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Simultaneous detection of classic and natural recombinant myxoma virus strains in European brown hare population in West Hungary

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Historically, Myxomatosis was a severe disease in the European wild and domesticated rabbits (*Oryctolagus cuniculus*) with only a few reported cases in hares. Recently, beside the classic myxoma virus strains, new natural recombinant strains have emerged. The latter ones caused several outbreaks, in European countries, in different *Lepus* species, indicating a species spillover of the virus. In our study, we isolated two distinct myxoma viruses from European brown hares (*Lepus europaeus*) that were found dead close to a hunting area in West Hungary, in October 2025. For the virus isolation RK-13 cell cultures were used and characteristic cytopathogenic effects were observed indicating successful replication. The harvested virus suspensions were tested with real-time PCR then full genome sequencing and were found positive for both the classic (MYXV) and hare-adapted natural recombinant myxoma virus (ha-MYXV), indicating the simultaneous presence of these two type. Our results are in line with recently published data regarding the circulating myxoma virus strains in the Central European region. Based on our finding, Myxomatosis is a significant disease in European brown hare populations in Central Europe. Further monitoring of wild living populations is needed to obtain more epidemiological data on the occurrence of different virus types/subtypes and understanding their specific role in the pathogenesis of this disease.

KEYWORDS

classic myxoma virus (MYXV), European brown hare, full genome sequencing, *Lepus europaeus*, Myxomatosis, natural recombinant hare-adapted myxoma virus (ha-MYXV)

1 Introduction

Myxomatosis is a disease with high mortality of lagomorphs. The causative agent is Myxoma virus (MYXV) which is a *Leporipoxvirus* and a member of the *Poxviridae* family (1). The genome of the myxoma virus is a large linear double-stranded DNA (2). The disease was first described in Uruguay in 1898 (3), but the virus was identified only later, in 1927 (4). In South America, the cottontail rabbits (*Sylvilagus brasiliensis*), while in North America the brush rabbits (*Sylvilagus bachmani*) are asymptomatic carriers of the virus (5). Deliberate introduction of myxoma virus

took place in the 1950's in Australia and in France for the reduction of the local rabbit populations (6, 7). Recently, the virus became enzootic in Europe among the wild European rabbits (*Oryctolagus cuniculus*) and in the domestic rabbits (*Oryctolagus cuniculus domesticus*). Myxomatosis virus can be spread both with direct contact and with mechanical vectors like mosquitoes, fleas and lice (8). For decades the infections of European brown hares (*Lepus europaeus*) were reported only rarely, for example in England in 2014 (9). However, there was a dramatic change in the number of cases starting from 2018, after the emergence of new hare-adapted natural recombinant myxoma virus strains (ha-MYXV). These recombinant strains have some distinct features compared to the classic ones (e.g., Lausanne strain), like an insertion of an approximately 2.8 kb long four-cassette region in the *M009L* gene and a presence of the *M060L* gene (10). The ha-MYXV infected the local *Lepus granatensis* population in Spain and in Portugal and the *Lepus corsicanus* in Sicily in 2018 and caused high mortality in these regions (11–13). In 2022, there were cases in Algeria in farmed rabbits (14). In 2023 and 2024, the recombinant strains were detected in the Netherlands and Germany (15). Recently, Myxomatosis was reported in European brown hares in Austria, in the Czech Republic and in Slovakia (16, 17). MYXV and ha-MYXV co-infections of *Lepus granatensis* and European wild rabbits were also described (18).

In Hungary, the first Myxomatosis cases in wild and domestic rabbits were described in 1959 (8).

In this report, we describe the results of a deep molecular investigation into the MYXV and ha-MYXV strains that detected in European brown hares in West Hungary in 2025. While the pathological and clinical findings of these cases were previously reported (19), this study provides novel insights through whole-genome sequencing and phylogenetic analysis.

2 Materials and methods

2.1 Samples

In October 2025, the carcasses of two adult female brown hares (*Lepus europaeus*) were submitted the Department of Exotic Animal, Wildlife, Fish, and Honeybee Medicine for postmortem examination. The animals originated from a hunting ground in West Hungary near to a busy public road (19, 20). The two hares originated from the same biotope, the distance between the two cadavers was approximately 20 meters. During the pathological and diagnostic examination, skin samples with characteristic Myxomatosis lesions of eyelids and lip regions were collected in the same plastic 1.5 mL Eppendorf tube and were frozen at -70°C . This pooled sample was used for all further investigations.

2.2 Virus isolation in cell cultures

RK-13 (rabbit kidney) cell line was used for the virus isolation according to established protocols with slight modifications (21, 22). Briefly, tissue homogenates were prepared from the frozen skin samples with mechanical homogenization (quartz sand) using 1 g tissue in 10 mL PBS supplemented with 1% antibiotic/antimycotic

solution (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) and 100 μL gentamycin. The homogenate was centrifuged at 2,000 g for 5 min (Hermle Z 300K centrifuge) in 50 mL centrifuge tubes.

RK-13 cell monolayer cultures, with approximately 80% confluency, were used for the inoculation; 500 μL supernatant of the tissue homogenate was added to 25 cm^2 tissue culture flasks and was placed for a 1-h-long adsorption in an incubator (ESCO Lifesciences, Cell Culture Incubator, CO_2 , Singapore) at 37°C . After the incubation time, 10 mL MEM with Earle's salts infection medium (Cat. No. MEM-A, Capricorn Scientific GmbH, Ebsdorfergrund, Germany) supplemented with 2% FBS (CAP-FBS-12A, Capricorn Scientific GmbH, Ebsdorfergrund, Germany) and 0.1% gentamycin was added to the flask and incubated for 6 days at 37°C in a 5% CO_2 atmosphere. As a negative control, non-infected RK-13 cells in the same 2% FBS containing maintenance medium were used. The cell cultures were investigated daily for cytopathogenic effects (CPE). Six days after the inoculation the flasks were frozen at -70°C , and after two freeze-thaw cycles, the cells were disrupted. The suspension was collected and centrifuged at 2,000 g for 5 min, then it was used for both PCR and for further inoculation of fresh confluent RK-13 cells. The cells were infected with 500 μL suspension according to the same protocol as described above. After 3 and 6 days this second passage was also frozen and after two freeze-thaw cycles the suspension was centrifuged and used for PCR and for full genome sequencing analyses.

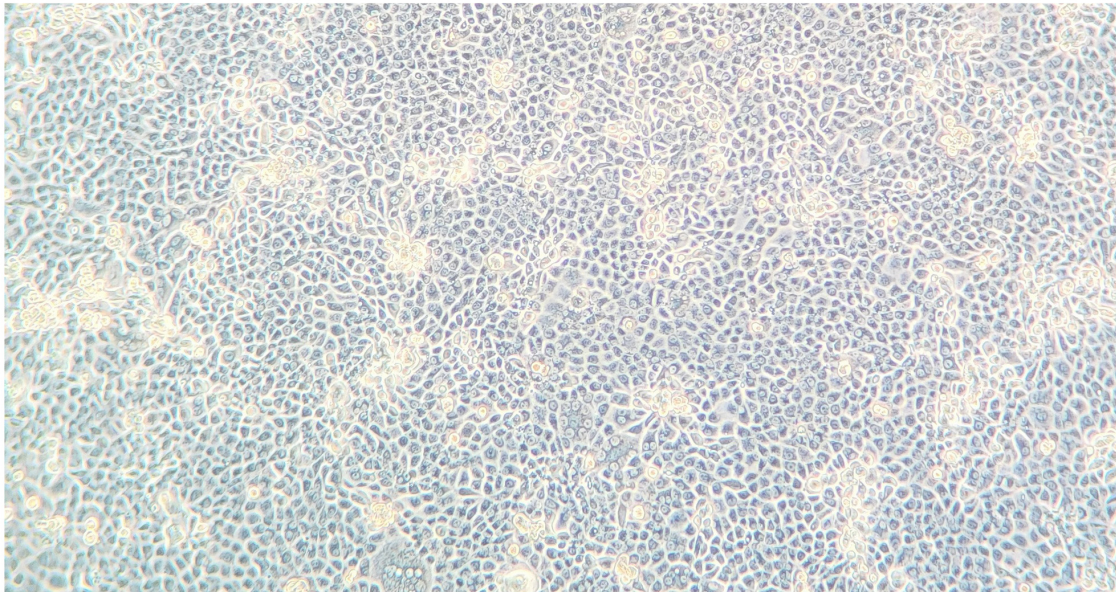
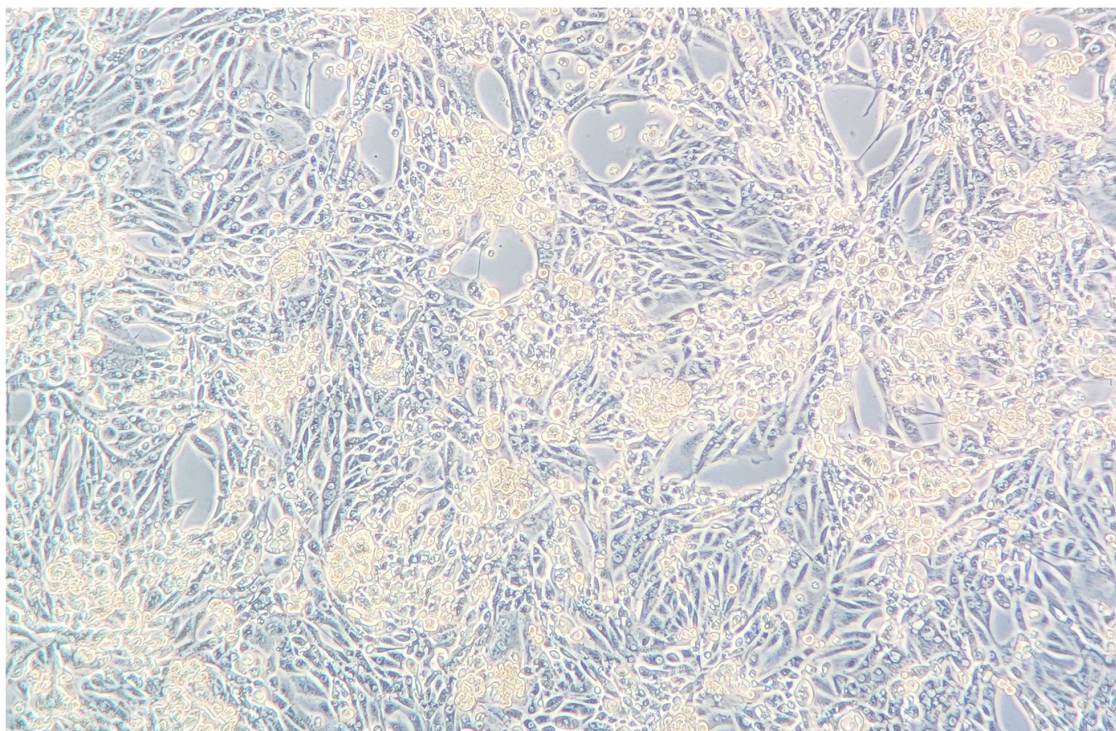
2.3 PCR

DNA was extracted from the harvested supernatants with the MagnifiQTM (A&A Biotechnology, Gdansk, Poland) DNA extraction kit according to the manufacturer's instructions. For the quantitative PCR, primer pairs designed for the following genes were used: M000.5L/R (for the detection of MYXV and ha-MYXV strains; M000.5L_F: 5'-CGACGTAGATTTATCGTATAC C-3' and M000.5L_R: 5'-GTCTGTCTATGT-ATTC-TATCTCC-3'), M009L (for the detection of MYXV strains; M009L_F: 5'-TCCATTTACGATACACGCCGACGC-3' and M009L_R: 5'-ACA ACGTTCTATACTGTTTAGGGGGTACG-3') and M060L (for the detection of ha-MYXV; M060L_F: 5'-GATTCTTTAATCTGGTTG AGGCAACTA-3' and M060L_R: 5'-GGATATTATTACGCTCCA TTATCGGAGG-3') according to previously published data (10).

Briefly, 4 μL extracted DNA samples and negative controls were added to 16 μL mix containing 10 μL 2X qPCR BIO SyGreen Blue Mix (Cat. No. PB20.17-05, PCR Bio-systems, London, United Kingdom), 1 μL of each forward and reverse primers and 4 μL distilled PCR grade water. The PCR profile was the following: 95°C for 10 min, then 42 cycles with 95°C for 15 s and 60°C for 1 min including plate read. Quantitative PCR was done with Bio-Rad CFX Opus 96, and the results were analyzed with Bio-Rad CFX Maestro software.

2.4 Whole genome sequencing

For the whole genome sequencing, the DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen N.V., Venlo, Netherlands)

A**B****FIGURE 1**

Representative photos of the RK-13 cell cultures (10x magnification). **(A)** Uninfected confluent control RK-13 cells (8-day-old cultures). **(B)** RK-13 cells 6 days after infection with tissue homogenate supernatant (8-day-old culture); cell elongation, rounding, detachment can be seen as cytopathogenic effects.

according to the manufacturer's instructions. The DNA quantity was measured using a Qubit 4.0 Fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, USA).

The sequencing library with 150 ng of DNA was prepared following the recommendations of the Ligation sequencing gDNA

by Native Barcoding Kit 24 V14 (SQK-NBD114.24, Oxford Nanopore Technologies, Oxford, UK), then loaded onto an R10.4.1. MinION flow cell (Oxford Nanopore Technologies). Raw data were generated for 72 h using a MinION mk1b instrument. Basecalling (with super accuracy basecalling mode) and demultiplexing of the

long-read raw sequences were performed with Dorado v7.6.8 as implemented in MinKNOW v 24.11.10.

As the first step of long read preprocessing, the reads of the DNA control strand were excluded using NanoLyse v1.2.1. NanoFilt v2.8.0 was used to trim 50 bp of reads at both the 5' and 3' ends to ensure that all adaptor sequences were removed and to exclude reads with a mean quality of less than 8 or with a length of less than 500 bp. Finally, the read quality metrics were evaluated and visualized using NanoPlot v1.43.0. (23).

De novo assembly was performed using Flye v2.9.5 (24). Assembly was successively polished using minimap2 v2.28 (25), racon v1.5.0 (26), medaka v2.0.1 (<https://github.com/nanoporetech/medaka>) using the r1041_e82_400bps_sup_v4.2.0 model. To confirm the results, the filtered raw reads were mapped to KP723390 (Myxoma virus strain FLI-H, complete genome) and to MK340973 (Myxoma virus strain Tol08-18, complete genome) using minimap2. Consensus sequence was extracted with samtools consensus from sam file. The final genome sequence was manually edited and annotated via Geneious Prime[®] 2026.0.2 (<https://www.geneious.com>).

MYXV complete genome sequences were downloaded from GenBank and aligned with MAFFT algorithm of Geneious Prime[®] 2026.0.2. Maximum likelihood (ML) tree was calculated using IQ-TREE version 3.0.1 for Linux x86 64-bit using the complete genome alignment as input, and automatic model selection turned on (-m MFP) with -bb 1000: ultrafast bootstrap (UFBoot), -alrt 1000: SH-aLRT support. The phylogenetic trees were visualized with FigTree v1.4.5 (<http://tree.bio.ed.ac.uk/software/figtree/>).

3 Results

3.1 Virus isolation

Six days after the inoculation of the RK-13 cells with the centrifuged, filtered tissue homogenate, a clear difference was seen between the negative control tissue culture flask and the infected ones. The non-infected culture remained confluent, but in the infected cultures detachment of the cells, cell elongation and cell rounding were observed as cytopathogenic effect (CPEs). For representative photos, see Figures 1A, B.

3.2 PCR results

Samples harvested after the first and second inoculation of the RK-13 cells were used for quantitative PCR analysis. Figures 2, 3 show the results. The blue labeled graphs belong to the first passage, while the orange and red ones to the second passages of the virus (3 and 6 days after inoculation). The lowest Ct-s were observed in the case of the second passage 6 days after inoculation, indicating a tendency of higher quantity of the specific target sequence in these samples. The PCR products were also run in a 1% agarose gel (Figure 4). Amplicons were detected in all cases indicating that both MYXV and ha-MYXV were amplified. In the case of the M000.5L/R and M060L PCRs the amplicon size was similar to published data, 125 and 178 bp (10). On the other hand, in the M009L PCR only

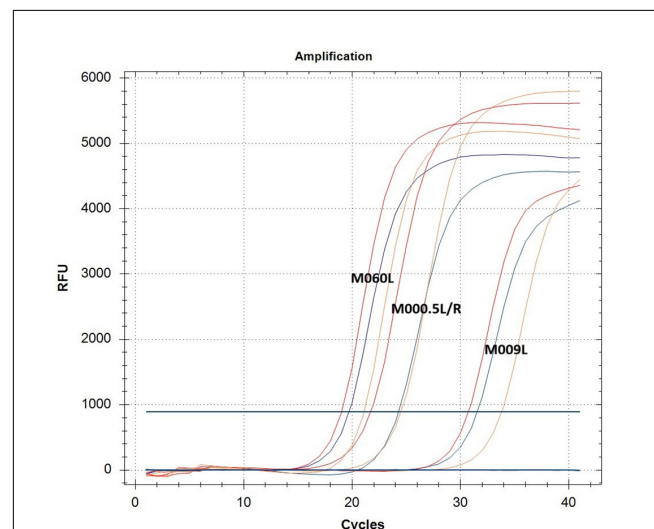


FIGURE 2

Results of the M000.5L/R (both MYXV and ha-MYXV detection), M060L (ha-MYXV detection) and M009L (MYXV detection) PCRs. Blue: sample of the first passage, yellow: sample of the second passage 3 days after inoculation, red: sample of the second passage 6 days after inoculation.

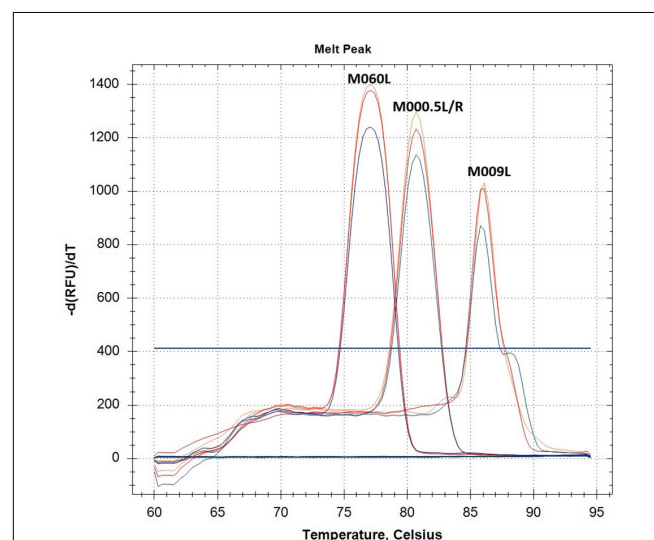


FIGURE 3

Results of the melting temperatures of the M000.5L/R (both MYXV and ha-MYXV detection), M060L (ha-MYXV detection) and M009L (MYXV detection) PCRs. Blue: sample of the first passage, yellow: sample of the second passage 3 days after inoculation, red: sample of the second passage 6 days after inoculation.

low amount of 146 bp long PCR fragments was amplified and the majority of the PCR amplicons was approximately 500 bp long.

3.3 Whole genome sequencing

The complete sequence of the Hun_UVMB_Myxv_Oct-2025 strain (GenBank accession number: PZ376974) consists of 161,779 bp, 170 genes, while the sequence of the Hun_UVMB_ha-Myxv_Oct-2025 strain (GenBank accession number: PZ376975)

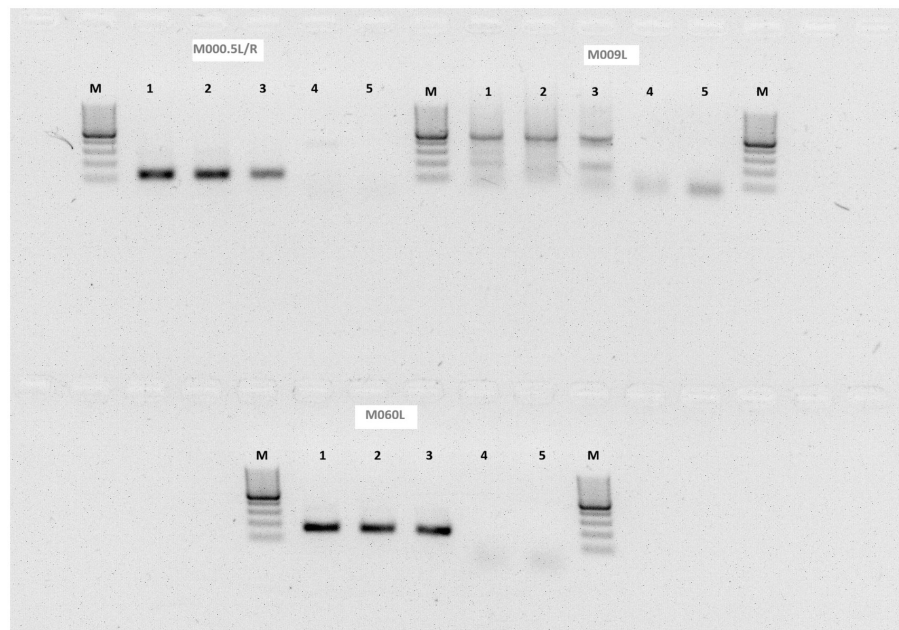


FIGURE 4

Photo of the gel electrophoresis. M000.5L/R PCR (for both MYXV and ha-MYXV detection): 125 bp long PCR amplicons; M060L PCR (for ha-MYXV detection only): 178 bp long PCR amplicons; M009L PCR (for MYXV detection only): the specific 146 bp amplicons only in low amount, while an approximately 500 bp fragment in higher amount. 1: 1st passage, 2: 2nd passage harvested 6 days after inoculation, 3: 2nd passage harvested 3 days after inoculation, 4: uninfected control culture, 5: negative control PCR, M: 100 bp marker.

consists of 164,566 bp, 175 genes. The Hun_UVMB_ha-Myxv_Oct-2025 genome is larger than the Hun_UVMB_Myxv_Oct-2025 due to an additional 2.8 Kbp insert within the M009L gene that disrupted it into ORFs M009L-a and M009L-b. The Hun_UVMB_ha-Myxv_Oct-2025 sequence contains multiple instances of gene duplication, including copies of genes *M060L*, *M061L*, *M064L*, and *M065L*, which are located on the right-hand side of the genome and have been transferred to the left-hand terminus of the inverted repeat (TIR). The nucleotide (nt) identity between the Hun_UVMB_Myxv_Oct-2025 strain and the Hun_UVMB_ha-Myxv_Oct-2025 strain is 98.2%. The comparison of the sequences in the *M009L* gene region is shown in Figure 5 below.

A comparison of newly sequenced Hun_UVMB_Myxv_Oct-2025 with the virulent reference MYXV Lausanne strain revealed a 99.9% identity. The comparison of Hun_UVMB_ha-Myxv_Oct-2025 with other ha-strains (e.g., strain Tol08-18, Algeria/El Affroun/2022/R1) showed 100% identity.

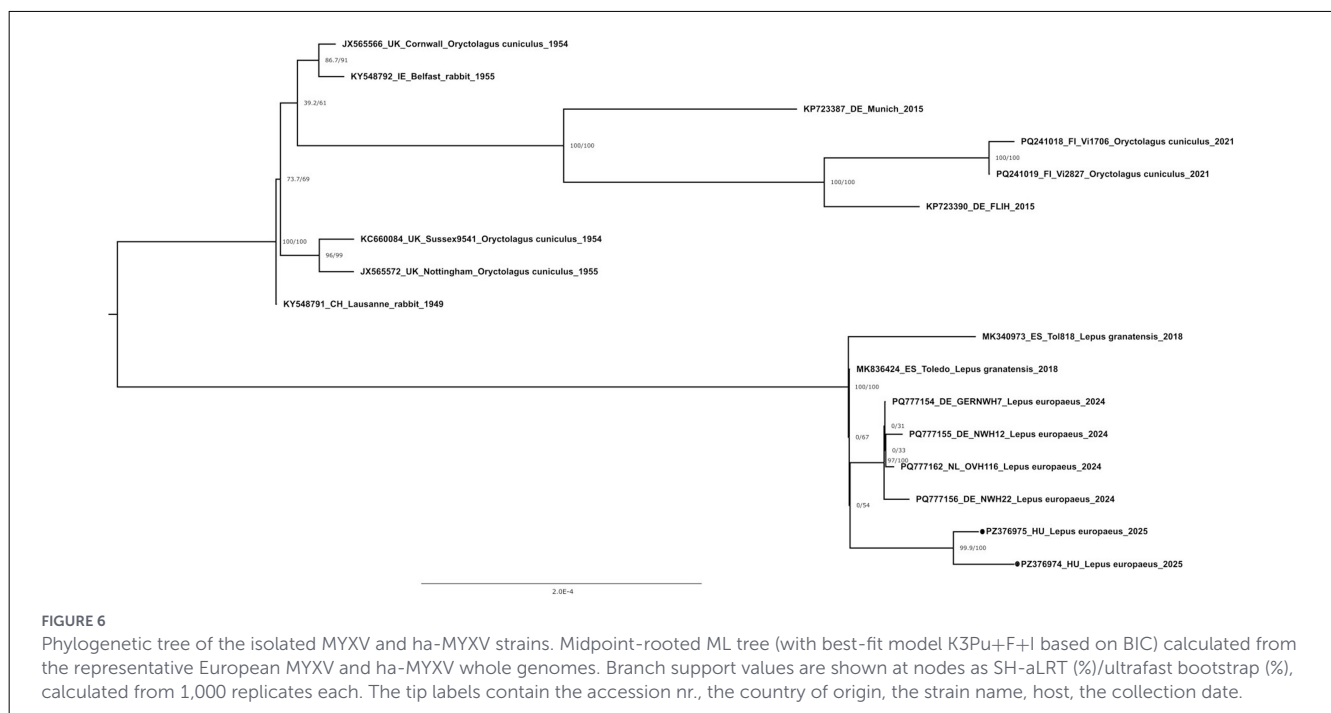
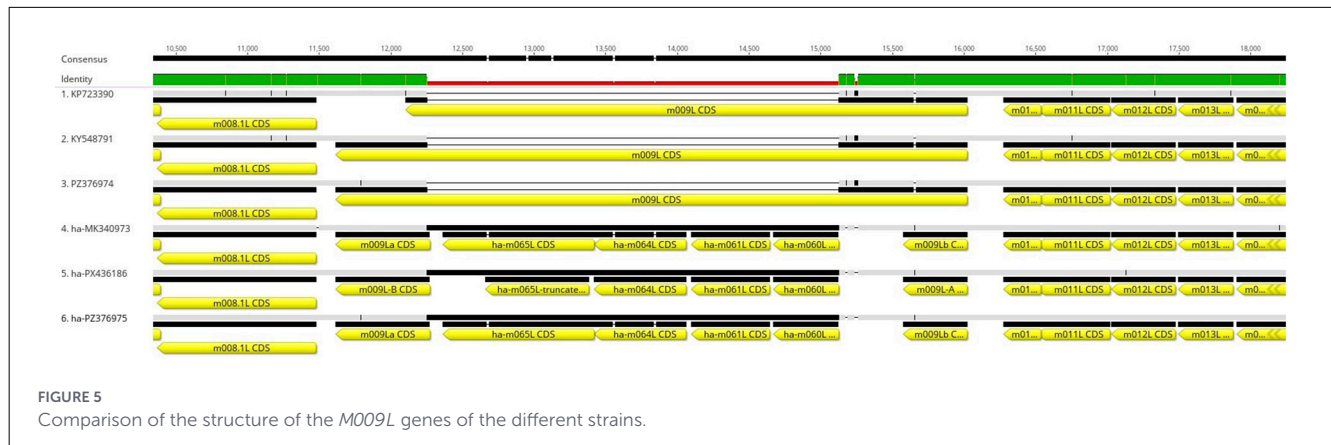
The phylogenetic analysis of the isolated classic and recombinant myxoma virus strains is shown in Figure 6 below. The strains Hun_UVMB_Myxv_Oct-2025 and Hun_UVMB_ha-Myxv_Oct-2025 clustered into a monophyletic group within ha-MYXV.

4 Discussion

In this study skin sample homogenates of two female brown hares were used. These animals were found dead in West-Hungary in October 2025. The details of the pathological examination and

the histopathological findings characteristic to Myxomatosis can be found in a previous publication (19). It was confirmed previously that a classic MYXV belonging to the Lausanne group caused the disease. In the previous study sequencing and phylogenetic analysis was performed based on the *M070R* and *M071L* capsid proteins only. As these genes are characteristic for both the classic and for the natural recombinant strains, based solely on these genes it is impossible to distinguish a possible co-infection case.

Our aim was to further investigate the causative agent behind this case with the help of molecular biological tools. We isolated the virus in RK-13 cell cultures, two passages were carried out, cytopathogenic effects were observed. Virus suspensions originating from the first and the second passage were used to confirm the multiplication of the virus using a published real-time PCR (10). Interestingly, the cultures were positive for both the classic and the recombinant strains suggesting a co-infection case. In addition, the PCR amplicons of correct base-pair size could also be detected with gel electrophoresis. In order to have a complete picture, whole genome sequencing was performed for further evaluation. The full genome sequencing data confirmed that the presence of two different strains behind this Myxomatosis case. There is an approximately 98.2% similarity between the two strains, but the Hun_UVMB_Myxv_Oct-2025 strain is similar to the classic and the ha-MYXV1 (Hun_UVMB_ha-Myxv_Oct-2025) to the recombinant strains. The isolated ha-MYXV (Hun_UVMB_ha-Myxv_Oct-2025) strain has the 2.8 kb-long insert in the *M009L* gene with a four-gene-cassette insertion (*M060L*, *M061L*, *M064L*, and *M065L*), similarly to previously published data (10). The *M060L* gene is unique to the recombinant strains and cannot be found in the classic ones. Previous studies



(10, 18) reported a 2.8 kbp insert containing the genes M060L–M066L in sequence KY548791. However, upon re-analysis of the GenBank record, we could not identify these genes in the deposited sequence or annotation. Furthermore, KY548791 appears to correspond to a classical MYXV isolate rather than a recombinant strain, suggesting a possible discrepancy in isolate attribution or genome annotation.

Although the whole-genome analysis clearly distinguished the classical and recombinant MYXV strains, both Hungarian isolates clustered within the same major lineage in the ML phylogeny. This likely reflects the overall high genomic similarity between the strains (~98.2%) and the largely conserved genomic backbone of recombinant ha-MYXV strains, in which only specific genomic regions differ substantially from classical MYXV genomes. Furthermore, midpoint rooting may also contribute to the observed topology, particularly among highly similar genomes with limited overall divergence.

Our findings are in line with the recently circulating recombinant myxoma virus strains in European brown hares (16, 17).

In our case the amount of the recombinant strain was lower compared to the classic strains (18). This phenomenon also highlights the fact that without thorough investigation one may easily overlook the presence of mixed virus populations in a certain area. We are aware of the fact that with our pooled sample it is not possible to draw conclusion on individual level, but it represents the status of the European brown hares in the region where the dead animals were found.

In the future, the close follow-up of the circulating strains is necessary in the hare populations. In addition, the surveillance of wild and domestic European rabbits is also important, as there are accumulating data regarding the cross infection of these species. It would be also important to map the immunological status of the hares and rabbits to predict the possible Myxomatosis

outbreaks in the next season. Currently, Myxomatosis in the Central European region raises concerns in brown hare populations.

Data availability statement

The original contributions presented in the study are publicly available. All assembled sequence data generated or analysed during this study were deposited to the GenBank, Hun_UVMB_Myxv_Oct-2025 strain (GenBank accession number: PZ376974, <https://www.ncbi.nlm.nih.gov/nuccore/PZ376974>), Hun_UVMB_ha-Myxv_Oct-2025 strain (GenBank accession number: PZ376975, <https://www.ncbi.nlm.nih.gov/nuccore/PZ376975>).

Author contributions

AK: Writing – original draft, Data curation, Investigation, Visualization, Methodology, Writing – review & editing. EK: Investigation, Writing – review & editing, Methodology, Data curation, Writing – original draft, Visualization. EF: Writing – review & editing, Methodology, Writing – original draft, Investigation, Visualization. JG: Supervision, Writing – review & editing, Resources, Funding acquisition, Validation. MT: Funding acquisition, Formal analysis, Project administration, Validation, Writing – review & editing, Conceptualization, Resources, Supervision.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Fenner F. Portraits of viruses: the poxviruses. *Intervirology*. (1979) 11:137–57. doi: 10.1159/000149027
2. Cameron C, Hota-Mitchell S, Chen L, Barrett J, Cao JX, Macaulay C, et al. The complete DNA sequence of myxoma virus. *Virology*. (1999) 264:298–318. doi: 10.1006/viro.1999.0001
3. Sanarelli G. Das myxomatogene Virus. Beitrag zum Studium der Krankheitserreger ausserhalb des Sichtbaren. *Zentralbl Bakteriol Parasitenk Infektiensk Abt I*. (1898) 23:9.
4. Aragão HB. Myxoma of rabbits. *Mem. Inst. Oswaldo Cruz*. (1927) 20:237–47. doi: 10.1590/S0074-02761927000200003
5. Fenner F, Ratcliffe FN. *Myxomatosis*. Cambridge: Cambridge University Press (1965).
6. Fenner F, Marshall ID. Occurrence of attenuated strains of myxoma virus in Europe. *Nature*. (1955) 176:782–3. doi: 10.1038/176782a0
7. Fenner F. Biological control, as exemplified by smallpox eradication and Myxomatosis. *Proc R Soc Lond B Biol Sci*. (1983) 218:259–85. doi: 10.1098/rspb.1983.0039
8. Vetési F. "Diseases of the domestic rabbit (in Hungarian)". In: *Mezőgazdasági Kiadó*. Budapest: Mezőgazdasági Kiadó (1990). p. 157–61.
9. Barlow A, Lawrence K, Everest D, Dastjerdi A, Finnegan C, Steinbach F. Confirmation of Myxomatosis in a European brown hare in Great Britain. *Vet Rec*. (2014) 175:75–6. doi: 10.1136/vr.g4621
10. Abade Dos Santos FA, Carvalho CL, Parra F, Dalton KP, Peleteiro MC, Duarte MD. A Quadruplex qPCR for detection and differentiation of classic and natural recombinant Myxoma virus strains of leporids. *Int J Mol Sci*. (2021) 22:12052. doi: 10.3390/ijms222112052
11. García-Bocanegra I, Camacho-Sillero L, Rialde MA, Dalton KP, Caballero-Gómez J, Agüero M, et al. First outbreak of Myxomatosis in Iberian hares (*Lepus granatensis*). *Transbound Emerg Dis*. (2019) 66:2204–8. doi: 10.1111/tbed.13289
12. Carvalho CL, Santos FAAD, Duarte MD, Monteiro M, Carvalho P, Mendonça P. First cases of Myxomatosis in Iberian hares (*Lepus granatensis*) in Portugal. *Vet Rec Case Rep*. (2020) 8:e001044. doi: 10.1136/vetreccr-2019-001044
13. Rossini E, Bazzucchi M, Trocchi V, Merzoni F, Bertasio C, Knauf S, et al. Identification and characterisation of a myxoma virus detected in the

- Italian hare (*Lepus corsicanus*). *Viruses* (2024) 16: 434. doi: 10.3390/v16030437
14. Maziz-Bettahar S, Montacq L, Caubet C, Gaide N, Yahouni-Slimani F, Sahraoui L, et al. Emergence of recombinant myxoma virus in Algerian farmed rabbits. Molecular and phylogenetic evidence. *Transbound Emerg Dis.* (2026) 2026:9374702. doi: 10.1155/tbed/9374702
15. Fischer L, de Bruin E, Jongepier E, Koffeman E, König P, Pfaff F, et al. Recombinant myxoma virus in European brown hares, 2023–2024. *Emerg Infect Dis.* (2025) 31:1608–12. doi: 10.3201/eid3108.241969
16. Auer A, Kübber-Heiss A, Sperser J, Dimmel K, Kugler S, Rümenapf T. “First outbreak of emerging ha-MYXV-associated Myxomatosis in European hare (*Lepus europaeus*) in Austria”. In: *7th International conference of European College of veterinary microbiology (ECVM)*. Berlin: Kaisern-Friedrich-Stiftung (2025). p. 10–12.
17. Sedlák K, Mikulášková K, Nagy A, Cukor J, Příhodová P, Cerníková L, et al. First evidence of recombinant Myxomavirus (ha-MYXV) in European hare (*Lepus europaeus*) in the Czech Republic and Slovakia. *Front Vet Sci.* (2026) 13:1754249. doi: 10.3389/fvets.2026.1754249
18. Abade Dos Santos FA, Dalton KP, Carvalho CL, Casero M, Álvarez ÁL, Parra F, et al. Co-infection by classic MYXV and ha-MYXV in Iberian hare (*Lepus granatensis*) and European wild rabbit (*Oryctolagus cuniculus algirus*). *Transbound Emerg Dis.* (2022) 69:1684–90. doi: 10.1111/tbed.14540
19. Gál J, Faragó S, Keszthelyi G, Vincze Z, Shen Y, Mándoki M, et al. First detection of Myxomatosis in European brown hares (*Lepus europaeus*) in West-Hungary. *Acta Vet Hung.* (2025) 74:26–33. doi: 10.1556/004.2025.01264
20. Gál J, Marosán M, Vincze Z, Tóth T. Myxomatosis patológiai elváltozásai mezei nyúlban (*Lepus europeus*) (in Hungarian). *Magy Állatorvosok Lapja* (2025) 147:727–37. doi: 10.56385/magyallorv.2025.12.727-737
21. World Organization for Animal Health. “Chapter 3.7.1: Myxomatosis”. In: *Terrestrial Manual 2021* (2021). Available online at: https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.07.01_MYXO.pdf (Accessed May 12, 2026).
22. Salem HM, Morsy EA, Hassanen EI, Shehata AA. Outbreaks of Myxomatosis in Egyptian domestic rabbit farms, 2019. *World Rabbit Sci.* (2019) 27:85–91. doi: 10.4995/wrs.2019.10585
23. De Coster W, D’Hert S, Schultz DT, Cruts M, Van Broeckhoven C. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics.* (2018) 34:2666–9. doi: 10.1093/bioinformatics/bty149
24. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. Assembly of long, error-prone reads using repeat graphs. *Nat Biotechnol.* (2019) 37:540–6. doi: 10.1038/s41587-019-0072-8
25. Li H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics.* (2018) 34:3094–100. doi: 10.1093/bioinformatics/bty191
26. Vaser R, Sović I, Nagarajan N, Šikić M. Fast and accurate de novo genome assembly from long uncorrected reads. *Genome Res.* (2017) 27:737–46. doi: 10.1101/gr.214270.116