Recombinant myxoma virus in European rabbit: emergence of a new threat

Context

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 “architect”, contributing to seed dispersal, and landscaping. Besides its ecological role, the wild rabbit is an important game species economically and socially.

Myxoma virus (MYXV) is one of the two major pathogen threats for the European rabbit (Oryctolagus cuniculus). The etiological agent of myxomatosis is MYXV, a double-stranded DNA Leporipoxvirus of the family Poxviridae.

Myxomatosis is an endemic disease of South American rabbits and was first described in laboratory rabbits in 1898 in Uruguay. The disease is usually characterised by the presence of nodules in the skin surrounding the eyes, nose, mouth, ears, and genitalia.

Until mid-2018, no Iberian hare (Lepus granatensis) was positive for any MYXV strain. The emergence of myxomatosis in the Iberian hare in mid-2018, was caused by a recombinant myxoma virus harbouring an insertion of about 2.8 kbp.

Material and Methods

Three wild rabbits (Oryctolagus cuniculus algerius) and five domestic rabbits that died with signals of myxomatosis were collected from Beja and Santarém, necropsied in the INAV, I.P. and submitted to virological analysis. For histopathology, skin and genitalia fragments were fixed in 10% neutral buffered formalin (w/v), routinely paraffin embedded, sectioned at 4 µm, and stained with Hematoxylin and Eosin (H&E).

Recombinant MYXV was investigated by conventional PCR directed to the insertion in the M009I gene, as previously described by Dalton, 2019. Samples of eyelid and lung were used to viral isolation in RK13 cells. Other samples were fixed and submitted to transmission electron microscopy. The PCR products were visualized in 2% horizontal electrophoresis agarose gel, purified and directly sequenced using the ABI Prism BigDye Terminator v3.1 Cycle sequencing kit on a 3130 Genetic Analyser. Sequencing by primer walking was carried out. The nucleotide sequences obtained were assembled using the Seqscape Software v2.7 and submitted to GenBank.

Results

The macroscopic and histopathological signs of the eight animals were compatible with myxomatosis. The presence of recombinant myxoma virus and not classic strains was confirmed by PCR and sequencing analysis. The presence of poxviruses were also observed by electron microscopy in the skin and lungs’ samples.

Discussion and Conclusion

The external signs of myxomatosis in eight rabbits found dead between June and August 2020 in Alentejo and Santarém, that arrived to the National Reference Laboratory for Animal Diseases (INAV, I.P.) for investigation corresponded to mild to moderate swelling of the eyelids and genitalia. All samples tested positive to MYXV-DNA by a generalist qPCR targeting the M0005 L/R gene, and by a conventional PCR targeting the 2.8 kbp insert, showing infection by the natural recombinant MYXV.

The high viral loads found in several tissues, and the good body condition of these rabbits, suggest that the disease developed rapidly.

During the Myoxide Project, 73% of the rabbits found dead with myxomatosis presented median/poor condition or even cachexia, reflecting the ability of the animals to survive infected for longer periods. All these animals were infected with classic MYXV strains. A lower adaptation of the recombinant MYXV strains to rabbits, compared to the MYXV classic strains with which rabbits have evolved for more than 50 years, may eventually account for these differences.

The detection of a recombinant MYXV circulating in hares, and its apparent segregation from MYXV circulating in rabbits, initially suggested the adaptation of recombinant MYXV to hares in order to efficiently multiply in this species. Sequencing of the full 2.8 kbp insert from two of the eight rabbits showed that both recombinant MYXV strains have the same poxvirus gene ‘cassette’ previously described in Iberian rabbits.

However, we described a putative truncated gene similar to the M066R gene of the MYXV that is also present, though not annotated, in the recombinant MYXV sequences obtained from Iberian hares.

The detection of the recombinant MYXV in wild rabbits raises serious concerns us as it constitutes an additional treat to the already fragile wild rabbit, which entered to the IUCN’s endangered conservation status last year.

The recombinant MYXV and classical MYXV strains behave as different viruses in rabbit, 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 favour 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 geographical dispersion and higher demographic densities compared to the Iberian hare, which will probably facilitate their maintenance in the 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.

Although vaccination is highly effective in the industry, inducing generally the seroconversion of almost 100% of the animals, parental vaccination of wild populations is almost impossible.

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