



Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump?

Pedro José Esteves, Joana Abrantes, Stéphane Bertagnoli, Patrizia Cavadini, Dolores Gavier-Widén, Jean-Sébastien Guitton, Antonio Lavazza, Evelyne Lemaitre, Jérôme Letty, Ana Margarida Lopes, et al.

► To cite this version:

Pedro José Esteves, Joana Abrantes, Stéphane Bertagnoli, Patrizia Cavadini, Dolores Gavier-Widén, et al.. Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump?. PLoS Pathogens, 2015, 11 (11), pp.e1005087. 10.1371/journal.ppat.1005087 . inserm-01243248

HAL Id: inserm-01243248

<https://inserm.hal.science/inserm-01243248>

Submitted on 14 Dec 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

OPINION

Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump?

Pedro José Esteves^{1,2,3*}, Joana Abrantes¹, Stéphane Bertagnoli^{4,5}, Patrizia Cavadini⁶, Dolores Gavier-Widén⁷, Jean-Sébastien Guitton⁸, Antonio Lavazza⁹, Evelyne Lemaitre^{10,11}, Jérôme Letty⁸, Ana Margarida Lopes^{1,2}, Aleksija S. Neimanis⁷, Nathalie Ruvoën-Clouet¹², Jacques Le Pendu¹², Stéphane Marchandeau⁸, Ghislaine Le Gall-Reculé^{10,11}



OPEN ACCESS

Citation: Esteves PJ, Abrantes J, Bertagnoli S, Cavadini P, Gavier-Widén D, Guitton J-S, et al. (2015) Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump? PLoS Pathog 11(11): e1005087. doi:10.1371/journal.ppat.1005087

Editor: Glenn F Rall, The Fox Chase Cancer Center, UNITED STATES

Published: November 5, 2015

Copyright: © 2015 Esteves et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors are grateful to the ERA-Net anihwa (Animal Health and Welfare), a Coordination Action funded under the European Commission's ERA-Net scheme within the Seventh Framework Programme (Contract No. 291815), for having retained the project ECALEP to come as part of the 2nd join call involving 15 European countries for the next three years. The ECALEP project is funded by the ANR (France, contracts ANR-14-ANWA-0004-01, ANR-14-ANWA-0004-02, ANR-14-ANWA-0004-03, ANR-14-ANWA-0004-04), the Ministry of Health, Dep. for Veterinary Public Health, Nutrition & Food Safety (Italy) and the Research council FORMAS (Sweden, contract FORMAS 221-2014-1841). The funders had no role in study design, data collection

1 InBIO—Research Network in Biodiversity and Evolutionary Biology, CIBIO, Campus de Vairão, Universidade do Porto, Vairão, Portugal, **2** Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal, **3** CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra, Portugal, **4** UMR 1225, INRA, Toulouse, France, **5** INP-ENVIT, University of Toulouse, Toulouse, France, **6** Proteomic Unit, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy, **7** Department of Pathology and Wildlife Diseases, National Veterinary Institute, Uppsala, Sweden, **8** Department of Studies and Research, National Hunting and Wildlife Agency (ONCFS), Nantes, France, **9** Virology Unit, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy, **10** Avian and Rabbit Virology Immunology Parasitology Unit, Ploufragan-Plouzané Laboratory, French Agency for Food, Environmental and Occupational Health & Safety (Anses), Ploufragan, France, **11** European University of Brittany, Rennes, France, **12** Inserm U892; CNRS, UMR 6299 University of Nantes, Nantes France

* pjesteves@cibio.up.pt

Introduction

Emergence of pathogenic viruses is of great concern, although the underlying mechanisms for emergence remain often poorly understood. RNA viruses are frequently implicated and recent examples include viruses within *Orthomyxoviridae*, *Flaviviridae*, or *Coronaviridae* [1–3]. Within *Caliciviridae*, the *Lagovirus* genus is particularly intriguing because it has generated viruses of exceptional pathogenicity on several occasions within the past 40 years. The genus *Lagovirus* encompasses two pathogenic viruses, Rabbit hemorrhagic disease virus (RHDV) affecting European rabbit (*Oryctolagus cuniculus*), and European brown hare syndrome virus (EBHSV) affecting Brown, Mountain, and Italian hare (*Lepus europaeus*, *L. timidus*, and *L. corsicanus*) [4]. These two viruses show a similar structure and ~70% homology [5–15]. They cause two distinct diseases, RHD (rabbit hemorrhagic disease) and EBHS (European brown hare syndrome), that emerged in the late 1970s to early 1980s [16–17]. Both cause high mortalities and impose a heavy economic burden on the rabbit industry and game animal management. They have also contributed to declines of wild leporid populations throughout Europe, resulting in major ecological impact in natural ecosystems where leporids are key species [18–25].

RHD was first detected in China in 1984, apparently in rabbits imported from Germany [16], suggesting that RHDV originated in Europe. Rabbit lagoviruses consist of pathogenic viruses (RHDV) and related, but genetically divergent, nonpathogenic viruses [26–34]. Phylogenetic analyses of pathogenic RHDV strains show three distinct groups: the classic RHDV with the genogroups G1–G5 [27,35–55], the antigenic variant RHDVa/G6 [35,56–61], and

and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

RHDV2/RHDVb [62–77]. The RHDV and RHDVa are phylogenetically related and differ from RHDV2 by more than 15% in nucleotide diversity. The RHDV strains have emerged at different times: RHDV was first isolated in 1984 [16] and RHDV2 in 2010 [62]. RHDV2 was identified in France and has since spread throughout other Western European countries, replacing the circulating strains in France and the Iberian Peninsula [64,67,71,76], while in Italy, it currently cocirculates with the “original” strains [64].

EBHSV was first reported in Sweden in 1980 [17] and later found in other European countries [11,78–85]. It may have emerged earlier, as suggested by descriptions of hares with lesions consistent with the disease in 1976 in England [86]. Otherwise, antibodies against the virus have also been found in archived sera [87], and the virus was detected by RT-PCR in samples collected in Sweden before 1980 [88].

Two competing hypotheses can be put forward to explain RHDV and EBHSV origin and the emergence of RHDV2: 1) the evolution from pre-existing nonpathogenic viruses circulating in European leporids; 2) a species jump. The first hypothesis is shared by several authors and originates from the detection of anti-RHDV antibodies in rabbit blood samples collected before the first documented outbreak in Europe and Australia [89–92] and later in the characterization of different nonpathogenic viruses in European rabbits [26–34]. However, this hypothesis has not been confirmed and fails to explain the abrupt emergence of high pathogenicity on several occasions in a short period of time. Notably, the pathogenic and nonpathogenic viruses are phylogenetically separated and display ~20% of nucleotide divergence in the capsid gene [26–34], suggesting that the pathogenic forms did not directly originate from the nonpathogenic ones. Nevertheless, nonpathogenic strains have not been exhaustively characterized in European leporids, which may explain why the ancestors of pathogenic strains have not yet been found. The second hypothesis involves a species jump from species sympatric with European leporids, either native or previously introduced. Among these species, Eastern cottontail (*Sylvilagus floridanus*), a leporid native to North America, would constitute a worthwhile species. Although no data is available on the presence of original lagoviruses, they likely would have caused asymptomatic infection in its natural host, with a course of infections similar to what occurs with myxoma virus, benign in *Sylvilagus* species, but lethal in the European rabbit following a species jump [93]. Indeed, it is intriguing that both RHDV and EBHSV emerged at around the same time, coinciding with the introduction of the Eastern cottontail in Europe. Large numbers of introductions of Eastern cottontails by hunters occurred in Europe from the 1960s, but because they were illegal, these introductions are poorly documented. The first known introduction attempt from the United States was in 1966 in Italy. This was followed by massive introductions involving thousands of animals, especially in Italy and France. It is highly likely that localized introductions still occur, as suggested by the existence of cottontail breeders in France.

In the Po valley in Italy, where Eastern cottontails are invasive and widespread, a serological study showed that 18% and 33% of them carry antibodies detected by both anti-EBHSV and anti-RHDV serological tests, proving the susceptibility of the species to lagoviruses [94]. Moreover, recent documentation of RHDV strains in Iberian hares (*L. granatensis*) with lesions compatible with RHD [95], demonstration of the capacity of RHDV2 to infect Sardinian Cape and Italian hares (*L. capensis mediterraneus* and *L. corsicanus*, respectively) causing RHDV-like disease [65,70], and the experimental infection of cottontails by EBHSV [94], show the feasibility of species jumps of lagoviruses between leporid species.

We therefore suggest that European leporids carry lagoviruses of two distinct origins (Fig 1): nonpathogenic strains that have evolved with these species for a long time and a second group including pathogenic strains that possibly emerged following species jumps from *S. floridanus* and that have then evolved in European leporids. Pathogenic strains may be pure

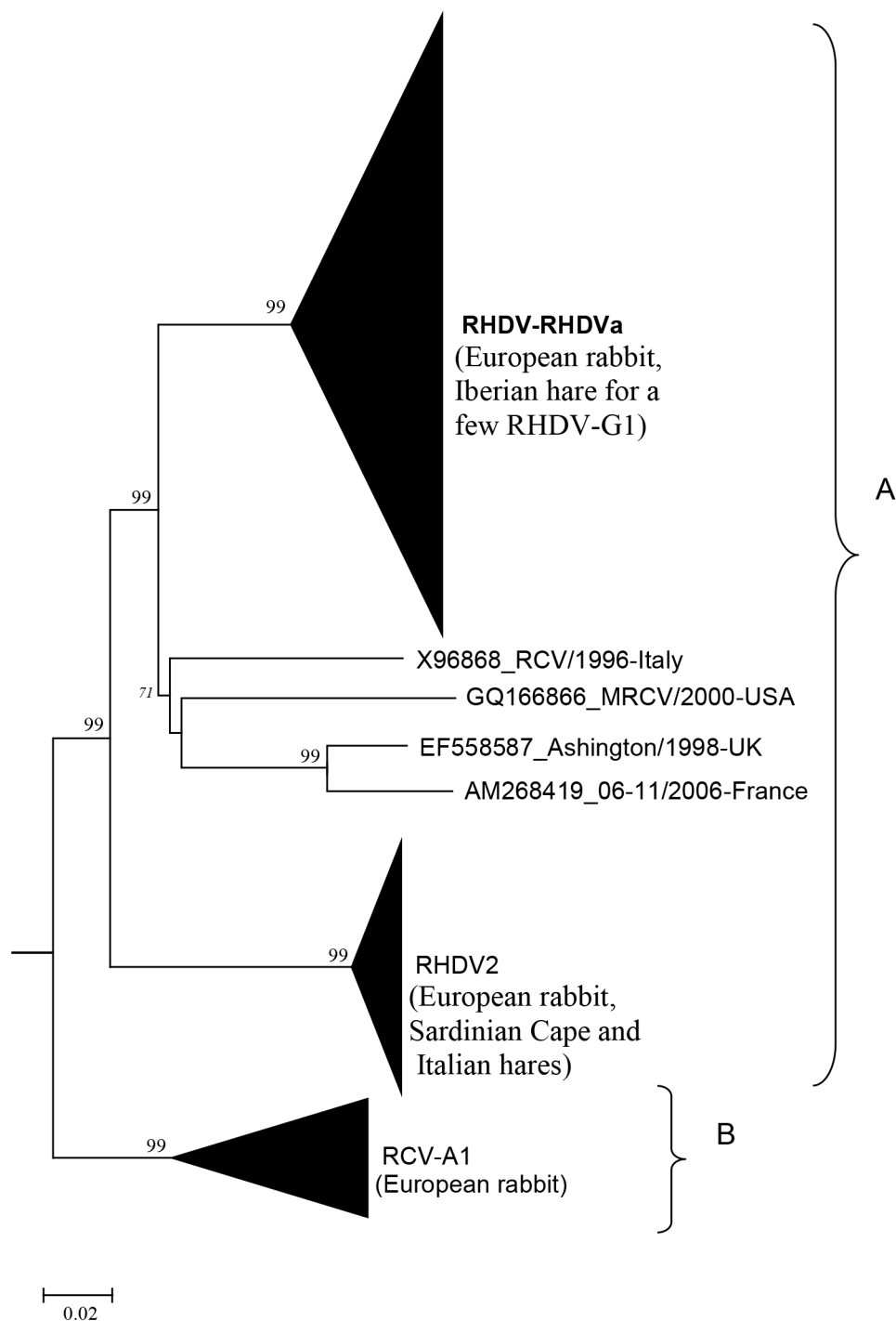


Fig 1. Possible origin of European rabbit (*O. cuniculus*) lagoviruses according to the hypothesis of a species jump. A) Lagoviruses that may share common ancestors following several species jump(s), B) Nonpathogenic viruses that have evolved in European rabbit for a long time. Phylogenetic tree (Neighbor-joining method) derived from 303 rabbit lagovirus sequences of the VP60 gene available on public databases (May 2015). The pathogenic RHDV, RHDVa, RHDV2, and the nonpathogenic RCV-A1 branches are collapsed; the name of the leporid species where these strains were isolated is given in brackets. X96868_RCV/1996-Italy, GQ166866_MRCV/2000-USA, EF558587_Ashington/1998-UK, and AM268419_06-11/2006-France are nonpathogenic strains isolated in the European rabbit. Percentage greater than 70% of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are given at major branch nodes. The EBHSV strain GD (Z69620) was used as an outgroup to root the tree. Similar clustering was observed in several recent works [63,64,66,70,74].

doi:10.1371/journal.ppat.1005087.g001

cottontail viruses or recombinants of cottontail viruses and nonpathogenic viruses of European leporid species. Indeed, recombination in RHDV is reported [96,97], and the recent documentation of recombination events between genome regions encoding the capsid and VP10 structural proteins of RHDV2 and the nonstructural proteins from nonpathogenic or pathogenic G1 lagoviruses suggests that recombination could have had an important role in the lagovirus evolution [74].

Evaluation of potential emergence of new pathogenic lagoviruses from the nonpathogenic strains circulating among native European leporid species and *Sylvilagus* species, as well as characterization of the genetic determinisms of pathogenicity, are key to identify the mechanisms of disease emergence and may help to evaluate the possibility of emergence of other highly pathogenic lagoviruses.

References

1. Bengis RG, Leighton FA, Fischer JR, Artois M, Mörner T, Tate CM. The role of wildlife in emerging and re-emerging zoonoses. *Rev Sci Tech*. 2004; 23: 497–511. PMID: [15702716](#)
2. Gautret P, Gray GC, Charrel RN, Odezulu NG, Al-Tawfiq JA, Zumla A, Memish ZA. Emerging viral respiratory tract infections—environmental risk factors and transmission. *Lancet Infect Dis*. 2014; 14: 1113–1122. doi: [10.1016/S1473-3099\(14\)70831-X](#) PMID: [25189350](#)
3. Mandl JN, Ahmed R, Barreiro LB, Daszak P, Epstein JH, Virgin HW, Feinberg MB. Reservoir host immune responses to emerging zoonotic viruses. *Cell*. 2015; 160: 20–35. doi: [10.1016/j.cell.2014.12.003](#) PMID: [25533784](#)
4. Abrantes J, van der Loo W, Le Pendu J, Esteves PJ. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. *Veterinary Research*. 2011; 43: 12.
5. Capucci L, Scicluna MT, Lavazza A. Diagnosis of viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev Sci Tech*. 1991; 10: 347–370. PMID: [1662098](#)
6. Marcato PS, Benazzi C, Vecchi G, Galeotti M, Della Salda L, Sarli G, Lucidi P. Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. *Rev Sci Tech*. 1991; 10: 371–392. PMID: [1760582](#)
7. Chasey D, Lucas M, Westcott D, Williams M. European brown hare syndrome in the U.K.; a calicivirus related to but distinct from that of viral haemorrhagic disease in rabbits. *Arch Virol*. 1992; 124: 363–370. PMID: [1318711](#)
8. Fuchs A, Weissenböck H. Comparative histopathological study of rabbit haemorrhagic disease (RHD) and European brown hare syndrome (EBHS). *J Comp Pathol* 1992; 107: 103–113. PMID: [1430343](#)
9. Wirblich C, Meyers G, Ohlinger VF, Capucci L, Eskens U, Haas B, Thiel HJ. European brown hare syndrome virus: relationship to rabbit hemorrhagic disease virus and other caliciviruses. *J Virol*. 1994; 68: 5164–5173. PMID: [7518531](#)
10. Lavazza A, Scicluna MT, Capucci L. Susceptibility of hares and rabbits to the European brown hare syndrome virus (EBHSV) and rabbit haemorrhagic disease virus (RHDV) under experimental conditions. *Zentralbl Veterinärmed B*. 1996; 43: 401–410. PMID: [8885705](#)
11. Le Gall G, Huguet S, Vende P, Vautherot J-F, Rasschaert D. European brown hare syndrome virus: molecular cloning and sequencing of the genome. *J Gen Virol*. 1996; 77: 1693–1697. PMID: [8760416](#)
12. Nowotny N, Bascunana CR, Ballagi-Pordany A, Gavier-Widen D, Uhlen M, Belak S. Phylogenetic analysis of rabbit haemorrhagic disease and European brown hare syndrome viruses by comparison of sequences from the capsid protein gene. *Arch. Virol*. 1997; 142: 657–673. PMID: [9170495](#)
13. Le Gall-Reculé G, Zwingelstein F, Laurent S, Portejoie Y, Rasschaert D. Molecular epidemiology of European brown hare syndrome virus in France between 1989 and 2003. *Arch. Virol*. 2006; 151: 1713–1721. PMID: [16596329](#)
14. Lopes AM, Gavier-Widén D, Le Gall-Reculé G, Esteves PJ, Abrantes J. Complete coding sequences of European brown hare syndrome virus (EBHSV) strains isolated in 1982 in Sweden. *Archives of Virology* 2013; 158: 2193–2196. doi: [10.1007/s00705-013-1714-7](#) PMID: [23640583](#)
15. Lopes AM, Capucci L, Gavier-Widén D, Le Gall-Reculé G, Brocchi E, Barbieri I, Quémener A, Le Pendu J, Geoghegan JL, Holmes EC, Esteves PJ, Abrantes J. Molecular evolution and antigenic variation of European brown hare syndrome virus (EBHSV). *Virology* 2014; 468–470:104–12. doi: [10.1016/j.virol.2014.08.002](#) PMID: [25155199](#)
16. Liu SJ, Xue HP, Pu BQ, Qian NH. A new viral disease in rabbit. *Anim Husb Vet Med*. 1984; 16: 253–255.

17. Gavier-Widén D, Mörner T. Epidemiology and diagnosis of the European brown hare syndrome in Scandinavian countries: a review. *Rev Sci Tech*. 1991; 10: 453–458. PMID: [1760585](#)
18. Xu WY. Viral haemorrhagic disease of rabbits in the People's Republic of China: epidemiology and virus characterisation. *Rev Sci Tech* 1991; 10: 393–408. PMID: [1760583](#)
19. Gregg DA, House C, Meyer R, Berninger M. Viral haemorrhagic disease of rabbits in Mexico: epidemiology and viral characterization. *Rev Sci Tech* 1991; 10: 435–451. PMID: [1760584](#)
20. Mitro S, Krauss H. Rabbit hemorrhagic disease: a review with special reference to its epizootiology. *Eur J Epidemiol*. 1993; 9: 70–78. PMID: [8386104](#)
21. Villafuerte R, Calvete C, Blanco JC, Lucientes J. Incidence of viral hemorrhagic disease in wild rabbit populations in Spain. *Mammalia*. 1995; 59: 651–660.
22. Marchandeau S, Chantal J, Portejoie Y, Barraud S, Chaval Y. Impact of viral haemorrhagic disease on a wild population of European rabbits in France. *Journal of Wildlife Diseases*. 1998; 34: 429–435. PMID: [9706551](#)
23. Marchandeau S, Chaval Y, Le Goff E. Prolonged decline in the abundance of wild European rabbits *Oryctolagus cuniculus* and high immunity level over three years following the arrival of rabbit haemorrhagic disease. *Wildlife Biology*. 2000; 6: 141–147.
24. Delibes-Mateos M, Delibes M, Ferreras P, Villafuerte R. Key role of European rabbits in the conservation of the Western Mediterranean basin hotspot. *Conserv Biol*. 2008; 22: 1106–17. doi: [10.1111/j.1523-1739.2008.00993.x](#) PMID: [18680504](#)
25. Delibes-Mateos M, Ferreira C, Carro F, Escudero MA, Gortázar C. Ecosystem effects of variant rabbit hemorrhagic disease virus, Iberian Peninsula. *Emerg Infect Dis*. 2014; 20: 2166–2168. doi: [10.3201/eid2012.140517](#) PMID: [25417710](#)
26. Capucci L, Fusi P, Lavazza A, Pacciarini ML, Rossi C. Detection and preliminary characterization of a new rabbit calicivirus related to rabbit hemorrhagic disease virus but nonpathogenic. *J Virol*. 1996; 70: 8614–8623. PMID: [8970986](#)
27. Moss SR, Turner SL, Trout RC, White PJ, Hudson PJ, Desai A, Armesto M, Forrester NL, Gould EA. Molecular epidemiology of Rabbit haemorrhagic disease virus. *J Gen Virol*. 2002; 83: 2461–2467. PMID: [12237428](#)
28. Forrester NL, Trout RC, Gould EA. Benign circulation of rabbit haemorrhagic disease virus on Lambay Island, Eire. *Virology*. 2007; 358: 18–22. PMID: [17049958](#)
29. Forrester NL, Boag B, Buckley A, Moureau G, Gould EA. Co-circulation of widely disparate strains of Rabbit haemorrhagic disease virus could explain localized epidemicity in the United Kingdom. *Virology*. 2009; 393: 42–48. doi: [10.1016/j.virol.2009.07.008](#) PMID: [19692104](#)
30. Strive T, Wright JD, Robinson AJ. Identification and partial characterization of a new Lagovirus in Australian wild rabbits. *Virology*. 2009; 384: 97–105. doi: [10.1016/j.virol.2008.11.004](#) PMID: [19049842](#)
31. Bergin IL, Wise AG, Bolin SR, Mullaney TP, Kiupel M, Maes RK. Novel calicivirus identified in rabbits, Michigan, USA. *Emerg. Infect. Dis*. 2009; 15: 1955–1962. doi: [10.3201/eid1512.090839](#) PMID: [19961675](#)
32. Abrantes J, Esteves PJ. Not-so-novel Michigan rabbit calicivirus. *Emerg Infect Dis*. 2010; 16:1331–1332. doi: [10.3201/eid1608.091803](#) PMID: [20678344](#)
33. Jahnke M, Holmes EC, Kerr PJ, Wright JD, Strive T. Evolution and phylogeography of the nonpathogenic calicivirus RCV-A1 in wild rabbits in Australia. 2010; 84: 12397–404.
34. Le Gall-Recule G, Zwingelstein F, Fages MP, Bertagnoli S, Gelfi J, Aubineau J, Roobrouck A, Botti G, Lavazza A, Marchandeau S. Characterisation of a non-pathogenic and non-protective infectious rabbit lagovirus related to RHDV. *Virology*. 2011; 410: 395–402. doi: [10.1016/j.virol.2010.12.001](#) PMID: [21195443](#)
35. Le Gall-Recule G, Zwingelstein F, Laurent S, de Boisseson C, Portejoie Y, Rasschaert D. Phylogenetic analysis of rabbit haemorrhagic disease virus in France between 1993 and 2000, and the characterisation of RHDV antigenic variants. *Arch Virol*; 2003; 148: 65–81. PMID: [12536296](#)
36. Gould AR, Kattenbelt JA, Lenghaus C, Morrissy C, Chamberlain T, Collins BJ, Westbury HA. The complete nucleotide sequence of rabbit haemorrhagic disease virus (Czech strain V351): use of the polymerase chain reaction to detect replication in Australian vertebrates and analysis of viral population sequence variation. *Virus Res*. 1997; 47: 7–17. PMID: [9037732](#)
37. Muller A, Freitas J, Silva E, Le Gall-Recule G, Zwingelstein F, Abrantes J, Esteves PJ, Alves PC, van der Loo W, Kolodziejek J, Nowotny N, Thompson G. Evolution of rabbit haemorrhagic disease virus (RHDV) in the European rabbit (*Oryctolagus cuniculus*) from the Iberian Peninsula. *Vet Microbiol*. 2009; 135: 368–373. doi: [10.1016/j.vetmic.2008.09.057](#) PMID: [18977620](#)
38. Milton ID, Vlasak R, Nowotny N, Rodak L, Carter MJ. Genomic 3' terminalsequence comparison of three isolates of rabbit haemorrhagic disease virus. *FEMS Microbiol Lett*. 1992; 72: 37–42. PMID: [1497750](#)

39. Boga JA, Casais R, Marin MS, Martin-Alonso JM, Carmenes RS, Prieto M, Parra F. Molecular cloning, sequencing and expression in *Escherichia coli* of the capsid protein gene from rabbit haemorrhagic disease virus (Spanish isolate AST/89). *J Gen Virol*. 1994; 75: 2409–2413. PMID: [8077941](#)
40. Parra F, Boga JA, Marin MS, Casais R. The amino terminal sequence of VP60 from rabbit hemorrhagic disease virus supports its putative subgenomic origin. *Virus Res*. 1993; 27: 219–228. PMID: [8488721](#)
41. Le Gall G, Arnauld C, Boilletot E, Morisse JP, Rasschaert D. Molecular epidemiology of rabbit haemorrhagic disease virus outbreaks in France during 1988 to 1995. *J Gen Virol*. 1998; 79: 11–16. PMID: [9460916](#)
42. Matiz K, Ursu K, Kecskemeti S, Bajmocy E, Kiss I. Phylogenetic analysis of rabbit haemorrhagic disease virus (RHDV) strains isolated between 1988 and 2003 in eastern Hungary. *Arch. Virol*. 2006; 151: 1659–1666. PMID: [16521047](#)
43. Tian L, Liao J, Li JW, Zhou WR, Zhang XL, Wang HN. Isolation and identification of a non-haemagglutinating strain of rabbit hemorrhagic disease virus from China and sequence analysis for the VP60 Gene. *Virus Genes*. 2007; 35: 745–752. PMID: [17705093](#)
44. Guittre C, Baginski I, Le Gall G, Prave M, Trepo C, Cova L. Detection of rabbit haemorrhagic disease virus isolates and sequence comparison of the N-terminus of the capsid protein gene by the polymerase chain reaction. *Res Vet Sci*. 1995; 58: 128–132. PMID: [7761690](#)
45. Rasschaert D, Huguet S, Madelaine MF, Vautherot JF. Sequence and genomic organization of a rabbit hemorrhagic disease virus isolated from a wild rabbit. *Virus Genes*. 1995; 9: 121–132. PMID: [7732658](#)
46. Viaplana E, Villaverde A. Microheterogeneity of p60 capsid protein and the encoding gene among contemporary isolates of rabbit hemorrhagic disease virus. *Virus Genes*. 1996; 12: 189–192. PMID: [8879136](#)
47. Asgari S, Hardy JR, Cooke BD. Sequence analysis of rabbit haemorrhagic disease virus (RHDV) in Australia: alterations after its release. *Arch. Virol*. 1999; 144: 135–145. PMID: [10076514](#)
48. van de Bildt MW, van Bolhuis GH, van Zijderveld F, van Riel D, Drees JM, Osterhaus AD, Kuiken T. Confirmation and phylogenetic analysis of rabbit hemorrhagic disease virus in free-living rabbits from the Netherlands. *J Wildl Dis*. 2006; 42: 808–812. PMID: [17255447](#)
49. Yang L, Wang F, Hu B, Xue J, Hu Y, Zhou B, Wang D, Xu W. Development of an RT-PCR for rabbit hemorrhagic disease virus (RHDV) and the epidemiology of RHDV in three eastern provinces of China. *J. Virol. Methods* 2008; 151: 24–29. doi: [10.1016/j.jviromet.2008.04.003](#) PMID: [18499276](#)
50. Hukowska-Szematowicz B, Pawlikowska M, Deptula W. Genetic variability of Czech and German RHD virus strains. *Pol. J. Microbiol*. 2009; 58: 237–245. PMID: [19899617](#)
51. Kerr PJ, Kitchen A, Holmes EC. Origin and phylodynamics of rabbit hemorrhagic disease virus. *J Virol*. 2009; 83: 12129–12138. doi: [10.1128/JVI.01523-09](#) PMID: [19759153](#)
52. Alda F, Gaitero T, Suárez M, Merchán T, Rocha G, Doadrio I. Evolutionary history and molecular epidemiology of rabbit haemorrhagic disease virus in the Iberian Peninsula and Western Europe. *BMC Evol Biol*. 2010; 10: 347. doi: [10.1186/1471-2148-10-347](#) PMID: [21067589](#)
53. Abrantes J, Lopes AM, Esteves PJ. Complete genomic sequences of rabbit hemorrhagic disease virus G1 strains isolated in the European rabbit original range. *J Virol*. 2012; 86: 13886. doi: [10.1128/JVI.02683-12](#) PMID: [23166278](#)
54. Esteves PJ, Lopes AM, Magalhães MJ, Pinheiro A, Gonçalves D, Abrantes J. Rabbit hemorrhagic disease virus detected in Pico, Azores, Portugal, revealed a unique endemic strain with more than 17 years of independent evolution. *Viruses*. 2014; 6: 2698–2707. doi: [10.3390/v6072698](#) PMID: [25025834](#)
55. Kovaliski J, Sinclair R, Mutze G, Peacock D, Strive T, Abrantes J, Esteves PJ, Holmes EC. Molecular epidemiology of Rabbit Haemorrhagic Disease Virus in Australia: when one became many. *Mol Ecol*. 2014; 23: 408–20 doi: [10.1111/mec.12596](#) PMID: [24251353](#)
56. Capucci L, Fallacara F, Grazioli S, Lavazza A, Pacciarini ML, Brocchi E. A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. *Virus Res*. 1998; 58: 115–126. PMID: [9879768](#)
57. Farnos O, Rodríguez D, Valdes O, Chiong M, Parra F, Toledo JR, Fernandez E, Leonart R, Suarez M. Molecular and antigenic characterization of rabbit hemorrhagic disease virus isolated in Cuba indicates a distinct antigenic subtype. *Arch Virol*. 2007; 152: 1215–1221. PMID: [17334949](#)
58. Schirmer H, Reimann I, Kollner B, Granzow H. Pathogenic, antigenic and molecular properties of rabbit haemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterization of antigenic variants. *Arch Virol*. 1999; 144: 719–735. PMID: [10365163](#)
59. McIntosh MT, Behan SC, Mohamed FM, Lu Z, Moran KE, Burrage TG, Neilan JG, Ward GB, Botti G, Capucci L, Metwally SA. A pandemic strain of calicivirus threatens rabbit industries in the Americas. *Virol J*. 2007; 4: 96. PMID: [17910765](#)

60. Oem JK, Lee KN, Roh IS, Lee KK, Kim SH, Kim HR, Park CK, Joo YS. Identification and characterization of rabbit hemorrhagic disease virus genetic variants isolated in Korea. *J Vet Med Sci* 2009; 71: 1519–1523. PMID: [19959905](#)
61. Abrantes J, Lopes AM, Dalton KP, Parra F, Esteves PJ. Detection of RHDVa on the Iberian Peninsula: isolation of an RHDVa strain from a Spanish rabbitry. *Arch Virol*. 2014; 159: 321–326. doi: [10.1007/s00705-013-1808-2](#) PMID: [23942953](#)
62. Le Gall-Reculé G, Zwingelstein F, Boucher S, Le Normand B, Plassiart G, Portejoie Y, Decors A, Bertagnoli S, Guérin JL, Marchandeau S. Detection of a new variant of rabbit haemorrhagic disease virus in France. *Vet Rec*. 2011; 168: 137–138.
63. Dalton KP, Nicieza I, Balseiro A, Muguerza MA, Rosell JM, Casais R, Álvarez ÁL, Parra F. Variant rabbit hemorrhagic disease virus in young rabbits, Spain. *Emerg Infect Dis*. 2012; 18: 2009–2012. doi: [10.3201/eid1812.120341](#) PMID: [23171812](#)
64. Le Gall-Reculé G, Lavazza A, Marchandeau S, Bertagnoli S, Zwingelstein F, Cavadini P, Martinelli N, Lombardi G, Guérin JL, Lemaitre E, Decors A, Boucher S, Le Normand B, Capucci L. Emergence of a new lagovirus related to Rabbit Haemorrhagic Disease Virus. *Vet Res*. 2013; 44: 81. doi: [10.1186/1297-9716-44-81](#) PMID: [24011218](#)
65. Puggioni G, Cavadini P, Maestrale C, Scivoli R, Botti G, Ligios C, Le Gall-Reculé G, Lavazza A, Capucci L. The new French 2010 Rabbit Hemorrhagic Disease Virus causes an RHD-like disease in the Sardinian Cape hare (*Lepus capensis mediterraneus*). *Vet Res*. 2013; 44: 96. doi: [10.1186/1297-9716-44-96](#) PMID: [24099575](#)
66. Abrantes J, Lopes AM, Dalton KP, Melo P, Correia JJ, Ramada M, Alves PC, Parra F, Esteves PJ. New variant of rabbit hemorrhagic disease virus, Portugal, 2012–2013. *Emerg Infect Dis*. 2013; 19: 1900–1902. doi: [10.3201/eid1911.130908](#) PMID: [24206671](#)
67. Dalton KP, Nicieza I, Abrantes J, Esteves PJ, Parra F. Spread of new variant RHDV in domestic rabbits on the Iberian Peninsula. *Vet Microbiol*. 2014; 169: 67–73. doi: [10.1016/j.vetmic.2013.12.015](#) PMID: [24461551](#)
68. Baily JL, Dagleish MP, Graham M, Maley M, Rocchi MS. RHDV variant 2 presence detected in Scotland. *Vet Rec*. 2014; 174: 411.
69. Simpson V, Everest D, Westcott D. RHDV variant 2 and *Capillaria hepatica* infection in rabbits. *Vet Rec*. 2014; 174: 486.
70. Camarda A, Pugliese N, Cavadini P, Circella E, Capucci L, Caroli A, Legretto M, Mallia E, Lavazza A. Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in Sicily from rabbit (*Oryctolagus cuniculus*) and Italian hare (*Lepus corsicanus*). *Res Vet Sci*. 2014; 97: 642–645. doi: [10.1016/j.rvsc.2014.10.008](#) PMID: [25458493](#)
71. Lopes AM, Correia J, Abrantes J, Melo P, Ramada M, Magalhães MJ, Alves PC, Esteves PJ. Is the new variant RHDV replacing genogroup 1 in Portuguese wild rabbit populations? *Viruses*. 2014; 7: 27–36. doi: [10.3390/v7010027](#) PMID: [25559218](#)
72. Dalton KP, Abrantes J, Lopes AM, Nicieza I, Álvarez ÁL, Esteves PJ, Parra F. Complete genome sequence of two rabbit hemorrhagic disease virus variant b isolates detected on the Iberian Peninsula. *Arch. Virol*. 2015; 160:877–881. doi: [10.1007/s00705-014-2329-3](#) PMID: [25577166](#)
73. Westcott DG, Choudhury B. Rabbit haemorrhagic disease virus 2-like variant in Great Britain. *Vet Rec*. 2015; 176: 74. doi: [10.1136/vr.102830](#) PMID: [25477331](#)
74. Lopes AM, Dalton KP, Magalhães MJ, Parra F, Esteves PJ, Holmes EC, Abrantes J. Full genomic analysis of new variant Rabbit Hemorrhagic Disease Virus (RHDVb) revealed multiple recombination events. *J Gen Virol*. 2015; 96: 1309–1319. doi: [10.1099/vir.0.000070](#) PMID: [25626685](#)
75. Duarte M, Henriques M, Barros SC, Fagulha T, Ramos F, Luís T, Fevereiro M, Benevides S, Flor L, Barros SV, Bernardo S. Detection of RHDV variant 2 in the Azores. *Vet Rec*. 2015; 176: 130. doi: [10.1136/vr.h497](#) PMID: [25634926](#)
76. Calvete C, Sarto P, Calvo AJ, Monroy F, Calvo JH. Could the new Rabbit haemorrhagic disease virus variant (RHDVb) be fully replacing classical RHD strains in the Iberian Peninsula? *World Rabbit Sci*. 2014; 22: 91.
77. Almeida T, Lopes AM, Magalhães MJ, Neves F, Pinheiro A, Gonçalves D, Leitão M, Esteves PJ, Abrantes J. Tracking the evolution of the G1/RHDVb recombinant strains introduced from the Iberian Peninsula to the Azores islands, Portugal. *Infection, Genetics and Evolution* 2015; 34: 307–313. doi: [10.1016/j.meegid.2015.07.010](#) PMID: [26165506](#)
78. Poli A, Nigro M, Gallazzi D, Siroli G, Lavazza A, Gelmetti D. Acute hepatitis in the European Brown Hare (*Lepus europaeus*) in Italy. *J Wild Dis*. 1991; 27: 621–629.
79. Zanni ML, Benassi ML, Scicluna MT, Lavazza A, Capucci L. Clinical evolution and diagnosis of an episode of European Brown Hare Syndrome (EBHS) in hares reared in captivity. *Rev. sci. tech. Off. int. Epiz*. 1993; 12: 931–940.

80. Scicluna MT, Capucci L, Lavazza A. European brown hare syndrome in northern Italy: results of a virological and serological survey. *Rev. sci. tech. Off. int. Epiz.* 1994; 13: 893–904.
81. Billinis C, Psychas V, Tontis DK, Spyrou V, Birtsas PK, Sofia M, Likotrafitis F, Maslarinou OM, Kanteres D. European brown hare syndrome in wild European brown hares from Greece. *J. Wildl. Dis.* 2005; 41: 783–786. PMID: [16456168](#)
82. Chasey D, Duff P. European brown hare syndrome and associated virus particles in the UK. *Vet. Rec.* 1990; 126: 623–624. PMID: [2165701](#)
83. Frölich K, Fickel J, Ludwig A, Lieckfeldt D, Streich WJ, Jurčík R, Slamečka J, Wibbelt G. New variants of European brown hare syndrome virus strains in free ranging European brown hares (*Lepus europaeus*) from Slovakia. *J. Wildl. Dis.* 2007; 43: 89–96. PMID: [17347397](#)
84. Frölich K, Meyer HHD, Pielowski Z, Ronsholt L, Seck-Lanzendorf Sv, Stolte M. European brown hare syndrome in free-ranging hares in Poland. *J. Wildl. Dis.* 1996; 32: 280–285. PMID: [8722266](#)
85. Lavazza A, Vecchi G. Osservazioni su alcuni episodi di mortalità nelle lepri. Evidenziazione al microscopio elettronico di una particella virale. Nota preliminare. *Selezione Veterinaria*. 1989; 30: 461–467.
86. Duff JP, Chasey D, Munro R, Wooldridge M. European brown hare syndrome in England. *Vet. Rec.* 1994; 134: 669–673. PMID: [7941275](#)
87. Duff JP, Whitwell K, Chasey D. The emergence and epidemiology of European brown hare syndrome in the UK, Proceedings of the First International Symposium on Caliciviruses, United Kingdom, 1997; pp. 176–181.
88. Ros Bascuñana C, Nowotny N, Belák S. Detection and differentiation of rabbit hemorrhagic disease and European brown hare syndrome viruses by amplification of VP60 genomic sequences from fresh and fixed tissue specimens. *J. Clin. Microbiol.* 1997; 35: 2492–2495. PMID: [9316895](#)
89. Rodak L, Smid B, Valicek L, Vesely T, Stepanek J, Hampl J, Jurak E. Enzyme-linked immunosorbent assay of antibodies to rabbit haemorrhagic disease virus and determination of its major structural proteins. *J. Gen. Virol.* 1990; 71: 1075–1080. PMID: [2161044](#)
90. O'Keefe JS, Tempero JE, Motha MXJ, Hansen MF, Atkinson PH. Serology of rabbit haemorrhagic disease virus in wild rabbits before and after release of the virus in New Zealand. *Vet Microbiol.* 1999; 66: 29–40. PMID: [10223320](#)
91. Cooke BD, Robinson AJ, Merchant JC, Nardin A, Capucci L. Use of ELISAs in field studies of rabbit haemorrhagic disease (RHD) in Australia. *Epidemiol Infect.* 2000; 124: 563–576. PMID: [10982081](#)
92. Robinson AJ, Kirkland PD, Forrester RI, Capucci L, Cooke BD, Philbey AW. Serological evidence for the presence of a calicivirus in Australian wild rabbits, *Oryctolagus cuniculus*, before the introduction of rabbit haemorrhagic disease virus (RHDV): its potential influence on the specificity of a competitive ELISA for RHDV. *Wildl Res.* 2002; 29: 655–662.
93. Fenner F, Ratcliffe FN. *Myxomatosis*; Cambridge University Press. 1965; Cambridge, UK.
94. Lavazza A, Cavadini P, Barbieri I, Tizzani P, Pinheiro A, Abrantes J, Esteves PJ, Grilli G, Gioia E, Zanoni M, Meneguz P, Guitton JS, Marchandeau S, Chiari M, Capucci L. Field and experimental data indicate that the eastern cottontail (*Sylvilagus floridanus*) is susceptible to infection with European brown hare syndrome (EBHS) virus and not with rabbit haemorrhagic disease (RHD) virus. *Vet Res.* 2015; 46: 13. doi: [10.1186/s13567-015-0149-4](#) PMID: [25828691](#)
95. Lopes AM, Marques S, Silva E, Magalhaes MJ, Pinheiro A, Alves PC, Le Pendu J, Esteves PJ, Thompson G, Abrantes J. Detection of RHDV strains in the Iberian hare (*Lepus granatensis*): earliest evidence of rabbit lagovirus cross-species infection. *Vet Res.* 2014; 45: 94. doi: [10.1186/s13567-014-0094-7](#) PMID: [25248407](#)
96. Abrantes J, Esteves PJ, van der Loo W. Evidence for recombination in the major capsid gene VP60 of the rabbit haemorrhagic disease virus (RHDV). *Archives of Virology* 2008; 153: 329–335. doi: [10.1007/s00705-007-1084-0](#) PMID: [18193156](#)
97. Forrester NL, Moss SR, Turner SL, Schirrmeyer H, Gould EA. Recombination in Rabbit haemorrhagic disease virus: possible impact on evolution and epidemiology. *Virology* 2008; 376: 390–396. doi: [10.1016/j.virol.2008.03.023](#) PMID: [18455748](#)