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Short communication

Tracking Pseudorabies: a case description in an Italian Hunting Dog

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Abstract

This study investigated the molecular features of Aujeszky's disease in a hunting dog from southern Italy. After consuming meat from infected wild boars, a 5-year-old male dog developed intense itching and died within 48 hours. Pseudorabies was suspected and necropsy was performed at the Experimental Zooprophylactic Institute of Apulia and Basilicata. SuHV-1 DNA was detected in the brain tissue, and the virus was isolated using Vero cells. Histological features and the analysis of genome sequencing revealed a non-suppurative meningoencephalitis caused by Aujeszky's disease. The yielded genome corresponded to a previously reported and unique genome (KU198433) isolated from a hunting dog in Italy. These findings improve understanding of pseudorabies's molecular epidemiology and help assess its epizootic potential.

Keywords

Aujeszky disease, Genome Sequence, Dog, Italy

Announcement

Pseudorabies (Aujeszky's disease) is a highly contagious viral infection caused by *Suid h erpesvirus 1* (SuHV-1), primarily affecting pigs, its natural host (Freuling et al., 2023). The virus can cause reproductive issues in sows, neurological disorders in piglets, and respiratory problems in growing pigs. SuHV-1 is neurotropic and lethal to non-swine species such as cattle, dogs, cats, and rodents, often leading to fatal neuropathy known as "mad itch." (Hansen, 1954). Though humans were once considered resistant, recent cases in China suggest possible zoonotic transmission, especially among individuals exposed to pigs (Guo et al., 2021).

In Italy, the disease persists in southern regions despite national vaccination efforts. Wild boars are key reservoirs, with a 23.85% antibody prevalence in Campania (Di Marco Lo Presti et al., 2021) and cross-species outbreaks in Sicily highlighting ongoing risks (Ferrara et al., 2021). Hunting dogs are particularly vulnerable, often exposed through consumption of infected wild boar tissues (Ciarello et al., 2020).

In November 2025, in the Potenza district (Basilicata region), a five-year-old male Maremma Hound developed acute clinical symptoms after ingesting the viscera of a wild boar killed during the hunt. Within hours, the dog exhibited intense pruritus localized to the face and muzzle, followed by pyrexia, myoclonus, and lethargy. The dog died naturally, and a postmortem examination was conducted. Brain tissue samples were processed for molecular and virological analyses. One portion was homogenized and used to infect Vero cell cultures route up to the 3rd passage. The homogenate was prepared in DMEM, centrifuged, and the supernatant was incubated on Vero cells at 37°C.

Genomic DNA was extracted from a brain portion using the GeneJET Genomic DNA Purification Kit. DNA concentration and quality were evaluated by a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) SuHV-1 DNA was successfully detected using a TaqMan-based real-time PCR assay (Sayler et al., 2017). Fourteen days post-infection, cytopathic effects indicative of viral replication were observed in the Vero

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cells. The real-time PCR assay confirmed the presence of SuHV-1 in the infected cultures, and viral DNA was subsequently extracted for sequencing. Quantification of the viral DNA was performed using a Qubit™ fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with a high-sensitivity assay. Library preparation was performed using the Native Barcoding Kit 24 V14 (Oxford Nanopore Technologies, Oxford, UK), according to the standard protocol provided by the manufacturer.

Sequencing was carried out on a MinION Mk1C device (Oxford Nanopore Technologies, Oxford, UK), employing an R10.4.1 flow cell (FLO-MIN114). The prepared library was loaded onto the flow cell, and sequencing was executed until sufficient raw sequencing data were produced (> 5Gb). Raw signal data were processed by means of MinKNOW v23.07 software, while bases were identified by the Guppy basecaller v6.5.7 (Oxford Nanopore Technologies, Oxford, UK) using "high-accuracy" mode and barcode trimming options. The raw fastq output underwent quality control, host (*Canis familiaris*) and possible contaminant (human, bacterial) genome depletion and read classification, by uploading the fastq file to the CZid.org web-resource (https://czid.org/ (Simmonds et al., 2024), accessed on 20 Jan 2025). Then, reads significantly (similarity e-value < 10^{e-10}) matching herpesvirus Genbank sequences (Sayers et al., 2025) were assembled together by using Raven v1.8.3 (Vaser et al., 2021) and Medaka (Seemann, 2014) tools. Then, the produced genome assembly was aligned to SuHV-1 reference genome (Genbank ID: NC_006151.1, *Suid herpesvirus 1*), to further refine the sequence (BioEdit v7.6 tool), while complete viral genome annotation was performed by using Prokka v1.14.6. (Seemann, 2014). Phylogenetic analysis was conducted for envelope glycoproteins D and C, among the most investigated phylogenetic markers for this clade.

First, homologous amino acid sequences for public envelope glycoproteins D and C were extracted from NCBI Virus database (access on 31 March, 2025, search by virus "*Suid alphaherpes virus* 1, taxid: 10345"; search option "Genome Organization" > "Has protein" > "envelope glycoprotein D" or synonyms) and aligned with corresponding sequences from the isolate, using ClustalOmega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo?stype=dna, accessed at 15th Sep 2025) (Madeira et al., 2024). Phylogenetic analysis (alignment revision, amino acid substitution model selection, consensus tree generation) for the two multiple alignments was done by IQTree webserver v1.6.3 (http://iqtree.cibiv.univie.ac.at/, accessed on 15 September 2025). The best-fit substitution model was JTT+F+G4, according to the Bayesian Information Criterion: thus, the obtained Maximum-Likelihood trees were manually edited using ITol (https://itol.embl.de/, accessed on 19 September, 2025) (Letunic et al., 2024).

A total of 2.23M reads (median read length = 1144 bp, SD = 1994.1, N50 = 3185) were produced and submitted to the NCBI SRA database (identifier: SRR35841438). Of these, 84.5% (1.88M reads) were above the nanopore Q10 quality score cut-off. The draft genome of the viral isolate (1 contig of 138802 bp, 73.7 % GC) was deposited in GenBank under accession number PX380308; mean and SD depth of coverage were 184.03 and 141.98, respectively, while horizontal coverage (with respect to the above mentioned reference genome) was 96.7% (138 over 143kbp, approximately). Among the 11 predicted envelope glycoproteins, phylogenetically relevant proteins C and D were 472 and 400 amino acid long, which is well consistent with reference protein lengths. After alignment revision, 252 and 108 homologous GlyC and GlyDpublic sequences were considered for phylogenetic analyses. Both phylogenetic analyses revealed that the isolate is consistently related to another one isolated in Abruzzo region (Central Italy) (Pizzurro et al., 2016), as observed from both glycoprotein trees (Figure 1).

A larger complexity is observable for GlyC phylogenesis, given the higher number of aligned sequences. However, both analyses highlight a relevant SuHV-1 genomic sampling bias, with most sequence data belonging to Chinese isolates (almost 50% of the total number, 566, of partial/full GlyC sequences in NCBI as of February 2025), and very few from Europe. Unfortunately, for the same sequence dataset, host/disease details are also scarce, with 50% of entries having no information about the host species. Furthermore, achieving a fully-covered high quality genome is biologically and technically challenging for such large and complex viral DNAs. To date, the NCBI virus database contains two complete reference assemblies (identifiers: NC_075689.1 and NC_006151.1), less than 80 nearly-complete assemblies and thousands of partial sequences. Unfortunately, these aspects make SuHV-1 phylogeographic studies quite incomplete.

This study supports clinical observations from other European reports documenting sporadic cases of Aujeszky's disease in domestic carnivores. The high seroprevalence of the Aujeszky's disease virus among wild boars in southern Italy, combined with scarce data on its incidence in wild carnivores and scavengers, indicates that viral circulation may be underestimated. By sequencing the viral genome from a canine brain isolate, our findings contribute to the molecular and epidemiological characterization of Aujeszky's disease within the Italian context. These results highlight the need for preventive strategies, including limiting exposure to raw meat and strengthening surveillance in endemic areas.

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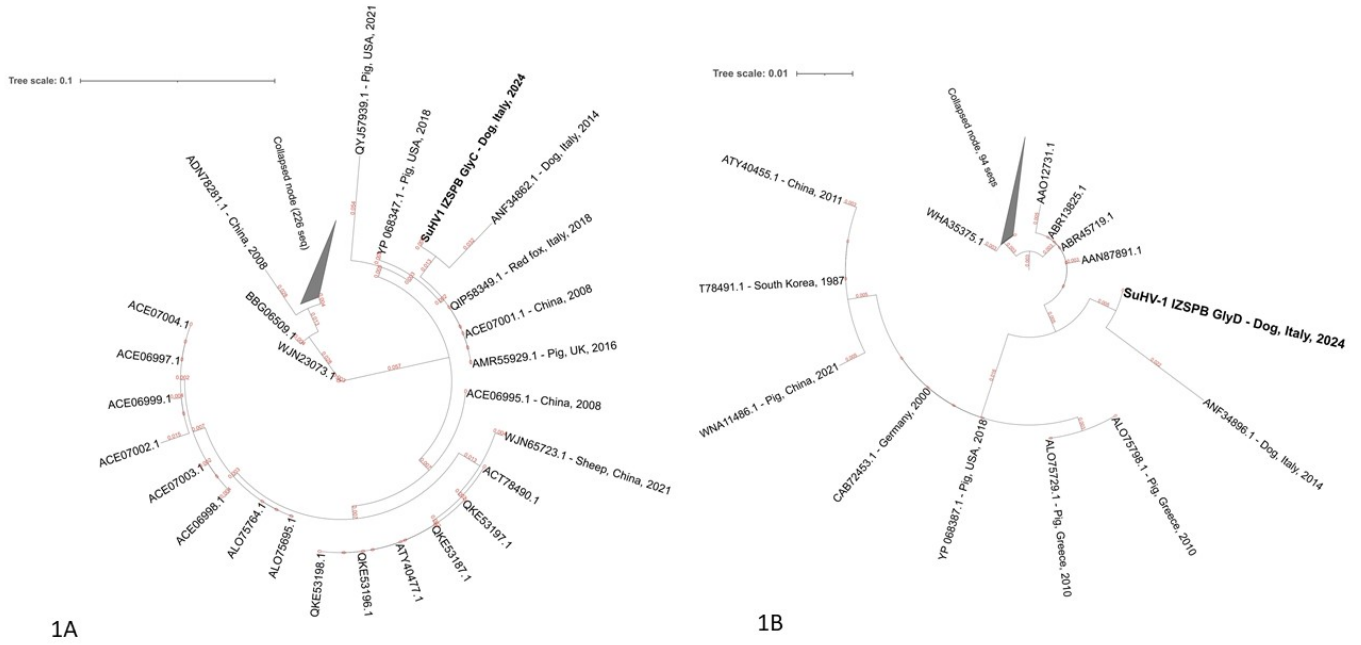

Figure 1. Maximum Likelihood Trees for envelope glycoprotein C amino acid sequences, with investigated proteins (GlyC, Figure 1A; GlyD, Figure 1B) in bold letters; red numbers at nodes: branch lengths; tree leaves: NCBI Genbank sequence identifiers. Host, geographical location and collection year are provided for most phylogenetically related viral proteins. Sequences with elevated phylogenetic affinity (very low branch lengths) are collapsed (grey triangles).

Ethical approval

The study was conducted in accordance with ethical principles for animal research and was carried out on material submitted to the IZSPB laboratories for diagnostic investigations that were aimed at determining the cause of death of the animal.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: DAR, SC; Methodology: DAR, SC, AP, LM, SM, NC, IP; Formal analysis: CL, SC, DL; Investigation: LP, RAC, TM, BE, AMD; Writing original draft preparation: DAR; Writing, review and editing: DAR, SC, AP, LM, LP, MS, AMD, AB; Visualization: DAR, SC, AP, LM; Supervision: DAR

All authors have read and agreed to the published version of the manuscript.

Data availability

The IZSDogSuHV-1 genomic sequence has been archived in GenBank under the accession number PX380308, while BioProject and BioSample ID are PRJNA1347598 and SAMN52861642, respectively.

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