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CASE REPORT

The First Human Infection Case of Macacine Alphaherpesvirus 1 Virus in China

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SUMMARY

Background: **Macacine alphaherpesvirus 1 (BV) was first reported in the 1930s and only about 60 cases have been diagnosed since then.**
Methods: **A 53-year-old male who worked as a veterinary surgeon, developed a fever with nausea and vomiting in April 2021 in Beijing, China. Real-time polymerase chain reaction (PCR) and metagenomics Next Generation Sequencing (mNGS) were used for diagnosis.**
Results: **BV DNA was confirmed by mNGS and PCR. The case died 51 days after onset, due to the damage to the brain and spinal cord caused by a viral infection and hypoxic-ischemic encephalopathy. The typical BV inclusion bodies in the brain were found for the first time.**
Conclusions: **Here we reported the first human infection case of BV in China. This fatal case highlights the potential threat of BV to occupational workers and the essential role of surveillance.**
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Supplementary Data

METHODS

Sample processing and DNA extraction
Approximately 3 mL cerebrospinal fluid (CSF), 0.5 mL blister fluid, 5 mL blood, 3 mL nasal swab, and 3 mL oropharyngeal swab sample of the patient was collected on 14 days after onset. Total RNA/DNA was extracted from 200 µL samples using an automated total nucleic acid extraction system according to the instructions (Tianlong, Xi'an, China).

Library preparation and metagenomic next-generation sequencing (mNGS)
The RNA/DNA was quantified using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The RNA was reverse-transcribed, then amplified with DNA using ULSEN MicroSpectrum Kit (MicroFuture, Beijing, China). The sequencing libraries were prepared using the Illumina Nextera XT Library Prep Kit (Illumina, San Diego, CA, USA) and were conducted by Illumina sequencing.

Data analysis
Quality control was carried out after high-quality sequencing data were obtained. Then the reads consistent with the human reference genome sequence were screened and removed [1]. After removing the low complexity reads, the remaining data were compared with microbial databases using a commercial pipeline (MicroFuture). The number of reads that could match a certain pathogen was obtained, and the possible pathogens were judged. Then the reads that match with alphaherpesvirus were extracted and mapped to the reference genome NC_004812.1.

Confirmatory assay by real-time PCR
Both in-house assay and commercial kits were used to verify the BV infection. For the in-house assay, real-time PCR was performed using Premix Ex Taq master mix for TaqMan real-time PCR (Takara, Dalian, China), according to the manufacturer's instructions. The following PCR conditions were used: denaturation at 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 60 seconds for 40 cycles. Twelve sets of primer-probe targeting BV, Monkeypox, pan-Orthopoxvirus, VZV (Varicella-Zoster Virus, Human Herpes Virus 3), EBV (Epstein-Barr Virus, Human Herpes Virus 4), CMV (Cytomegalovirus, Human Herpes Virus 5), HHV-6a/b, HHV-7, and HHV-8 were applied according to previous reports (supplementary table 1). For commercial assays, herpes simplex virus 1 (HSV-1, HHV-1), HSV-2 (HHV-2), VZV, EBV, CMV, and HHV-6 were tested according to the manufacturers' instructions (Mabsky, Shenzhen, China and Altona Diagnostics, Hamburg, Germany).

Virus isolation
Vero and HEp-2 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% calf bovine serum. The virus was propagated at 37°C as described previously [2].

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Table S1. Oligonucleotide primers and probes for qPCR.

Pathogen	Gene target	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Probe (5' to 3')	Reference
Macacine Alphaherpes-virus 1	US3	TGGCCTACTACCGCGTGG	TGGTACGTGTGGGAGTAGCG	FAM-CCGCCCTCTCCGAGCACGTG-TAMRA	[1]
Monkeypox	F3L	CTCATTGATTTTTCGCGGGATA	GACGATACTCCTCCTCGTTGGT	FAM-CATCAGAATCTGTAGGCCGT-MGBNFQ	[2]
Monkeypox	N3R	AACAACCGTCCTACAATTAAACAACA	CGCTATCGAACCATTTTTGTAGTCT	FAM-TATAACGGCGAAGAATATACT-MGBNFQ	[2]
pan-Orthopox-virus	HA (J7R)	GATGATGCAACTCTATCATGTA	GTATAATTATCAAAATACAAGACGTC	FAM-AGTGCTTGGTATAAGGAG-MGBNFQ	[3]
pan-Orthopox-virus	DNA poly-merase (E9L)	GAACATTTTTGGCAGAGAGAGCC	CAACTCTTAGCCGAAGCGTATGAG	FAM-CAGGCTACCAGTTCAA-MGBNFQ	[3]
VZV (Varicella-Zoster Virus, HHV-3)	Gene 28	AACTTTTACATCCAGCCTGGCG	GAAAACCCAAACCGTTCTCGAG	FAM-TGTCTTTCACGGAGGCAAACACGT-TAMRA	[4]
EBV (Epstein-Barr Virus, HHV-4)	BALF 5	TCTGCTTTTCAACGGAGCTCA	ACTTCTGGTAGACGGCCAGGT	FAM-GCTCTCAGCCTACAAGAGCACCCAGATG-TAMRA	[5]
CMV (Cytomegalo-virus, HHV-5)	UL54	CCTGGTGCTTTCGTCGGT	CAATCGCTTAATGACGGCCAAT	FAM-AAGGACATCTCGCTGTACCGTCAATCTAACC-TAMRA	[5]
HHV-6a	U38	GTTGTCCTCTGTGCTTTCCAAA	GCCAATAGATGGTGCTATTAACACG	FAM-TCTGCGCCAACCTTCTAATGACGCTAA-TAMRA	[5]
HHV-6b	U65/66	TGTTGTCTTCTGTGCTTTCCAAG	TTACCAGTAGATGGTGCTATTAACACA	FAM-TCTGCGCCAACCTTCTAATGACGCTAA-TAMRA	[5]
HHV-7	U10	TCTGCCTACAAGCAAGCAAATTT	CTATGTTTGGCAACTCTTCCTTTCT	FAM-TGAGCTAACCTTTTAACAACAGCCAAATGTGG-TAMRA	[5]
HHV-8	ORF73	CGTTTACCACCGAGCTAAGCC	GTAGCTTTTGGTACACGGTCAGG	FAM-CGCTGGCGGACTACAAGACGCA-TAMRA	[5]

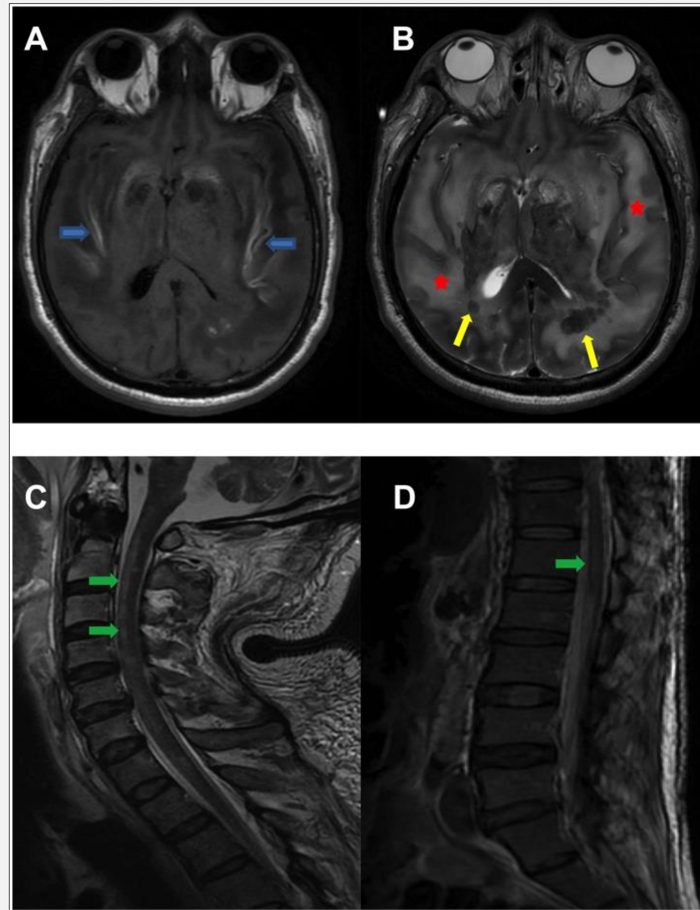

Figure S1. Head axial MRI T1WI, T2WI, cervical and lumbar sagittal T2WI of the case.

A, The Head axial MRI T1WI. The cortical laminar necrosis after ischemia and hypoxia was indicated by blue arrows. B, the Head axial MRI T2WI. The multiple low signal foci and hyperintensity in the brain were indicated by yellow arrow and red star, respectively. C and D, MRI T2WI of cervical and lumbar spine, respectively. The multiple abnormal signals in spine were indicated by green arrow.

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