinal fluid. Protein gB is a conserved protein of PRV and present in all kinds of pseudorabies virus. The principle of this kit is that the PRV gB antibodies in the sample will bind to the antigen coated in the microwell, competing with enzyme-conjugated monoclonal antibodies. If no PRV gB antibodies are present in the test sample, the conjugated antibodies are free to react with the antigen. In the presence if enzyme, the substrate is converted to a product that reacts with the chromophore to generate a blue color.

Samples diluted by sample diluent were added to a PRV antigen-coated plate. After incubation and elution, OD values of each microwell were evaluated by spectrophotometer at 650 nm. Results are calculated by dividing the A(650) of the sample by the mean A(650) of the negative control, resulting in a sample/negative (S/N) value. The quantity of antibodies to PRV is inversely proportional to the A(650) and, thus, to the S/N value. If the S/N value is no higher than 0.60, than the result is considered positive.

Serologic tests of EBV/CMV/HSV-1/HSV-2/VZV were conducted by KingMed Diagnostics (Shanghai) laboratory. Besides plasma collected from this patient, control samples used were plasma from the same 20 patients who have been the control group in PRV serologic test. EBV IgG, HSV-1&2 antibodies and VZV antibodies were tested through euzymelinked immunosorbent assay. CMV IgG were tested through electrochemiluminescence detection method.

The results were shown in Technical Appendix Table 2. Serologic tests of HSV 1/2 and VZV were conducted on this patient and the results were negative. As for EBV and CMV antibodies, IgG for both viruses were positive while IgM were both negative. In the control group, 19 of 20 patients had positive plasma EBV IgG and all 20 patients had positive plasma CMV IgG while their PRV antibodies were all negative, which ruled out the possibility of false-positive serologic test of PRV due to cross-reaction with EBV or CMV serologic tests. Besides, the serologic test results of EBV and CMV were in accordance with the global epidemiologic data: EBV infects more than 90% of the world's adult human population (4) while CMV seroprevalence rates ranging 40–100% of the adult population (5). Therefore, we believe that the above data will further support the PRV serologic tests results in our study.

Phylogenetic Analysis

We inferred the evolutionary history of the Suid herpesvirus by using the Neighbor-Joining method in making a phylogenetic tree (Main article, Figure 2B). The optimal tree with the sum of branch length = 171.41397980 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed by using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 21 nt sequences. All positions containing gaps and missing data were eliminated. There were a total of 56899 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. The scale bar represents 0.2 aa substitutions per site.

The result revealed that the sequences of the PRV (Suid herpesvirus 1) in our case has a close connection with the Suid herpesvirus 1 isolate HB1201, Suid herpesvirus 1 strain ADV32751/Italy2014 (6), Suid herpesvirus 1 strain TJ (7) and Suid herpesvirus 1 isolate HLJ8

(8), among which three were observed in China. The Suid herpesvirus 1 strain TJ is an emergent and highly pathogenic PRV variant with unique molecular signatures clustering in one clade with Suid herpesvirus 1 isolate HLJ8. Both strains were isolated from the northern regions of China and were related to the recent years' PRV -like outbreaks occurred in numerous Bartha-K61-vaccinated pig farms in multiple regions in China.

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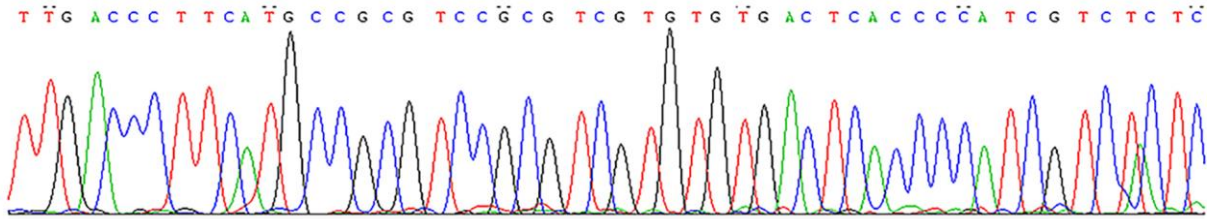
Technical Appendix Table 1. Primers used in polymerase chain reaction

Target gene	Primers sequence (5'-3')	Amplicon (bp)
PRV -gB-718F	ACAAGTTCAAGGCCACATCTAC	94
PRV -gB-812R	GTCYGTGAAGCGGTTTCGTGAT	94
PRV -gB-778F	ACCAACGACACCTACACCAAG	79
PRV -gB-856R	CCTCCTCGACGATGCAGTTG	79
PRV -gE-Sybr-R	GGAGAGACGATGGGGTGAGT	116
PRV -gE-Sybr-F	GACGGATGTGATGTTGCTGA	116

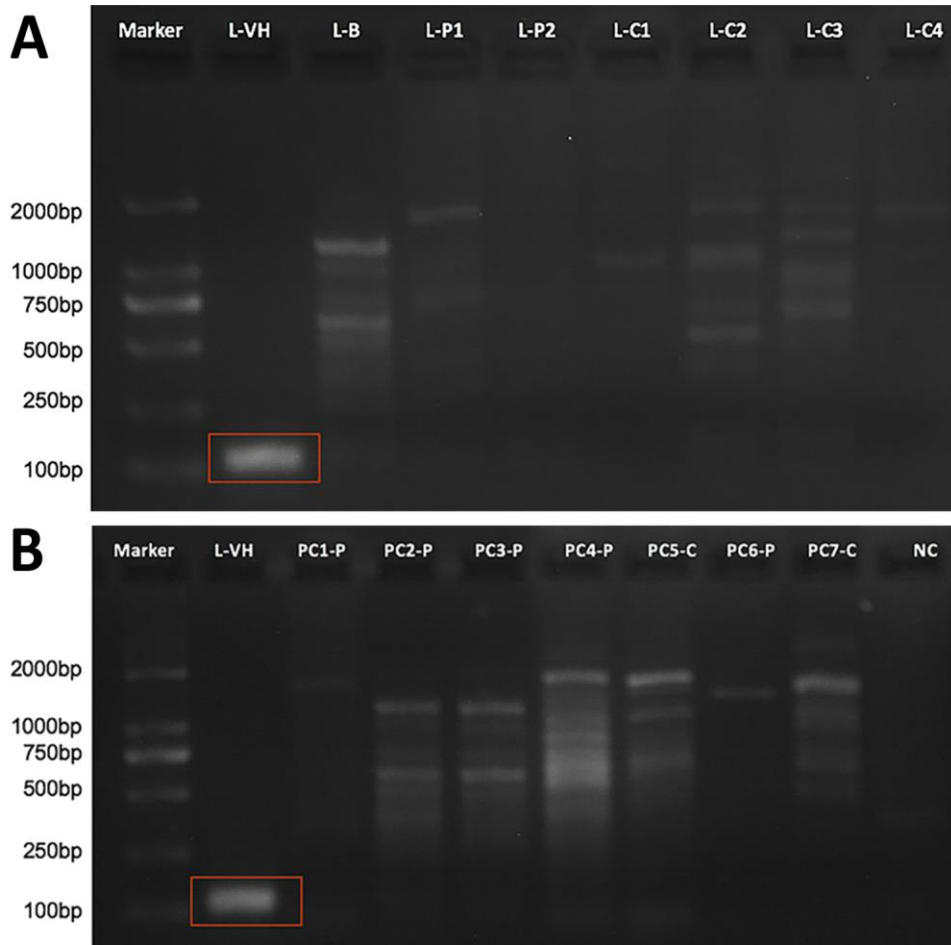
Technical Appendix Table 2. Serologic tests result of PRV, EBV and CMV for the target patient and control group*

Sample	PRV-antibodies	EBV-IgG	CMV-IgG
Target Patient	+	+	+
Control 1	-	+	+
Control 2	-	+	+
Control 3	-	+	+
Control 4	-	+	+
Control 5	-	+	+
Control 6	-	+	+
Control 7	-	+	+
Control 8	-	+	+
Control 9	-	+	+
Control 10	-	+	+
Control 11	-	+	+
Control 12	-	+	+
Control 13	-	+	+
Control 14	-	+	+
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Control 17	-	+	+
Control 18	-	+	+
Control 19	-	-	+
Control 20	-	+	+

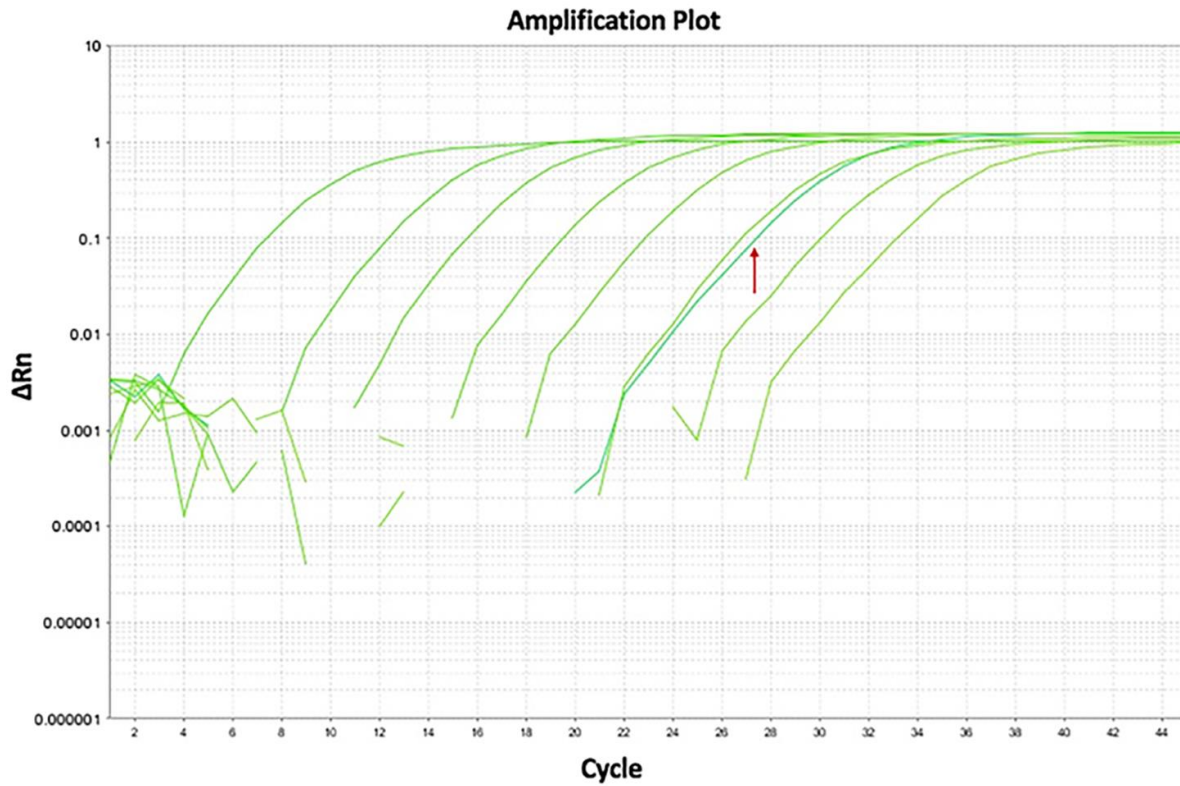
*PRV, pseudorabies virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; +, positive; -, negative.



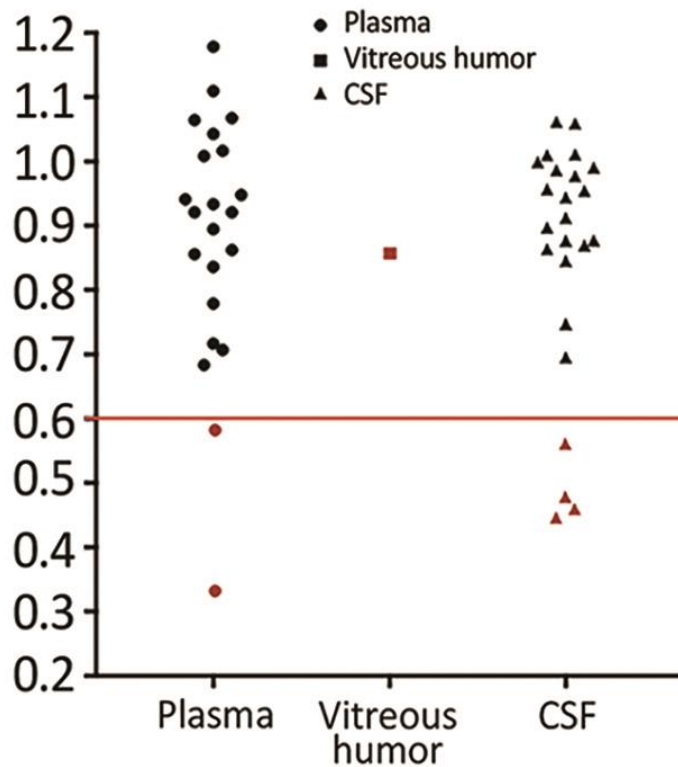
Technical Appendix Figure 1. Sanger sequencing of PCR product identified Suid herpesvirus-1.



Technical Appendix Figure 2. Electrophoretogram of PCR; the image outlined in red in panels A and B is the PrV amplified product: A) PCR conducted among vitreous humor, plasma, and cerebrospinal fluid obtained from the patient during follow-up exams: L represents this patient, VH represents vitreous humor, B represents blood, P represents plasma, C represents cerebrospinal fluid. B) PCR conducted among samples collected from other patients as control group during the same period: L represents this patient, PC1–7 represents different patient control, VH represents vitreous humor, P represents plasma, C represents cerebrospinal fluid, NC represents negative control.



Technical Appendix Figure 3. Amplification curve of the real-time PCR. The dark dotted line with arrow indicates vitreous humor sample; light dotted lines indicate standard curves with different concentration gradients. ΔRn , amount of probe degradation during PCR, which represents the amount of PCR product.



Technical Appendix Figure 4. The result of serologic testing reveals that there were antibodies to pseudorabies virus in the patient's plasma and cerebrospinal fluid. The red dots represent samples from this patient; black dots represent 20 different control samples obtained from other patients randomly chosen from databases during the same period. S/N, sample/negative value; results are considered positive when S/N is ≤ 0.60 (Technical Appendix). The 2 plasma samples collected 4 months and 5 months after disease onset from this patient and the 4 cerebrospinal fluid samples collected during 2 weeks to 2 months after disease onset obtained positive results; the vitreous sample collected during the operation obtained a negative result. All control samples obtained negative results.