WILDLIFE

Myxoma virus and rabbit haemorrhagic disease virus 2 coinfection in a European wild rabbit (*Oryctolagus cuniculus algirus*), Portugal

Carina Luisa Carvalho ,[1] Fábio Alexandre Abade dos Santos ,[1,2] Teresa Fagulha,[1] Paulo Carvalho,[1] Paula Mendonça,[1] Madalena Monteiro,[1] Margarida Dias Duarte [1,2]

[1]Instituto Nacional de Investigação Agrária e veterinária (INIAV), Av. da República, Quinta do Marquês (edifício sede), Oeiras, Portugal
[2]CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, Lisboa, Portugal

Correspondence to
Dr Carina Luisa Carvalho; carina.carvalho@iniav.pt

Received 30 October 2019
Revised 17 February 2020
Accepted 19 February 2020

To cite: Carvalho CL, Abade dos Santos FA, Fagulha T, *et al*. *Vet Rec Case Rep* Published Online First: [*please include* Day Month Year]. doi:10.1136/vetreccr-2019-001002

SUMMARY

Myxoma virus (MYXV) and rabbit haemorrhagic disease virus 2 (RHDV2) are two major pathogens that affect the European rabbit (*Oryctolagus cuniculus*). Between August 2017 and August 2019, 1166 wild rabbits (971 legally hunted and 195 found dead) were tested by PCR-based methods for MYXV and RHDV2 within the scope of an ongoing surveillance programme on wild leporids in Portugal. Despite never having been reported before and being considered a rare event, coinfection by RHDV2 and MYXV was detected in one juvenile wild rabbit found dead in the Évora district located in Alentejo. The relative frequency of coinfection in the group of diseased rabbits (found dead in the field) was 0.52 per cent (1/195). The positivity percentage of each single virus was much higher, namely, 14.36 per cent (28/195) for MYXV and 55.38 per cent (108/195) for RHDV2, within the 2 years of sample collection considered.

BACKGROUND

Myxoma virus (MYXV) and rabbit haemorrhagic disease virus 2 (RHDV2) are the two major pathogen threats for the European rabbit (*Oryctolagus cuniculus*).[1 2]

MYXV is a large double-stranded DNA *Leporipoxvirus* of the Poxviridae family.[3] In the European rabbit, it causes myxomatosis, a systemic and usually lethal disease[4] endemic in Iberia since 1953.[5] The disease still downregulates the wild rabbit populations, affecting mostly newborn and juvenile rabbits.[2]

RHDV2, a *Lagovirus* of the Caliciviridae family, emerged in France in 2010[6] and induces an acute, highly contagious and often lethal systemic disease in wild and domestic rabbits.[1 6] RHDV2 rapidly replaced the former rabbit haemorrhagic disease virus (RHDV),[7–9] first reported in 1984 in China.[10] Soon after their emergences, nearly 24 years apart, both viruses quickly spread throughout Europe,[11 12] causing high economic losses in the industry[6 12] and high mortality rates in wild rabbit populations.[1 12]

Serological surveys have demonstrated the concomitant presence of MYXV and RHDV/RHDV2 antibodies in wild rabbits.[13–15] Intriguingly, coinfections by these viruses were never reported in any of the many countries affected by the two diseases and were therefore considered rare or unexpected events.[16]

This study reports a coinfection event by MYXV and RHDV2 in a juvenile wild rabbit (*O cuniculus algirus*) found dead in Portugal.

CASE PRESENTATION

A male juvenile wild rabbit from the Évora district, Alentejo region, South of mainland Portugal, was found dead in the field on 20 February 2019. The animal was tested within the scope of an ongoing national surveillance programme on wild leporids (dispatch 4757/17, 31 May, Portuguese Ministry of Agriculture).

INVESTIGATIONS

Postmortem examination and histopathology

Necropsy of this juvenile wild rabbit was carried out at the National Reference Laboratory for Animal Diseases. During the necropsy, the animal presented poor body condition and skin lesions in the eyelids, ears, upper lips and genitals, suggestive of the nodular form of myxomatosis. Hepatic discoloration and multifocal congestion of the lungs, characteristic of rabbit haemorrhagic disease (RHD), were also observed.

For histopathological examination, skin samples were fixed in 10 per cent buffered formalin and embedded in paraffin using standard procedures. Five micrometre-thick sections were stained with H&E and examined using light microscopy.[17]

Histopathological examination of the eyelids and the upper lips showed typical myxoid tumours (myxomas) (figure 1).

Virological examination

Liver, spleen and lung samples were tested for the presence of RHDV, RHDV2 and MYXV by the PCR-based methods detailed further below. Skin lesions were also used for MYXV investigation.

For the virological screening, each tissue sample was homogenised with phosphate-buffered saline to a final concentration of 20 per cent (w/v) and clarified at 3000*g* for 5 minutes. Total DNA and RNA were extracted from 200 µl of the clarified supernatant, in a BioSprint 96 nucleic acid extractor (Qiagen, Hilden, Germany), using the MagAttract 96 Cador Pathogen kit (Qiagen), according to the manufacturer's instructions.

Samples were tested for RHDV2 RNA by the RT-quantitative PCR (qPCR) by Duarte *et al*[18]

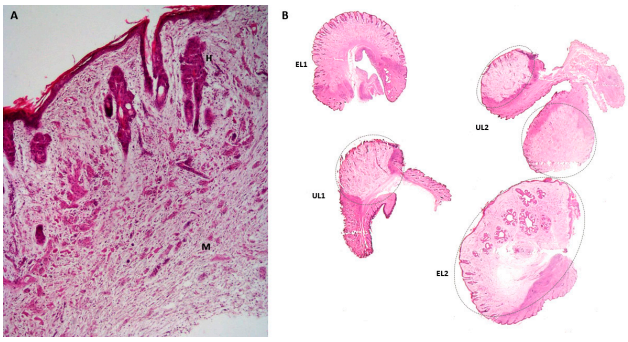

Figure 1 (A) Cross section of the eyelid showing the replacement of dermis by M and moderate H. H&E staining (×40 magnification). (B) Cross sections of the EL (EL1 and EL2) and ULs (UL1 and UL2) showing M nodules, pointed by dashed grey circles. H&E staining, original magnification. EL, eyelid; H, hyperplasia of the epidermis; M, myxoid tissue; UL, upper lip.

referred in the World Organisation for Animal Health (OIE) manual (https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.06.02_RHD.pdf), using the One-Step RT-PCR Kit (Qiagen). The presence of MYXV DNA was investigated by the qPCR by Duarte *et al*,[19] also recommended in the OIE manual (https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.06.01_MYXO.pdf), using the FastStart TaqMan Probe Master Kit (Roche, Roche Diagnostics GmbH, Manheim, Germany). For the real-time systems described, undetectable Cq or Cq values of >38.00 were considered negative. Positive controls were included in each reaction. Negative controls contained PCR-grade water.

Both MYXV DNA and RHDV2 RNA were detected in the liver, spleen and lungs of the juvenile wild rabbit. For RHDV2, as expected, the viral loads found in a pool of liver and spleen (9.09×10^8 copies/mg) were higher than those in the lungs (7.37×10^7 copies /mg).

MYXV DNA was detected in a skin lesion, where higher viral loads (2.49×10^7 copies/mg) were found, when compared with a pool of liver and spleen (6.87×10^5 copies/mg) or lungs (7.47×10^5 copies/mg) (figure 2). The lower viral loads detected in the internal organs suggest that MYXV replicates to low titres in the liver and lungs or may represent the contamination of organs with blood during the viraemic phase.

To rule out any contamination, samples from the juvenile wild rabbit were extracted *de novo* and retested, generating similar results.

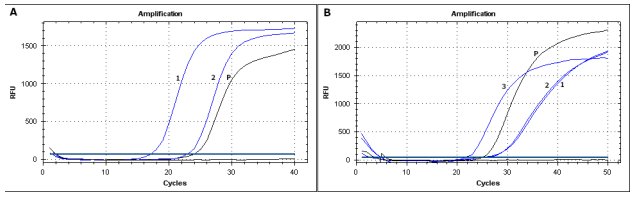

Figure 2 Amplification curves of (A) RHDV2 detection in the liver and spleen (1) (Cq value of 17.14) and lungs (2) (Cq value of 22.97), and (B) MYXV detection in the liver and spleen (1) (Cq value of 29.09), lungs (2) (Cq value of 28.98) and skin (3) (Cq value of 23.37). The negative controls (without nucleic acid) are the flat black lines seen below the thresholds. Cq, quantification cycle; P, positive control; RHDV2, rabbit haemorrhagic disease virus 2; MYXV, myxoma virus; RFU, Relative Fluorescence Units,

Screening for the classical strains of RHDV was performed by conventional PCR with primers RC-9 and RC-10[20] using the One-Step RT-PCR Kit (Qiagen). As expected, since the classical strains no longer circulate, the animal tested negative.

The full RHDV2 VP60 gene was amplified using the pairs of primers 27F[21] and 986R[22] and 717 F[22] and 10 R.[20] The fragment was excised from agarose gel after electrophoresis, purified using the NZYGelpure kit (NzyTech, Genes and Enzymes, Lisbon, Portugal) and sequenced in an automated 3130 Genetic Analyser system (Applied Biosystems, Foster City, California, USA) using the BigDye Terminator Cycle sequencing kit (Applied Biosystems). The sequence was submitted to GenBank database and given the accession number MN894670. Using the Basic Local Alignment Search Tool BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi), our sequence revealed high similarity with RHDV2 strains circulating in the Alentejo region in previous years (namely, with strain MG763940).

Parasitological and bacteriological examinations

Parasitological examination revealed light coccidian infections with *Eimeria stidae* in the liver and *E perforans* in the faeces, as well as the intestinal nematodes *Graphidium strigosum* and *Passalurus ambiguous*.

Liver, spleen and lung samples collected from this rabbit cadaver were analysed using standard bacteriological methods. *Staphylococcus xylosus*, a commensal bacterium found in mammals' skin and mucous membranes,[23] was isolated from a pool of organs, including the liver, spleen and lung.

None of these infections explains the animal death.

DISCUSSION

In this study, we report the detection of a juvenile wild rabbit coinfected with RHDV2 and MYXV. This animal was found dead in the South of mainland Portugal in late winter 2019.

Since RHDV, RHDV2 and MYXV have emerged, hundreds of animals were tested worldwide for *Leporipoxvirus* and *Lagovirus*. Interestingly, although both RHD and myxomatosis are mechanically transmitted by arthropod vectors and their dissemination is facilitated by an increase in the wild rabbit population density, there are no previous reports of coinfection. However, interaction between RHD and myxomatosis has been described before.[13–15] Mutze *et al*[13] proposed, regarding the possible mechanisms of the interaction between RHD and myxomatosis, that when RHD is most active during spring, RHD outcompetes myxomatosis due to the longer incubation time of the latter, which could explain the infrequency of such coinfection events. Assuming similar likelihood of transmission from infective to susceptible animals, RHD would kill most rabbits before they became infective for myxomatosis.[13] In fact, in Australia, the introduction of RHD caused an important change in the timing of myxomatosis outbreaks by displacing these occurrences to autumn and delaying or eliminating the spring–summer outbreaks.[13] Additionally, Marchandeau *et al*[14] hypothesised, based on serological data, that exposure to one virus could increase the probability of developing the other disease. García-Bocanegra *et al*[24] showed that seropositivity to lagoviruses was associated with seropositivity to myxomatosis, as 70 per cent of the rabbits that were seropositive to lagoviruses had been also exposed to MYXV.

The coinfection event described here required the interaction between myxomatosis and RHD either by the contemporary overlapping of the two epidemics or a scenario of an endemic situation of myxomatosis plus an epidemic wave of RHDV2.

None of the nine wild rabbit cadavers collected in the same geographical area of the index case between 20 February and 10 May 2019 were simultaneously positive for both viruses, with most of the rabbits testing positive only for RHDV2 (7/9, 77.78 per cent), which suggests an ongoing RHDV2 outbreak.

The occurrence of an RHDV2 outbreak at that time of the year is in accordance with the seasonality of the disease in Iberia. In Portugal, RHDV2 mortality is higher in the cooler months (December–March), in association with the annual inflow of susceptible young rabbits during the breeding season that takes place during the winter and spring, reaching a peak of incidence in January and February.[25] Regarding myxomatosis, the disease can occur throughout the whole year in Europe, although seasonality is observed in Iberia.[24 26 27] As with RHDV2, myxomatosis outbreaks frequently synchronise with the appearance of a large number of susceptible young rabbits, such as the juvenile of this case report, and with the abundance of arthropod vectors.[2] Fleas and mosquitoes are mechanical vectors of MYXV[28 29] and RHDV2,[30–33] and in Portugal, the 2018–2019 winter was mild, favouring to the presence of these arthropods.

Although the clinical history of the juvenile wild rabbit is irretrievable, its poor body condition, compatible with a longer-lasting infection, is more plausible to be caused by MYXV. The severity of the lesions observed in the liver and lungs suggests that RHDV2 infection took place when the animal was already suffering from myxomatosis. The immunosuppressive effect of MYXV increases the risk of other infections,[34] making it possible that infection with MYXV may have favoured RHD virus infection.

In Portugal, viral surveillance of RHD and myxomatosis, both endemic in the entire national territory, was recently strongly intensified within the scope of a national surveillance programme on wild leporids. Since its implementation in August 2017 until August 2019, 1166 wild rabbits legally hunted (n=971) and found dead (n=195) were systematically tested by PCR-based methods for RHDV, RHDV2 and MYXV. For this 2-year period, RHDV2 and MYXV infections were responsible for the death of 70.83 per cent of the animals found in the field, and no coinfections were detected. This may suggest a viral interference phenomenon, in accordance with Mutze *et al*[13] results. Apart from the coinfected animal, the only wild rabbit from the affected area that tested MYXV positive (1/9, 11.11 per cent) was RHDV2 negative.

Data produced by the ongoing surveillance programme on wild leporids show that the number of positive cases for each disease has been higher in Alentejo and Algarve[35] (NUT 2 regions in the South), compared with the rest of the national territory. In the district of Évora, where the coinfected wild rabbit was collected, the percentages of positivity (PP) in the sample of diseased animals (found dead) for MYXV and RHDV2 were 9.09 per cent (5/55) and 76.37 per cent (42/55), respectively, indicating the contemporary circulation of both viruses in the wild populations. For apparently healthy hunted animals, the PPs found in that district for MYXV and RHDV2 were 6.91 per cent (11/159) and 0 per cent (0/159), respectively.

From August 2017 onwards, apart from the case reported here, no other wild rabbits were found simultaneously infected by both viruses, confirming that this event is extremely rare when compared with single infections by MYXV and RHDV2. In the group of diseased wild rabbits found dead in the field, sampled between August 2017 and August 2019, coinfection represents 0.52 per cent (1/195), much lower than single infections by MYXV (14.35 per cent, 28/195) and RHDV2 (55.38 per cent, 108/195). Our results reflect the high pathogenicity of both viruses, showing that more than half of the animals found in the field died of RHDV2 infection, while MYXV accounted for one in seven of the deaths.

As expected, the PPs for the group of hunted animals are much lower for both viruses, namely 4.33 per cent (42/971) for MYXV and 0.62 per cent (6/971) for RHDV2. The differences in the PP ratios (found dead/hunted), 89.9 per cent for RHDV2 and 3.3 per cent for MYXV, indicate a shorter course disease for RHDV2 infections and higher mortality compared with myxomatosis.

To our knowledge, viral coinfections were never reported in wild rabbits, despite in other species, this event is common. Examples of coinfections are provided by MYXV and leporid herpesvirus 5 in the Iberian hare,[36] feline herpesvirus 1 and feline calicivirus in cats,[37] or peste des petits ruminants virus and foot-and-mouth disease virus in goats.[38]

Generally, coinfections are believed to exert a negative effect on the hosts' health, but little is yet known about the effect that one pathogen has on the other and on the implications of coinfection to the host. Viral interference, where one virus competitively suppresses replication of the other coinfecting viruses, is the most common outcome of coinfection. It may be mediated by various factors, such as interferons, defective interfering particles and cellular factors, among others. Nevertheless, coinfections of certain viruses may also promote an increase or may have no effect on virus replication, allowing, in the latter, for coinfecting viruses to coexist (accommodation), (Reviewed in Kumar *et al*).[39]

Although from an epidemiological point of view the impact of the coinfection by RHDV2 and MYXV in the wild rabbit populations appears minor since it is a rare event, this possibility must be taken into account, especially in the context of diagnosis, where it is common to attribute a viral disease to the infection by a single agent. The contribution of multiple-agent infections in the clinical outcome is rarely considered, which may lead to failure in the detection of additional agents.[39] In the case reported here, the molecular diagnosis for MYXV and RHDV2 were also corroborated by the observation of histopathological lesions typical of both diseases.

More than reporting an RHDV2–MYXV coinfection event in a wild rabbit, this paper aimed to raise awareness of the need to perform both diagnoses in areas where the two diseases are endemic, even when skin lesions are not yet present.

Learning points

- During a 2-year period, starting in August 2017, a national ongoing surveillance programme on wild leporids tested 1166 wild rabbits for myxoma virus (MYXV) and rabbit haemorrhagic disease virus 2 (RHDV2).
- In the sampling group diseased animals found dead, 14.35 per cent (28/195) and 55.38 per cent (108/195) were found positive for MYXV and RHDV2, respectively.
- A coinfection with MYXV and RHDV2 was detected in one juvenile wild rabbit found dead in Alentejo, Évora district, South Portugal.
- MYXV and RHDV2 coinfection is a rare event compared with single infections, representing a relative frequency of 0.52 per cent (1/195) in the group of diseased animals found dead.

Contributors CLC carried out the experimental work regarding the virological screening and sequencing analysis and wrote the manuscript. FAAdS assisted in the necropsies and, along with TF, helped in writing the manuscript. PC, PM and MM carried out the anatomohistopathological examinations. MDD conceived the

experiments and wrote and revised the manuscript. All authors discussed the results and contributed critically to the final document.

Funding This study was supported by Project +Coelho 2, financed by the Fundo Florestal Permanente, Portuguese Ministry of Agriculture and by the Portuguese Foundation for Science and Technology (FCT) (Project Fight-two: PTDC/CVT-CVT/29062/2017-PT2020). FAAdS holds a PhD grant from FCT (SFRH/BD/137067/2018).

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository.

ORCID iDs
Carina Luisa Carvalho http://orcid.org/0000-0002-6648-9254
Fábio Alexandre Abade dos Santos http://orcid.org/0000-0002-0696-7322
Margarida Dias Duarte http://orcid.org/0000-0003-1488-9659

REFERENCES

1 Delibes-Mateos M, Ferreira C, Carro F, *et al*. Ecosystem effects of variant rabbit hemorrhagic disease virus, Iberian Peninsula. *Emerg Infect Dis* 2014;20:2166–8.
2 Villafuerte R, Castro F, Ramírez E, *et al*. Large-scale assessment of myxomatosis prevalence in European wild rabbits (*Oryctolagus cuniculus*) 60years after first outbreak in Spain. *Res Vet Sci* 2017;114:281–6.
3 Murphy FA, Fauquet CM, Bishop DHL, *et al*. - *virus taxonomy: classification and nomenclature of viruses. sixth report of the International Committee for the taxonomy of viruses. Archives of virology, 1995*. Vienna: Springer Verlag: 586.
4 Fenner F, Ratcliffe FN. *Myxomatosis*. Cambridge University Press, 1965.
5 Guixeras J. *Tratado de la mixomatosis*. Graf. Bat, Salt, Girona, 1957: 126 pp.
6 Le Gall-Reculé G, Zwingelstein F, Boucher S, *et al*. Detection of a new variant of rabbit haemorrhagic disease virus in France. *Vet Rec* 2011;168:137–8.
7 Le Gall-Reculé G, Lavazza A, Marchandeau S, *et al*. Emergence of a new lagovirus related to rabbit haemorrhagic disease virus. *Vet Res* 2013;44:81.
8 Lopes A, Correia J, Abrantes J, *et al*. Is the new variant RHDV replacing genogroup 1 in Portuguese wild rabbit populations? *Viruses* 2015;7:27–36.
9 Mahar JE, Hall RN, Peacock D, *et al*. Rabbit hemorrhagic disease virus 2 (RHDV2; GI.2) is replacing endemic strains of RHDV in the Australian landscape within 18 months of its arrival. *J Virol* 2018;92:e01374–17.
10 Liu SJ, Xue HP, BQ P, *et al*. A new viral disease of rabbits. *Animal Husbandry Vet Med* 1984;16:253–5.
11 Rouco C, Aguayo-Adán JA, Santoro S, *et al*. Worldwide rapid spread of the novel rabbit haemorrhagic disease virus (GI.2/RHDV2/b). *Transbound Emerg Dis* 2019;66:1762–4.
12 Abrantes J, van der Loo W, Le Pendu J, *et al*. Rabbit haemorrhagic disease (RhD) and rabbit haemorrhagic disease virus (RHDV): a review. *Vet Res* 2012;43:12.
13 Mutze G, Bird P, Kovaliski J, *et al*. Emerging epidemiological patterns in rabbit haemorrhagic disease, its interaction with myxomatosis, and their effects on rabbit populations in South Australia. *Wildlife Research* 2002;29:577–90.
14 Marchandeau S, Bertagnoli S, Peralta B, *et al*. Possible interaction between myxomatosis and calicivirosis related to rabbit haemorrhagic disease affecting the European rabbit. *Vet Rec* 2004;155:589–92.
15 García-Bocanegra I, Astorga RJ, Napp S, *et al*. Factors affecting the seroprevalence of lagovirus infection in wild rabbits (*Oryctolagus cuniculus*) in southern Spain. *Vet J* 2011;189:89–94.
16 Fulford GR, Lee XJ, Berman D, *et al*. *Interaction of myxomatosis and rabbit haemorrhagic disease in wild rabbit*. 19th international Congress on modelling and simulation, Perth, Australia, 2011.
17 Cook HC. Origins of … tinctorial methods in histology. *J Clin Pathol* 1997;50:716–20.
18 Duarte MD, Carvalho CL, Barros SC, *et al*. A real time TaqMan RT-PCR for the detection of rabbit hemorrhagic disease virus 2 (RHDV2). *J Virol Methods* 2015;219:90–5.
19 Duarte MD, Barros SC, Henriques AM, *et al*. Development and validation of a real time PCR for the detection of myxoma virus based on the diploid gene M000.5L/R. *J Virol Methods* 2014;196:219–24.
20 Tham KM, Barnes SM, Hunter SN. Polymerase chain reaction amplification and gene sequence analysis of a calicivirus from a feral rabbit. *Virus Genes* 1999;18:235–42.
21 Le Gall G, Arnauld C, Boilletot E, *et al*. Molecular epidemiology of rabbit haemorrhagic disease virus outbreaks in France during 1988 to 1995. *J Gen Virol* 1998;79:11–16.
22 Duarte M, Carvalho C, Bernardo S, *et al*. Rabbit haemorrhagic disease virus 2 (RHDV2) outbreak in Azores: disclosure of common genetic markers and phylogenetic segregation within the European strains. *Infect Genet Evol* 2015;35:163–71.
23 Dordet-Frisoni E, Dorchies G, De Araujo C, *et al*. Genomic diversity in *Staphylococcus xylosus*. *Appl Environ Microbiol* 2007;73:7199–209.
24 García-Bocanegra I, Astorga RJ, Napp S, *et al*. Myxomatosis in wild rabbit: design of control programs in Mediterranean ecosystems. *Prev Vet Med* 2010;93:42–50.
25 Rouco C, Abrantes J, Serronha A, *et al*. Epidemiology of RHDV2 (Lagovirus europaeus/GI.2) in free-living wild European rabbits in Portugal. *Transbound Emerg Dis* 2018;65:e373–82.
26 Ross J, Tittensor AM. The establishment and spread of myxomatosis and its effect on rabbit populations. *Philos Trans R Soc Lond B Biol Sci* 1986;314:599–606.
27 Simón MC, Ortega C, Maynar P, *et al*. Studies in wild rabbit (Oryctolagus cuniculus) populations in Navarra (Spain). II. epidemiology of myxomatosis. *Gib Faun Sauv Gam* 1998;15:103–21.
28 Fenner F, Day MF, Woodroofe GM. Epidemiological consequences of the mechanical transmission of myxomatosis by mosquitoes. *J Hyg* 1956;54:284–303.
29 Flowerdew JR, Trout RC, Ross J. Myxomatosis: population dynamics of rabbits (Oryctolagus cuniculus Linnaeus, 1758) and ecological effects in the United Kingdom. *Rev Sci Tech* 1992;11:1109–13.
30 Lenghaus C, Westbury H, Collins B, *et al*. Overview of the RHD project in Australia. In: *Rabbit haemorrhagic disease: issues for assessment for biological control (R.K. Munro and R.T. Williams*. Canberra: Australian Government Printing Service, 1994: 104–29.
31 Asgari S, Hardy JR, Sinclair RG, *et al*. Field evidence for mechanical transmission of rabbit haemorrhagic disease virus (RHDV) by flies (Diptera:Calliphoridae) among wild rabbits in Australia. *Virus Res* 1998;54:123–32.
32 Cooke BD. Rabbit haemorrhagic disease: field epidemiology and the management of wild rabbit populations. *Rev Sci Tech* 2002;21:347–58.
33 McColl KA, Merchant JC, Hardy J, *et al*. Evidence for insect transmission of rabbit haemorrhagic disease virus. *Epidemiol Infect* 2002;129:655–63.
34 Marlier D, Mainil J, Linde A, *et al*. Infectious agents associated with rabbit pneumonia: isolation of amyxomatous myxoma virus strains. *Vet J* 2000;159:171–8.
35 Duarte M, Carvalho CL, Santos FA, *et al*. *"+Coelho: Avaliação Ecossanitária das Populações Naturais de Coelho Bravo Visando o Controlo da Doença Hemorrágica Viral". Relatório de atividades na época venatória 2017-2018*. INIAV I.P., DGAV, CIBIO, ANPC, CNCP, FENCAÇA. Fundo Florestal Permanente. ICNF., 2018.
36 Abade dos Santos FA, Monteiro M, Pinto A, *et al*. First description of a herpesvirus infection in genus *Lepus*. *BioRxiv.* 2020 https://doi.org/
37 Zicola A, Saegerman C, Quatpers D, *et al*. Feline herpesvirus 1 and feline calicivirus infections in a heterogeneous cat population of a rescue shelter. *J Feline Med Surg* 2009;11:1023–7.
38 Kumar N, Barua S, Riyesh T, *et al*. Complexities in isolation and purification of multiple viruses from mixed viral infections: viral interference, persistence and exclusion. *PLoS One* 2016;11:e0156110.
39 Kumar N, Sharma S, Barua S, *et al*. Virological and immunological outcomes of coinfections. *Clin Microbiol Rev* 2018;31:e00111–7.