

Article

Detection of Recombinant Hare Myxoma Virus in Wild Rabbits (*Oryctolagus Cuniculus Algirus*)

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Abstract:

In late 2018, an epidemic myxomatosis outbreak emerged on the Iberian Peninsula leading to high mortality in Iberian hare populations. A recombinant myxomas virus (MYXV-Tol or ha-MYXV) was rapidly identified, harboring a 2.8 kb insertion containing evolved duplicates of M060L, M061L, M064L, and M065L genes from MYXV.

Since 2017, 1616 rabbits and 125 hares were tested by a qPCR directed to M000.5L/R gene, conserved in MYXV and MYXV-Tol/ ha-MYXV strains. A subset of the positive samples (20%) from both species was tested for the insert with MYXV being detected in rabbits and the recombinant MYXV in hares. Recently, two wild rabbits found dead in South of mainland Portugal, showing skin edema and pulmonary lesions tested positive for the 2.8 Kb insert. Sequencing analysis showed 100% similarity with the insert sequences described in Iberian hares from Spain. Viral particles were observed in the lungs of both rabbits by electron microscopy, and isolation in RK13 cells attested virus infectivity.

Despite the analysis of complete genomes may predict the recombinant MYXV strains ability to infect rabbit, routine analyses showed species segregation for the circulation of MYXV and recombinant MYXV in wild rabbit and in Iberian hares, respectively. This study demonstrates, however, that recombinant MYXV can effectively infect and cause myxomatosis in wild rabbits and domestic rabbits, raising serious concerns for the future of the Iberian wild leporids while emphasizes the need of the continuous monitoring of MYXV and recombinant MYXV in both species.

Keywords: Myxomatosis; recombinant myxoma virus; ha-MYXV; European rabbit; *Oryctolagus cuniculus algirus*; species jump; spillover

1. Introduction

In the Mediterranean ecosystems, wild rabbit is an important prey for more than 40 predatory aerial and terrestrial species, some of which are endangered. It also plays a crucial role as a soil “architecture”, contributing to seed dispersal, and landscaping [1]. Besides its ecological role, the wild rabbit has an economic and social importance as a favorite game species in Europe.

Myxoma virus (MYXV) and rabbit haemorrhagic disease virus 2 (RHDV2) are the two major pathogen threats for the European rabbit (*Oryctolagus cuniculus*), and may occasionally be found simultaneously [2]. The etiological agent of myxomatosis is MYXV, a double-stranded DNA *Leporipoxvirus* of the Poxviridae [3].

Myxomatosis is an endemic disease of South American rabbits and was first described in laboratory rabbits in 1898 in Uruguay [4]. The disease is characterized by the presence of nodules in the skin surrounding the eyes, nose, mouth, ears, and genitalia. Conjunctivitis, accompanied by purulent discharge is frequently found as a signal of disease [5].

Despite these signs being the most commonly found in the classic, nodular or typical form of disease, myxomatosis can also be found as a respiratory form (amyxomatous form), with variable degrees of severity, where cutaneous signals are minor or not observed [6–8]. The origin of this amyxomatous virus is still unclear. Viral mutations and reactivation of subclinical infections are two of the hypotheses proposed [9].

Regardless the two disease presentations, myxomatosis was considered a rabbit disease during many decades, with some scarce reports in hares [10,11].

Since mid-2017, 125 hares and 1616 rabbits collected within the scope of a National Leporid Surveillance Program (Project +Coelho, Dispatch 4757/17, 31th may), were analyzed for MYXV-DNA using a qPCR directed to the diploid gene M000.5L/R, which is conserved in MYXV and recombinant MYXV strains.

After the recent emergence of myxomatosis in the Iberian hare in 2018, caused by a recombinant myxoma virus (first designated as MYXV-Tol, and subsequently ha-MYXV considering its modified tropism towards hares), harboring an insertion of about 2.8 kb of bases [12–15], health surveillance within the scope of Project +Coelho in the Iberian hare (investigating MYXV [16], RHDV2 [17] and LeHV-5 [18]) and in the wild rabbit (investigating MYXV and RHDV2[17]) was extended to include screening for ha-MYXV as described by Dalton et al [14].

The detection of a recombinant MYXV in hares, and the apparent segregated circulation of classical MYXV in rabbits and ha-MYXV in hares, initially suggested the adaptation of MYXV to hares in order to efficiently multiply in this species. Given that hare MYXV, originally considered hare specific, is also being detected in rabbits, a more generalist designation, geographic and species independent, such as rec-MYXV (for recombinant myxoma virus) may be preferable for the future.

Until the cases reported here, in all tested samples, MYXV was only found in wild rabbits and recombinant MYXV in Iberian hares. To our knowledge, we are reporting for the first time, the detection of myxomatosis in European rabbit caused by the recombinant MYXV, raising another concern, to the present conservation state of the wild rabbit, taking into account its threat of extinction [19].

2. Materials and Methods

2.1. Sample, necropsy and histopathology

Two adult wild rabbits (*Oryctolagus cuniculus algirus*), in good body condition, found dead in June 2020 (male, 15758PT20) and July 2020 (female, 20545PT20) in the same hunting reserve in Moura, Alentejo, were collected and investigated within the scope of a national surveillance program in action since August 2017.

Necropsy was performed according to routine procedures, and samples were collected for bacteriology (liver, spleen and lung), parasitology (gastrointestinal tract and liver), histopathology (lung, liver, spleen, kidney, eyelid and genitalia) and virology (liver, spleen, lung, kidney, eyelid, genitalia and duodenum).

For histopathology, skin and genitalia fragments were fixated in 10% neutral buffered formalin (w/v), routinely paraffin embedded, sectioned at 4 µm, and stained with Hematoxylin and Eosin (H&E).

2.2. Parasitological and bacteriological examination

Parasitological examination of the intestine was carried out resorting to direct wet mount, sedimentation and filtration techniques. Liver, spleen and lung samples were analysed using standard bacteriological methods. Enterobacteriaceae and non-Enterobacteriaceae were tested using the ID 32E (Biomerieux®) test and the API 20NE kit (Biomerieux®) test respectively. The presence of Streptococcus and Staphylococcus, was investigated using the ID 32 STREPT (Biomerieux®) and the ID 32 STAPH kits (Biomerieux®), respectively. The API CORYNE (Biomerieux®) kit was used for the identification of Corynebacteria and coryne-like organisms. For Salmonella, peptone water and Rappaport Vassiliadis semi solid culture media were used. Whenever there was a suspicion of Salmonella, the agarose SMID2 and XLD culture media were used. Other culture media for bacterial identification in the samples included the MacConkey agar and the Blood agar culture media.

2.5. Virological and serological examinations

For nucleic acid extraction, fresh samples of liver and spleen, kidney, lung, eyelid and genitalia were homogenised at 20% (w/v) with phosphate buffered saline (PBS) and clarified at 3000g for 5 min. Total DNA and RNA were extracted from 200µl of the clarified supernatants, using the MagAttract 96 cadon Pathogen Kit in a BioSprint 96 nucleic acid extractor (Qiagen, Hilden, Germany), according to the manufacturer's protocol.

Both rabbits were tested for rabbit haemorrhagic disease virus 2 (RHDV2) and MYXV by real time PCR targeting the M0005L/R gene [17,20]. The 2.8 bp insert was investigated by the system described by Dalton et al. [14] using primers 9A/9B and 9E/9F, that flank the insertion, allowing the amplification of a 3,1 or 4,6 Kb region in recombinant MYXV or 300bp region (absence of insert, using the oligomers 9E/9F) in MYXV. Amplification reactions were carried out in a Bio-Rad CFX96™ Thermal Cycler (Bio-Rad Laboratories Srl, Redmond, USA), using the One Step RT-PCR kit (Qiagen, Hilden, Germany) for RHDV2, and the HighFidelity PCR Master Mix (Roche Diagnostics GmbH, Mannheim, Germany), for MYXV and recombinant MYXV.

A commercial kit (Civtest® Cuni Mixomatosis - Hipra) developed for the detection of rabbit MYXV antibodies was validated for hare sera [21] and used to detect hare MYXV antibodies, following the manufacturer's instructions. For the female, serosanguinolent thoracic fluid was used instead of serum due to blood coagulation.

2.6. Sequencing analysis

The initial PCR products (≈3100 bp or ≈4600 bp) encompassing the 2.8 kb insert, were visualised in 2% horizontal electrophoresis agarose gel, purified using the NZYGelpure kit (Nzytech), and directly sequenced using the ABI Prism BigDye Terminator v3.1 Cycle sequencing kit on a 3130 Genetic Analyser (Applied Biosystems, Foster City, CA, U.S.A). Sequencing by primer walking was carried out, with a total of 12 additional oligomers designed for the purpose (Table 1).

The two nucleotide sequences obtained were assembled using the Seqscape Software v2.7 (Applied Biosystems, Foster City, CA, USA), and submitted to GenBank (MT940239 and MT940240).

2.7. Isolation

Isolation of MYXV from the two rabbits' tissues was achieved separately from eyelid, genitalia and lung. Samples were homogenized at 20% (w/v) in PBS containing penicillin, streptomycin and amphotericin B (antibiotic-antimycotic), used according to the manufacturer (Gibco, Life Technologies Corporation). Following centrifugation (3000g, 10min), the supernatant was filtered through a 0.45-µm-pore-size filter (Millipore Express) and used to inoculate sub confluent (70%) Rabbit Kidney (RK13) cells (ATCC-CCL-37). RK13 cells were grown in Eagle's medium supplemented with 5% foetal calf serum (Gibco), penicillin, streptomycin and amphotericin B (antibiotic-antimycotic used at 1:100), 50µg/ml gentamicin (Gibco). Cells were maintained at 37°C under humidified atmosphere with 5% CO₂ and observed daily for cytopathic effect (CPE) by phase contrast microscopy. The supernatant and cell pellet of each passage were tested for the presence of myxoma virus by qPCR [20].

The isolation of the virus allowed verifying its viability in the rabbit tissues, inferred by the cytopathic effect and in-house immunofluorescence protocol using MYXV positive hare serum (*protocol available on request*).

161

162

163

Table 1. Summary of oligomers used for amplification and sequencing

Primer name	Sequence (5'-3')	Position in MK340973 (nt)	Reference
9B (forward)	CGCAGGTCCACGTATAAACC	11458-11477 and 153103-153084	[14]
9A (reverse)	CGAACGTATCATTAGACAATG	16060-16040	
9E (forward)	CTTCGTCTACGCCTCCTACG	12116-12135	
9F (reverse)	GCGTCGTTGTGGTCAGACAGAG	15256-15243	
305R (reverse)	AACCCGCACAACGTAAAGTACC	12420-12399	
448F (forward)	GTCATATTCCTGATTTGGGTAATC	12563-12587	
796R (reverse)	AGGAGGAAAAGAACCTATGACAC	12911-12889	
1003F (forward)	GTGTGTACCTGGTGCAGAACC	13118-13138	
1302R (reverse)	TGAAGATGATAATGATGATGAATATCG	13417-13391	
1467F (forward)	TTCATCGTTTATGGGAAAATCTATG	13582-13606	
1819R (reverse)	GAGGGGACAGTTATGGATGTAC	13934-13913	
2028F (forward)	AAGATGCGTCTGTGTAACAATCC	14143-14165	
2325R (reverse)	AACAATGTATACACTCATGACAGTAC	14440-14415	
2458F (forward)	ATGGCCATCGTAAGTTGCCATG	14573-14594	
2847R (reverse)	CAGAGTACTTAGATTTTCTGCTAG	14962-14939	
2954F (forward)	ATCCATTGTTTCGTCAGTAGATCG	15069-15091	

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2.8. Electron microscopy

The fragments selected (eyelid and lung) for transmission electron microscopy (TEM) were placed in 10% buffered formalin (w/v). Samples were then washed and transferred to 0.05M cacodylate buffer containing 2.5% glutaraldehyde, and post-fixed with aqueous 1% osmium tetroxide (EMS) for 1 hour, fragments were then stained in block with ready-to-use UA-zero (Agar Scientifics), after which they were dehydrated in increasing concentrations of ethanol, infiltrated and embedded in Araldite resin (Agar Scientifics). Polymerization was performed at 60°C for 2 days. Ultrathin sections were cut using a Reichert ultracut E ultramicrotome (Leica), collected to 1% 200 mesh copper grids (Agar scientific), and examined in a Jeol 1400plus transmission electron microscope at an accelerating voltage of 120 kV. Digital images were obtained using a AMT XR16 bottom mount digital camera (AMT©). The sections were systematically analyzed using AMT© software and several high and low magnifications were acquired.

3. Results

3.1. Necropsy and Histopathology

The male wild rabbit had mild swelling of the eyelids (Fig. 1) and focal alveolar edema with hyaline substance deposits in the alveolar septa (Fig. 3). The female wild rabbit presented mild swelling of eyelids and vulva (Fig. 2), infiltration of alveolar septa by mononucleated cells and focal necrosis of alveolar septa with deposits of hyaline substance. The eyelid presented edema with epidermal detachment (Fig. 4). There was bacterial infiltration in the lung parenchyma of male rabbit.



Figure 1. Mild edema of the eyelid and presence of serous discharge (male rabbit).



Figure 2. Edema of the vulva (female rabbit).

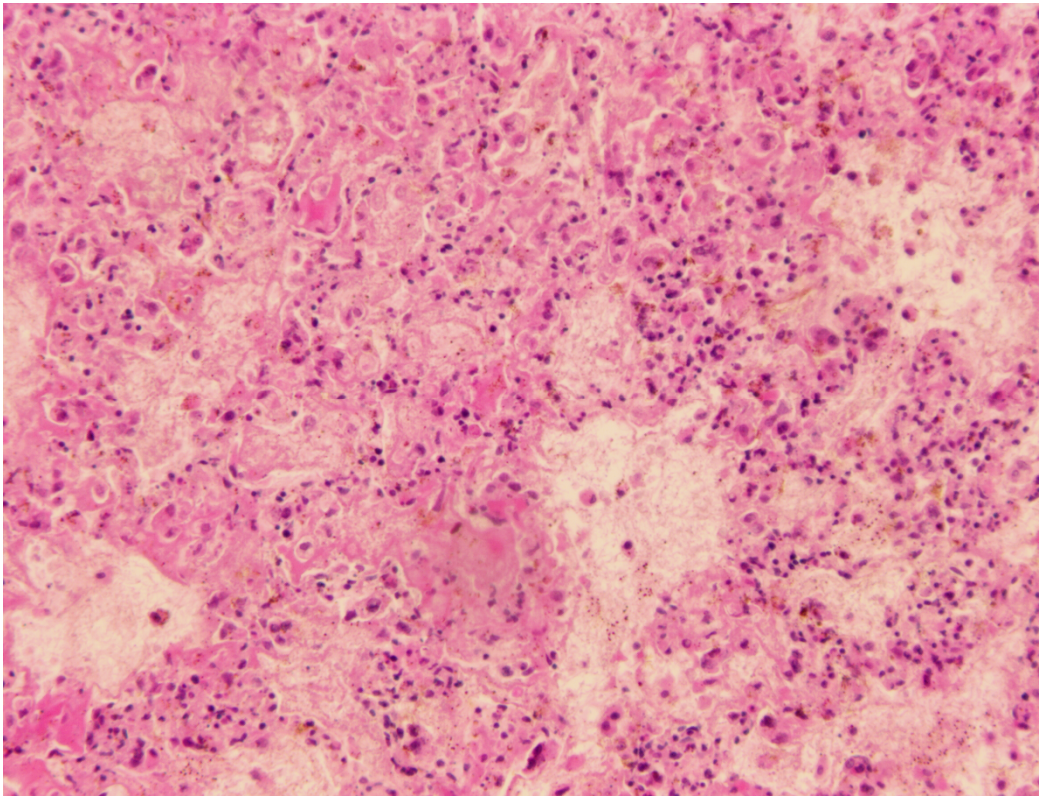


Figure 3. Infiltration of alveolar septa by mononucleated cells and focal necrosis of alveolar septa with deposits of hyaline substance (H&E, 100x).

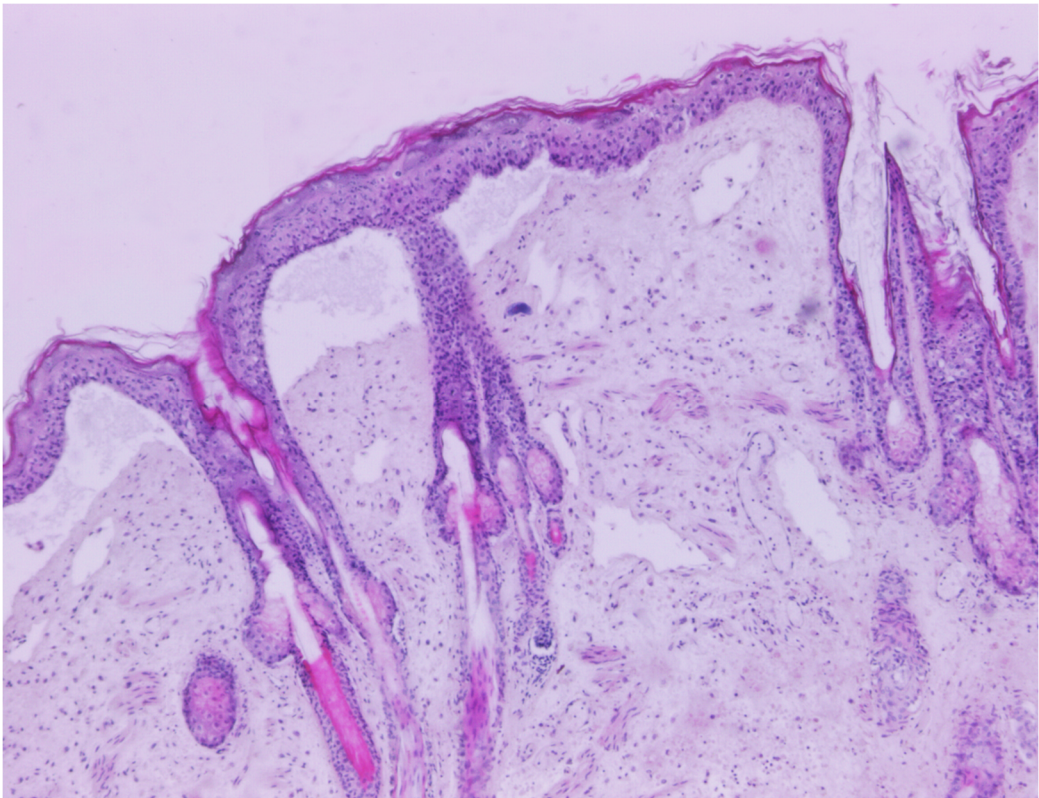


Figure 4. Eyelid presented edema with epidermal detachment (H&E, 40x).

3.2. Virological, bacteriological and parasitological results

Both animals tested negative for RHDV, RHDV2 and LeHV-5. The Cq values obtained with the qPCR targeting the diploid MYXV gene M0005L/R in the tissues from both rabbits revealed high viral charges in the liver/spleen (male=2.01E+09 copies/mg; female=1.88E+09 copies/mg), lung (male=1.3E+10 copies/mg; female=1.53E+09 copies/mg), eyelid (male=1.13E10 copies/mg; female=2.41E+10 copies/mg), genitalia (penis =7.47E+09 copies/mg, vulva=1,30E+10 copies/mg) and kidney (male=not tested; female=2,92E+08 copies/mg). Only the 4.6 Kb fragment was obtained with the PCR directed [18] to the 2.8 Kb insertion, indicating the presence of recombinant MYXV in the tissues of the rabbits and the absence of classical MYXV.

Serology using a commercial kit (Civtest® Cuni Mixomatosis-Hipra) according to the manufacturer's instructions, showed a high antibody titer in the male rabbit (RI10 about 20), similar to the RI values of hare positive control serum. Despite the RI value obtained for the female suggests no seroconversion, considering that blood serum was not available no robust conclusions can be taken.

Bordetella bronchiseptica and *Escherichia coli* were isolated from lungs of the male and female, respectively. Feces from both rabbits showed small infestations of *Eimeria* spp. oocysts.

3.3. Molecular characterization

Around position ≈61,000 nt of the complete MYXV genome, ORFs M060R, M061R, M062R, M063R M064R, M065R and M066R are sequentially located and close together, in different frames, with ORFs M065R and M066R overlapping by 100 nt.

Sequencing analysis of the 4.6 kb amplicon confirmed the presence of a 2,800 bp insert disrupting the M009L gene around nucleotide position 12,336 in the reference MYXV strain AF170726. M009L split into ORF M009L-a containing the original 5' end, and ORF M009L-b corresponding to the original 3' end.

In silico analysis of the 4,600/2,800 bp sequence showed the presence of 5 ORFs with some degree of similarity with genes M060R, M061R, M064R, M065R and M066R of MYXV strains, but with inverted orientation (Figure 5 B). ORF M066R encodes a 185 aa long protein and is found in MYXV (AAF14954.1). ORF M066L (the remaining of the complete gene M066R) encodes a putative protein of 70 amino acids in the recombinant MYXV from Portugal. Despite present in MYXV-Tol and ha-MYXV strains from Spain [14] [12], this ORF was not annotated previously. M066L is recognised between ORF M065L and ORF M009L-b, sharing 80% similarity with the homologous sequence of ORF M066R. This small ORF overlaps the M009L-b ORF by 21 nucleotides and M065 by 101 nucleotides (Fig. 5 C).

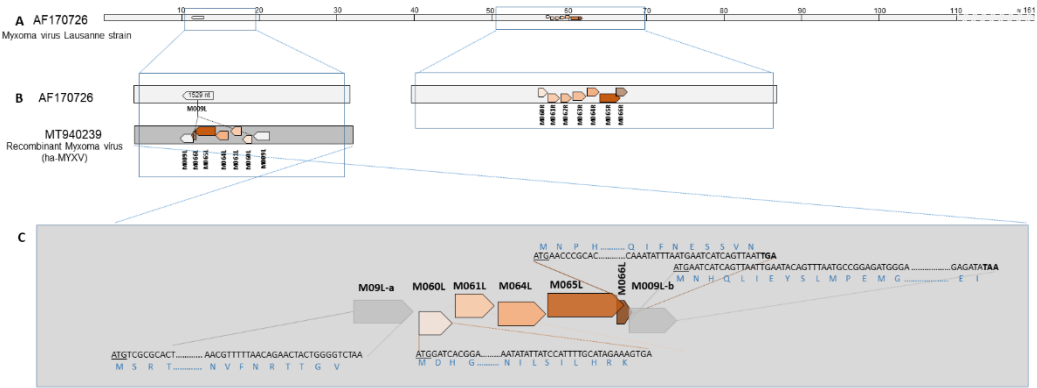


Figure 5– A- Linear genomic organization of the natural recombinant MYXV insert according with reference strain. B- Organization of the insert sequence and most similar genes in the normal region. C. B- Organization of the insert sequence with emphasis on the "connecting" zones with M009 disrupted gene.

The two nucleotide sequences obtained from the male and female wild rabbits were identical to each other and to the homologous sequences from other MYXV-Tol/ha-MYXV (MK836424 and MK340973). The differences between the truncated ORF M066L and the homologous M066R ORFs from MYXV-Tol and ha-MYXV obtained from hares (MK836424 and MK340973), and classical MYXV obtained from rabbits (MK388144, MK388143, MK388142 and MK388141 (MYXV) are shown in Fig. 6 In particular, the percentage of similarity between ORF M066L and ORFs M066R despite its species of origin is around 79%.

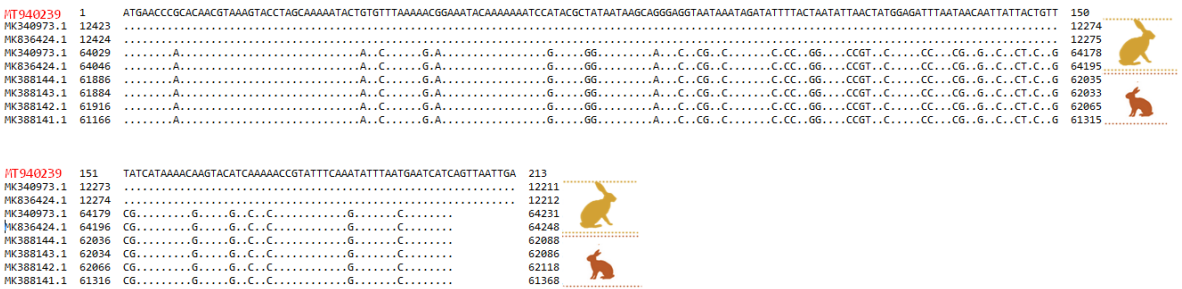


Figure 6. Nucleotide alignment analysis comparing the natural recombinant MYXV M066 CDS with the homologous sequence in other MYXV-Tol and ha-MYXV strains (MK836424 and MK340973) and classic rabbit MYXV strains. The hare (yellow icon) delimits sequences from strains isolated in this specie, and the rabbit (orange icon) delimits rabbit strains. The nucleotide position relative to the query (MT940239) indicates the insertion (12423 or 12424), and the location in the genome of M066R sequence (611884 to 64046).

The M066L sequences obtained are 100% similar to correspondent sequences of recombinant MYXV strains described before (MK836424 and MK340973) in Spain. Similarity was observed between the M066L and the M066R sequences from other ha-MYXV (79% of identity) and MYXV strains (79.43% of identity with many classic MYXV strains).

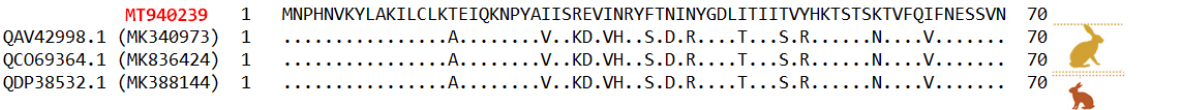


Figure 7. Alignment of natural recombinant MYXV M066L (protein sequence deduced from MT940239) putative truncated protein with the homologous M066R proteins deduced by recombinant MYXV strains (MK836424 and MK340973) and classic rabbit MYXV strain (MK388144).

The putative M066L protein obtained presented 80% identity with M066R protein of MYXV-Tol and ha-MYXV strains described before (MK340973 and MK836424). The same similarity was also observed between the M066L and the M066R sequences from classic rabbit MYXV strains (Fig. 7).

3.4. Isolation of the virus in cell culture

The successful isolation of the recombinant MYXV in RK13 cells from a separate eyelid, genitalia and lung samples from the male rabbit and from the eyelid of the female rabbit, confirmed its viability and infectiousness, proving that the virus was multiplying in the rabbit's tissues. Viral isolation was confirmed by cytopathic effect (CPE) at day 5 in RK13 subconfluent infected cells, by indirect immunofluorescence (*protocol available on request*), by qPCR of the cell supernatant and by indirect immunofluorescence of the cells.

The characteristic CPE at late stage of infection was observed 6 days after inoculation (Fig. 8 A). By qPCR we demonstrated the progressive decrease of the Cq value in DNA samples extracted from supernatant aliquots, collected at day 1, 5 and 10 (*results not shown*). To demonstrate the presence of

viral protein in the RK13 cells, an indirect immunofluorescence test was performed in an 10 days infected culture plate after fixation, allowing to confirm myxoma virus foci (Fig. 8 B).

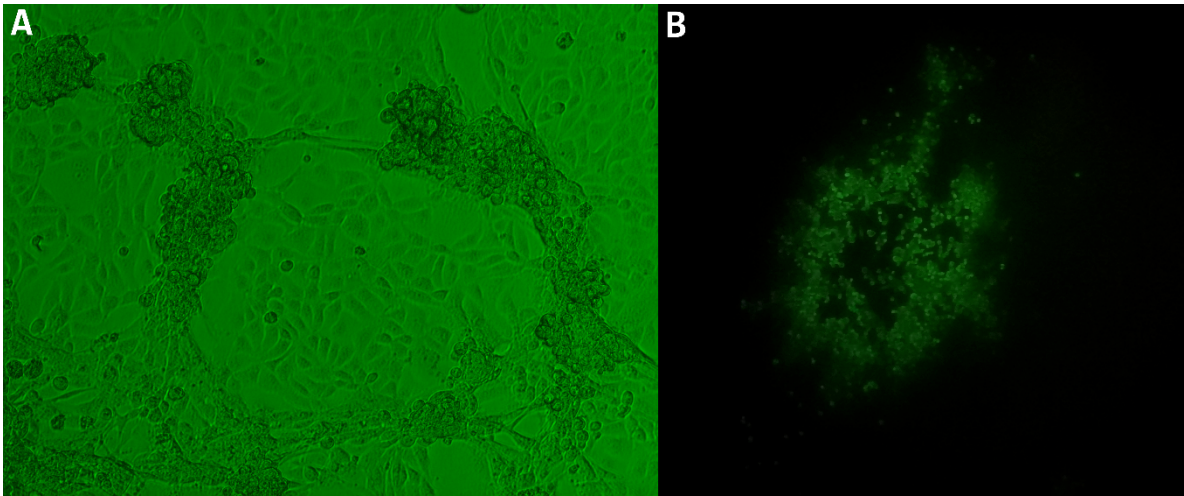


Fig. 8 (A) Cytopathic effect in RK13 cells infected with an eyelid homogenate of the rabbit male. 6 days after the infection, large aggregates of round and refringent cells within normal cells. Inverted research microscope, Nikon Eclipse Ti, 100×. (B) Positive immunofluorescence staining of the recombinant MYXV infected RK13 cells 10 days after the inoculation. Inverted research microscope, Nikon Eclipse Ti, 200×.

3.5. Electron microscopy

Analysis by electron microscopy allowed observing poxvirus-compatible particles in the lung and eyelid. In the eyelid, a higher number of viral particles was observed, especially in epithelial cells (Figure 9). The degree of autolysis of advanced tissues did not allow a more detailed evaluation of the type of most infected cells.

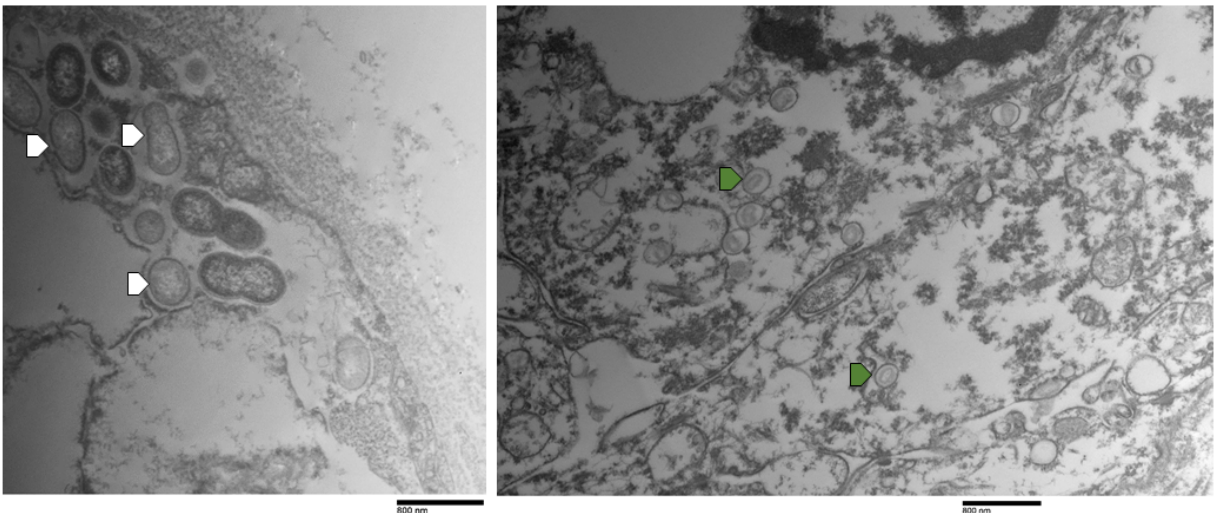


Fig.9. At left, a lung photograph. At right, an eyelid epithelium photograph. White arrow heads indicate immature viral particles and green arrow heads indicate apparently mature viral particles. The black bar indicates the scale (800 nm).

4. Discussion

The external signs of myxomatosis in two adult rabbits found dead in June and July 2020 in Alentejo that arrived to the National Reference Laboratory for Animal Diseases (INIAV, I.P.) for investigation were quite moderate, with mild to moderate swelling of the eyelids and genitalia.

Both male and female tested positive to MYXV-DNA by the M0005L/R gene qPCR, and by the 2.8 Kb insert PCR, showing to be infected by the natural recombinant MYXV. None of the rabbits were co-infected with classical MYXV strains, RHDV2, RHDV or LeHV-5.

Most of the wild rabbits that died of myxomatosis, generally arrive to the laboratory with severe swelling of the eyelids and genital, often accompanied by ocular purulent discharge and very frequently in a state of thinness or cachexia. The high viral loads found in several tissues, and the good body condition of the wild rabbits, suggest a short course of the disease therefore a possible higher virulence of the natural recombinant MYXV strain towards wild rabbits, although further testing is necessary to support this relation. With regards to the MYXV strains, the national surveillance plan of wild leporids in action in Portugal since 2017 allowed testing more than 57 infected wild rabbits. 73% of the rabbits found dead with myxomatosis still presented median/poor corporal condition or even cachexia, reflecting the ability of the animals to survive infected for longer periods. A lower adaptation of the recombinant MYXV strains to rabbits, comparing the MYXV classic strains with which rabbits have evolved for more than 50 years [21], may eventually account for these apparent differences.

The detection of a recombinant MYXV circulating in hares, and its apparent segregation from MYXV circulating in rabbits, initially suggested the adaptation of MYXV-Tol and ha-MYXV to hares in order to efficiently multiply in this species.

Sequencing of the 2.8 Kb insert from the two rabbits showed that both recombinant MYXV strains have the same poxvirus gene “cassette” previously described in Iberian hares [2,12,14]

However, we described a putative truncated gene similar to M066R gene of myxoma virus, that is also present, but not annotated, in available myxoma virus sequences obtained previously from Iberian hare. As in the MYXV-Tol (MK836424) and ha-MYXV genomes (MK340973), M062R and the M063R are not found in the insert.

The origin of this insert was discussed previously by other authors, and is not within the aims of this work. However, the putative protein encoded by ORF M066L is 65.22 to 76.81% similar to homologous ORFs in capripoxviruses, cervidpoxviruses, suipoxviruses, yatapoxviruses but not with BeAn 58058 virus, appointed previously [12] as potential donor or sharing an ancestral donor of the genetic material found in the insert. On the other hand, the higher similarity of M066L with rabbit fibroma virus and with classical myxoma virus strains, suggests that the insert may have originated from one of these viruses, or a similar virus, not yet described.

During the two months in which the two rabbits were collected, only a small number of found dead rabbits with myxomatosis arrived at the laboratory from mainland Portugal, limiting any inference about the prevalence, frequency and distribution of the recombinant MYXV in the wild. However, since the natural recombinant MYXV emergency in 2018, and according to data collected under the +Coelho project, Beja was the district from which more hares were sampled (52 out of 170) and tested, and was also one of the districts mostly affected by myxomatosis (34.6% of positivity in the sample).

Very recently, we also detected this recombinant MYXV in a wild rabbit with signs of myxomatosis (*results not shown*), from Samora Correia (Benavente, Portugal), about 200 km north from Moura, indicating that there are most likely multiple unknown outbreaks or spillover events of the recombinant MYXV in wild rabbit.

The detection of the recombinant MYXV in wild rabbits raises serious concerns at different levels, constituting an additional treat to the already fragile wild rabbit, which entered to the IUCN's endangered conservation status last year [19]. If the recombinant MYXV and MYXV strains behave as different viruses in rabbits, with no full cross protection between the two, the jump of a recombinant MYXV into the rabbit populations will eventually accelerate the decline of these already diminished wild populations. On the other hand, the fact that the recombinant MYXV affects both

the Iberian hare and the wild rabbit, may favor the maintenance of the virus as more hosts are available for virus replication and circulation. The recombinant MYXV may therefore become endemic in the same way that classic strains did, allowing the co-evolution in both species. However, the ability to infect the wild rabbit, may lead the recombinant MYXV to prefer the rabbit host, taking into account the greater dispersion and higher density compared to the Iberian hare, which would favor their maintenance in environment. Further concerns include the rabbit industry, and the need to evaluate if MYXV or Shope Fibroma virus attenuated vaccines are protective against the recombinant MYXV.

Since the complete genome sequences were not obtained in this study, there are no certainties that the recombinant MYXV strains found in the two rabbits are identical to MYXV-Tol or ha-MYXV. Therefore, we cannot exclude the existence of other mutations that may have contributed to the ability of the recombinant MYXV to cause disease in rabbits.

5. Conclusions

Almost two years after the emergence of a recombinant MYXV in Iberian hares, our finding brings one new piece into the model of host-myxoma virus co-evolution by demonstrating the pathogenicity of this recombinant virus towards rabbits. It is important to continue monitoring the disease in rabbits and hares in order to ascertain the geographic dimension of the spillover phenomena or the spread of this species jump of recombinant MYXV back to the European rabbit.

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Declaration: This study did not use live animals and was carried out within the scope of a National Plan for the Control of Rabbit Haemorrhagic Disease Virus 2 in rabbits (Dispatch no. 4757/2017 of 31 May), with the legal authorizations from the National Authority-the Institute for Nature Conservation and Forests (Instituto da Conservação da Natureza e das Florestas, I.P., ICNF).

Conflicts of Interest: The authors declare no conflict of interest.

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