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Letter

# Isolation and phylogenetic analysis of swinepox virus from pigs in China

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*Dear Editor,*

Swinepox virus (SWPV) belongs to the family *Poxviridae*, subfamily *Chordopoxvirinae*, and genus *Varicellovirus* (Lefkowitz et al., 2018). It exhibits the typical brick-shaped morphology and envelope structure characteristic of poxviruses. Its genome is a 146 kbp linear double-stranded DNA encoding approximately 150 genes (Moorkamp et al., 2008; Schwarz et al., 2024). SWPV is highly host-specific, infecting only domestic pigs and wild boars. Transmission occurs through direct contact with infectious scabs, congenital transmission, or mechanical transmission by arthropods such as pig lice and house flies. The disease is more likely to occur in pig farms with poor sanitation conditions (Borst et al., 1990; Jindal, 2015; Kaiser et al., 2021; Kumar et al., 2023; Riyesh et al., 2016).

Upon infection, SWPV enters host cells via membrane fusion. Clinical manifestations include widespread bag-like lesions on the skin, often accompanied by mild fever and localized lymphadenopathy (Medaglia et al., 2011, 2015). Adult pigs typically exhibit a self-limiting disease, while piglets may develop severe symptoms, even leading to death, resulting in significant economic losses (Koltsov et al., 2023). The disease was first reported in North America in 1929 and is now globally distributed (Aasdev et al., 2021). Recent outbreaks have been reported in Russia (2007) (Koltsov et al., 2023), India (2011–2021) (Mittal et al., 2011), and Germany (2021) (Kaiser et al., 2021). Although the actual incidence rate may be underestimated, it remains relatively rare compared to other poxviruses (Riyesh et al., 2016). Diagnosis is mainly based on characteristic skin lesions and epidemiological features, with attention needed to differentiate from vaccinia virus infections. Notably, the virus has clear seasonal epidemiological patterns, with higher incidence during the rainy season, suggesting that environmental factors play a significant role in disease transmission (Kumar et al., 2023).

In 2010, a significant outbreak of SWPV was reported at a pig farm in Jiangxi Province, China. The infected pigs were mostly piglets and pregnant sows, exhibiting clinical symptoms such as pox lesions on the back and shoulders, accompanied by loss of appetite, listlessness, *etc*. The infection was diagnosed as SWPV based on clinical manifestations, pathological analysis, virus isolation, and molecular characterization (Zhang et al., 2012). In 2024, an outbreak of swine pox virus occurred in Suzhou (Anhui Province), and in 2025, it occurred in Chenzhou (Hunan Province), China (Fig. 1A). The outbreaks primarily affected Yorkshire-Large White cross piglets, with clinical symptoms of bag-like abdominal skin lesions, accompanied by lethargy, fever, and anorexia, ultimately leading to death (Fig. 1B). The skin scab samples were collected, with six (Samples a–f) from Anhui Province and five (Samples g–k) from Hunan Province. Subsequently, virus isolation and culture experiments revealed that when the skin scab suspension was inoculated into swine tesis (ST) cells and pig kidney PK-15 cells, respectively. ST cells exhibited cell degeneration 36 hours after infection, showing typical cytopathic effects (CPE) including cell rounding, detachment, nuclear vacuolation, and cytoplasmic inclusion body formation. In contrast, PK-15 cells displayed CPE at 48 hours post-infection (Fig. 1C). Electron microscopy revealed virus particles with a typical brick or oval shape, approximately 310 nm by 240 nm in size, and possessing the characteristic dumbbell-shaped core structure of poxviruses (Fig. 1D). The virus's titer was determined in PK-15 cells, calculated to be $1 \times 10^6$ $TCID_{50}$/mL by the Reed-Muench method (Supplementary Fig. S1). These results suggest that SWPV can effectively replicate in ST and PK-15 cell lines, with ST cells exhibiting greater sensitivity, providing an important experimental basis for future viral research.

To ensure the identification precision, two pairs of specific primers (SWPV-JC-1 F/R and SWPV-JC-2 F/R) were used for PCR amplification of DNA extracted from porcine scab samples, yielding specific bands of 555 bp and 961 bp, respectively (Fig. 1E–H). Further PCR amplification was performed on the genes of the viral late transcription factor 3 (*VLTF-3*), kelch-like protein (*ORF006/136*), extracellular membrane-associated viral protein (*ORF119/120*), and anchoring protein repeat sequence (*ORF143/144*). *VLTF-3* gene alignment analysis showed that the Hunan

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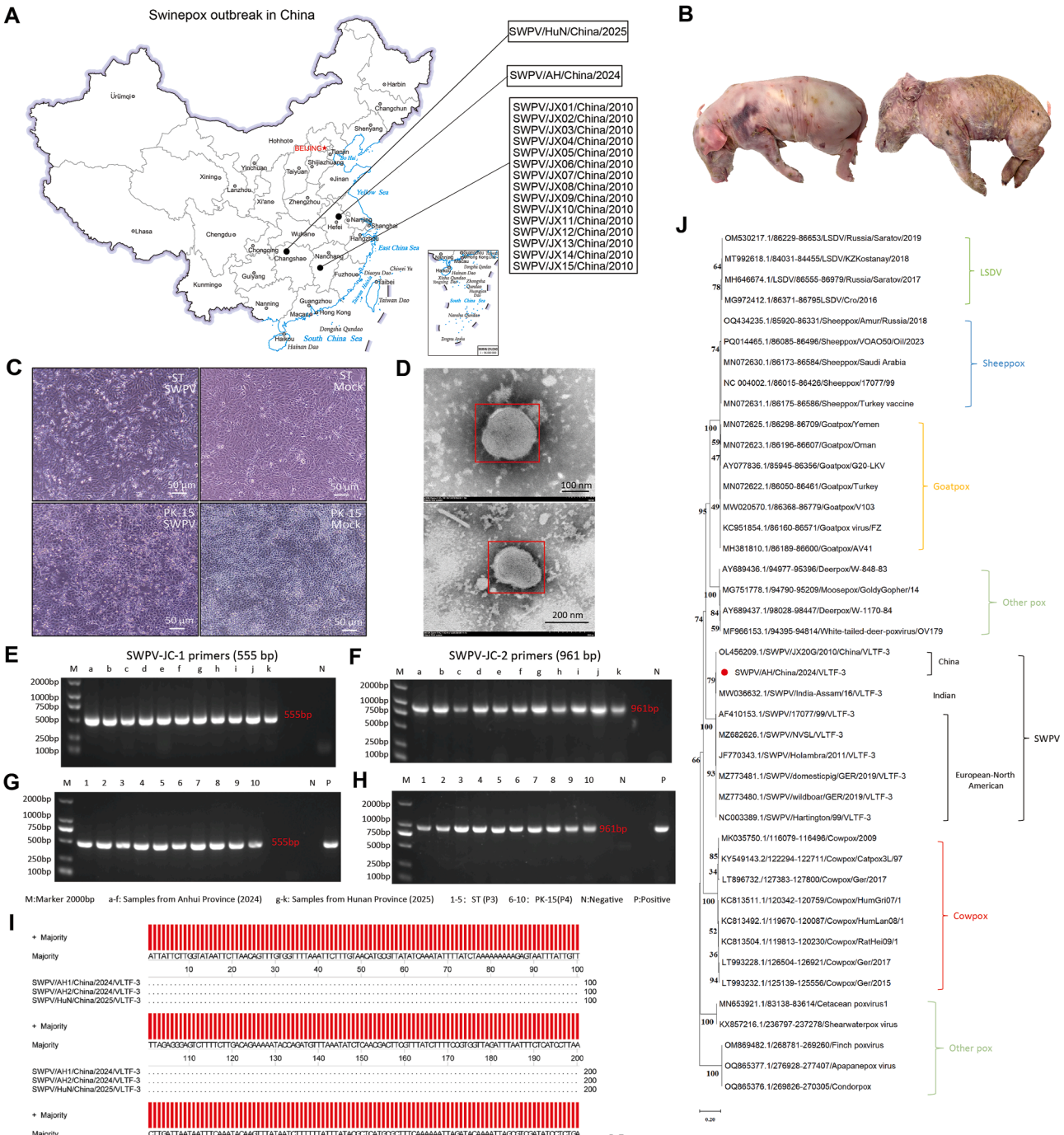


K

| | Swinepox | Cowpox | Goatpox | Sheeppox | LSDV | Otherpox |
|---|---|---|---|---|---|---|
| Swinepox | *97.5%-100%* | *12.3%* | *10.9%-11.7%* | *12.4%* | *12.3%* | *10.9%-58.5%* |
| Cowpox | | *97.8%-100%* | *70.1%-71.5%* | *70.5%-72.3%* | *70.5%-71.9%* | *14.6%-97.1%* |
| Goatpox | | | *99.3%-100%* | *97.1%-97.8%* | *97.1%-97.8%* | *12.4%-80.3%* |
| Sheeppox | | | | *100%* | *100%* | *13.1%-80.3%* |
| LSDV | | | | | *100%* | *13.1%-78.6%* |

(caption on next page)

**Fig. 1.** Isolation and phylogenetic analysis of swinepox virus from pigs in China. **A** The epidemic distribution map swinepox in China. **B** Piglets infected with swinepox. Piglets infected with swinepox exhibit generalized pock lesions across the body. **C** SWPV isolation in ST and PK-15 cells line. The infected ST cell monolayer exhibited characteristic CPE at 36 hours, including cell rounding, detachment, nuclear vacuolation, eosinophilic granule formation, cytoplasmic inclusions, disrupted intercellular connections, and localized cell death. Mock-treated ST cells retained normal morphology at 36 hours. In PK-15 cells, the infected monolayer displayed cell rounding morphology, increased granules, cell degeneration and detachment at 48 hours, whereas mock-control PK-15 cells maintained normal morphology. Scale bar 50 μm. **D** Negative-stain-TEM image analysis of SWPV. SWPV was subjected to TEM imaging after negative staining. The virus particles were marked in red boxes. **E–H** PCR analysis of piglet skin pox scab and viral fluid samples. **E/G** PCR amplification of SWPV-specific genomic region gene using specific SWPV-JC-1 primers (555 bp); **F/H** PCR amplification of SWPV-specific genomic region gene using specific SWPV-JC-2 primers (961 bp). **I** Isolation, identification, and comparative analysis of the SWPV strain *VLTF-3* gene sequence. **J** The phylogenetic tree of *Poxviridae* family members based on *VLTF-3* gene sequences. **K** Amino acid homology analysis of *Poxviridae* family members based on *VLTF-3* gene.

strain (SWPV/HuN/China/2025) was highly homologous to the Anhui isolate strain (SWPV/AH/China/2024) (Fig. 1I). In addition, we conducted a phylogenetic analysis of SWPV/AH/China/2024. The maximum likelihood phylogenetic tree was constructed, and amino acid homology analysis was performed. The results showed that the *VLTF-3* gene (482 bp/160 aa) of the SWPV virus formed a unique cluster, with the Anhui strain (SWPV/AH/China/2024) showing 100% amino acid homology to the Jiangxi strain (SWPV/JX20G/China/2010) (Fig. 1J, Supplementary Fig. S2). Additionally, the VLTF-3 amino acid homology among all SWPV strains ranged from 99.2% to 100%, demonstrating a close evolutionary relationship (Supplementary Fig. S3). In contrast, the homology with other poxvirus members was only 10.9%–58.5%, indicating a distant relationship (Fig. 1K). Analysis of the *ORF006* (1593 bp/530 aa) and *ORF136* (1725 bp/574 aa) genes showed that the Anhui strain (SWPV/AH/China/2024) clustered with the Jiangxi strain (SWPV/JX20G/China/2010) and the U.S.A. strain (SWPV/Hartington/99), with amino acid homologies of 98.7% and 99.8%, respectively (Supplementary Fig. S4). The analysis of *ORF119* (558 bp/185 aa) and *ORF120* (510 bp/169 aa) revealed that the Anhui strain formed an independent branch with the Jiangxi strain (SWPV/JX20G/China/2010), with 100% amino acid homology (Supplementary Fig. S5). *ORF143* (1458 bp/485 aa) and *ORF144* (1482 bp/493 aa) showed high conservation, with 100% homology to the Jiangxi strain (SWPV/JX20G/China/2010) and 97.6%–99% homology to other reference SWPV strains (Supplementary Fig. S6). We classified these Chinese strains, including SWPV/JX20G/China/2010, SWPV/AH/China/2024, and SWPV/HuN/China/2025, as part of the SWPV China lineage.

Currently, SWPV has been classified into the India lineage and the Europe-North America lineage. To investigate the genetic characteristics of the Chinese SWPV lineage, one must conduct a comparative analysis using sequence data from the United States, India, and Germany. Our results revealed significant regional differences between Chinese SWPV isolates and those from India and Europe-North America lineages. Based on the phylogenetic analysis of indicator genes and amino acid homology, we could clearly distinguish three different SWPV lineages: Indian SWPV lineage (including SWPV reference strains), the European-North American lineage (including reference strains from the USA, Germany, and Russia), and the Chinese SWPV lineage (including reference strains from China). This finding expands the known classification of SWPV beyond the previously recognized two lineages. However, due to the lack of comprehensive epidemiological data on SWPV globally, the full distribution and prevalence of this virus remain unclear. Consequently, it is challenging to fully understand its transmission routes and other related information.

The phylogenetic tree constructed using the *VLTF-3* gene indicates that SWPV forms a distinct branch (Fig. 1J), demonstrating that SWPV's genetic evolution is significantly different from that of other poxviruses, classifying it as an independent poxvirus. Furthermore, the sequential emergence of swinepox infection cases in Anhui and Hunan Provinces between 2024 and 2025 suggests that the virus may have acquired cross-regional transmission capabilities. Notably, both provinces documented cross-provincial transportation of live animals prior to the outbreaks, providing a plausible transmission pathway for viral geographic dissemination via transport vectors. The currently circulating SWPV in China may have undergone adaptive evolution driven by environmental pressures, which could have enhanced the virus's transmission capability or host adaptability. Given that there is no commercially available vaccine for SWPV, the periodic outbreaks of the disease not only pose direct economic losses to the swine industry but may also present potential public health threats through cross-species transmission. Therefore, there is an urgent need to prioritize the implementation of proactive monitoring and control strategies to curb the spread of the outbreak and prevent its further escalation into a regional epidemic.

This study for the first time systematically elucidates the molecular characteristics and epidemiological patterns of the Chinese SWPV lineage. The findings suggest that the SWPV strains circulating in China exhibit distinct spatiotemporal distribution features, with the latest isolates showing potential for cross-regional transmission. This highlights the need for enhanced molecular epidemiological surveillance of swinepox virus to prevent further spread of the disease.

## FOOTNOTES

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The SWPV host gene sequences obtained in this study have been deposited in the Science Data Bank (https://doi.org/10.57760/sciencedb.24817). Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2025.07.006.

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