New Variant of Rabbit Hemorrhagic Disease Virus, Portugal, 2012–2013

To the Editor: During November 2012–February 2013, rabbit hemorrhagic disease virus (RHDV) strains belonging to the new variant RHDV were isolated in Portugal from wild European rabbits (Oryctolagus cuniculus) as part of a surveillance program. Before 2011, RHDV outbreaks in wild European rabbit (O. cuniculus) populations in the Iberian Peninsula were exclusively caused by strains belonging to genogroup 1. RHDV had been previously detected in Portugal in 1989 (1). Before 2011, RHDV outbreaks in wild European rabbits were associated with the H5N1 virus in China, and a recent report on a novel strain of avian influenza A virus in China (2) raised concern that RHDVs could be susceptible to H5N1 infection on the European mainland. A phylogenetic comparison of the new variant of RHDV to different RHDV subgroups and nonpathogenic RHDV-related strains may help to better understand the potential risk of new RHDV strains or other avian-origin influenza A virus strains in Europe.

In the Iberian Peninsula, 2 subspecies of European rabbits are found, O. cuniculus subsp. cuniculus and O. cuniculus subsp. algirus. These subspecies are equally susceptible to RHDV (3). In 2011, a new variant was isolated in young rabbits belonging to O. cuniculus subsp. cuniculus from a rabbitry in the province of Navarra, Spain (4). The topology of the phylogenetic tree that included this variant and the susceptibility of kits <2 months old suggest that this strain is similar to that described in France in 2010 (5).

Before the new variant of RHDV emerged and on the basis of phylogenetic relationships, RHDV strains had been divided into 6 genogroups (G1–G6) (6), with strains of G6, or RHD-Va, having a distinct antigenic profile (6). All of these strains replicate in the liver and are responsible for causing death in rabbits >2 months of age. Nonpathogenic and weakly pathogenic RHDV-related strains have also been described. The nonpathogenic and weakly pathogenic strains are phylogenetically distinct from the G1–G6 strains with ≈20% of nucleotide divergence (7); they typically replicate in the intestines (8,9). New variant RHDV causes death in kits as young as 30 days old and affects vaccinated and unvaccinated animals (4). Phylogenetically, this new variant falls between the nonpathogenic groups (4,5).

During November 2012–February 2013, our laboratory, CIBIO, Universidade do Porto, Portugal, received liver samples from wild adult rabbits and kits, belonging to O. cuniculus subsp. algirus, from 3 areas of Portugal, Valpaços, Barrancos, and Algarve. The rabbits had appeared dead and had clinical signs suggesting rabbit hemorrhagic disease (RHD). We analyzed the samples for RHDV by reverse transcription PCR. For this process, total RNA was extracted by using the RNeasy Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer’s instructions. Reverse transcription was performed by using oligo(dT) as primer (Invitrogen, Carlsbad, CA, USA) and SuperScript III reverse transcriptase (Invitrogen) as recommended by the manufacturer. Screening of the samples consisted of PCR with a pair of primers as described by Dalton et al. (4). This pair amplifies a 738-bp fragment of the gene encoding the capsid protein, VP60 (PCR conditions are available on request). After purification, PCR products were sequenced on an automatic sequencer ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) with the same pair of primers. The virus was detected in 15 samples, 5 from each locality. The obtained sequences were aligned with those available from public databases. Retrieved sequences represent the RHDV groups G1–G6, the nonpathogenic groups, and the new variant (GenBank accession nos. KF442960–KF442964). A phylogenetic tree was inferred in MEGA5 (10) by using a maximum-likelihood (ML) approach. Reliability of the nodes was assessed with a bootstrap resampling procedure consisting of 500 replicates of the ML trees. The best-fit nucleotide substitution model was determined by using MEGA5.

Address for correspondence: Cheng-Yi Li, Institute of Disease Control and Prevention, 20 Dong-Da St, Fengtai District, Beijing 100071, People’s Republic of China; email: licy_60@163.com

References

1. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med. 2013;368:1888–97. http://dx.doi.org/10.1056/NEJMoa1304459
2. Lam WY, Yeung AC, Ngai KL, Li MS, To KF, Tai SK, et al. Effect of avian influenza A H5N1 infection on the expression of microRNA-141 in human respiratory epithelial cells. BMC Microbiol. 2013;13:104. http://dx.doi.org/10.1186/1471-2180-13-104
3. Zhang W, Wang L, Hu W, Ding F, Sun H, Li S, et al. Epidemiological characteristics of cases for influenza A (H7N9) virus infections in China. Clin Infect Dis. 2013;57:619–20. http://dx.doi.org/10.1093/cid/cit277
4. Cowling BJ, Jin L, Lau EH, Liao Q, Wu P, Jiang H, et al. Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: a population-based study of laboratory-confirmed cases. Lancet. 2013;382:129–37. http://dx.doi.org/10.1016/S0140-6736(13)61717-X
5. World Health Organization. WHO case definitions for human infections with influenza A(H5N1) virus. 2006 [cited 2013 May 10]. http://www.who.int/influenza/resources/documents/
6. Kulldorf M. SaTScan user guide for version 9.0. 2010 [cited 2013 May 10]. http://www.satscan.org
7. Olsen SJ, Unghesuak K, Sovann L, Uyeki TM, Dowell SF, Cox NJ, et al. Family clustering of avian influenza A (H5N1). Emerg Infect Dis. 2005;11:1799–801. http://dx.doi.org/10.3201/eid1111.050646
Our sequences exhibit the highest nucleotide sequence identity with the RHDV N11 strain from Spain (99%; GenBank accession no. JX133161.1), which corresponds to the new RHDV variant. Thirteen nucleotide substitutions were detected in comparison to the Spanish sequence, 3 of which were nonsynonymous. The inferred ML phylogenetic tree is in agreement with those published (1,3,9). G1–G6 (pathogenic) RHDV strains and nonpathogenic and weakly pathogenic RHDV-related strains (generally referred to as RCV) form 2 groups (Figure). The nonpathogenic strain from Australia (RCV_A1_Australia_MIC-07) does not cluster with other nonpathogenic groups and European brown hare syndrome virus (EBHSV_France) appears in a basal position in the tree. As described, the new variant (N11_Spain) appears between RCV and the nonpathogenic Australian strain (4,5). The strains isolated from rabbits in Portugal cluster with the new variant and form a highly supported group (bootstrap value 1.00). These results support the conclusion that the virus recovered in Portugal belongs to the new variant RHDV described in Spain and France.

This confirms the presence of the virus in wild rabbits on the Iberian Peninsula. We also confirm that both European rabbit subspecies are susceptible to the new variant. The appearance and rapid spread of the new variant RHDV into the Iberian wild rabbit populations raise concern for the survival of these populations in this region. These conservation concerns are particular highlighted for the O. cuniculus subsp. algirus, because it only occurs in the southwestern part of the Iberian Peninsula, and it is a key prey species for several carnivores, namely, for the most endangered feline, the Iberian Lynx (Lynx pardinus). Therefore, monitoring the spread and evolution of this new variant is crucial in determining the most appropriate conservation measures.

Acknowledgments

The samples from Valpaços and Algarve were provided by Jorge Pires and Vitor Palmilha, respectively.

The Portuguese Foundation for Science and Technology supported the doctoral fellowship of A.M.L. (SFRH/BD/78738/2011) and the postdoctoral fellowship of J.A. (SFRH/BPD/73512/2010). The Portuguese Foundation for Science and Technology Projects PTDC/CVT/108490/2008 and FCT-ANR/BIA-BIC/0043/2012 supported this work. Also, project “Genomics Applied to Genetic Resources,” cofinanced by North Portugal Regional Operational Programme 2007/2013 (ON.2–O Novo Norte), under the National Strategic Reference Framework, through the European Regional Development Fund, supported this work. F.P. and K.P.D. gratefully acknowledge the financial support of Organización Interprofesional Cunicola.
Joana Abrantes, Ana M. Lopes, Kevin P. Dalton, Pedro Melo, Jorge J. Correia, Margarida Ramada, Paulo C. Alves, Francisco Parra, and Pedro J. Esteves

Author affiliations: Centro de Investigação em Biodiversidade e Recursos Genéticos/InBIO Laboratório Associado—Universidade do Porto (CIBIO/UP), Vairão, Portugal (J. Abrantes, A.M. Lopes, P.C. Alves, P.J. Esteves); Université de Nantes, Nantes, France (J. Abrantes, A.M. Lopes); Faculdade de Ciências da Universidade do Porto, Porto, Portugal (A.M. Lopes, P.C. Alves); Universidade da Oviedo, Asturias, Spain (K.P. Dalton, F. Parra); VetNatura, Lisbon, Portugal (P. Melo, M. Ramada); Faculdade de Medicina Veterinária—Universidade Técnica de Lisboa, Lisbon (J.J. Correia); University of Montana, Melo, M. Ramada); Faculdade de Medicina Veterinária—Universidade Técnica de Lisboa, Lisbon (J.J. Correia); University of Montana, Missoula Montana, USA (P.C. Alves); and Instituto de Investigación y Formación Avanzada en Ciencias y Tecnologías da Saúde (CESPU), Gandra, Portugal (P.J. Esteves).

DOI: http://dx.doi.org/10.3201/eid1911.130908

References

1. Abrantes J, van der Loo W, Le Pendu J, Esteves PJ. Rabbit hemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. Vet Res. 2012;43:12. http://dx.doi.org/10.1186/1297-9716-43-12

2. Alda F, Gaitero T, Suarez 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. http://dx.doi.org/10.1186/1471-2148-10-347

3. Muller A, Freitas J, Silva E, Le Gall-Recule G, Zwingelstein F, Abrantes J, et al. Evolution of rabbit hemorrhagic disease virus (RHDV) in the European rabbit (Oryctolagus cuniculus) from the Iberian Peninsula. Vet Microbiol. 2009;135:368–73. http://dx.doi.org/10.1016/j.vetmic.2008.09.057

4. Dalton KP, Nicieza I, Balseiro A, Muguerza MA, Rosell JM, Casais R, et al. Variant rabbit hemorrhagic disease virus in young rabbits, Spain. Emerg Infect Dis. 2012;18:2009–12. http://dx.doi.org/10.3201/eid1812.120341

5. Le Gall-Recule G, Zwingelstein F, Boucher S, Le Normand B, Plassart G, Portejoie Y, et al. Detection of a new variant of rabbit hemorrhagic disease virus in France. Vet Rec. 2011;168:137–8. http://dx.doi.org/10.1136/vr.d697

6. 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;58:115–26. http://dx.doi.org/10.1016/S0168-1702(98)00106-3

7. Abrantes J, Esteves PJ. Not-so-novel Michigan rabbit calicivirus. Emerg Infect Dis. 2010;16:1331–2. http://dx.doi.org/10.3201/eid1608.091803

8. 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;70:8614–23.

9. Strive T, Wright JD, Robinson AJ. Identification and partial characterization of a new Lagovirus in Australian wild rabbits. Virology. 2009;384:97–105. http://dx.doi.org/10.1016/j.virol.2008.11.004

10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011;28:2731–9. http://dx.doi.org/10.1093/molbev/msr121

To the Editor: Mycobacterium yongonense is a recently described species (1) that belongs to the M. avium complex (MAC) and is associated with pulmonary infection. The strain on which the description of species was based was isolated in South Korea from the sputum of a patient with unspecified pulmonary disease. We describe 2 M. yongonense strains isolated from patients in Italy.

Patient 1 was a 74-year-old woman who had experienced fatigue, diarrhea, and weight loss. Her medical history included liver cirrhosis resulting from hepatitis C virus infection and surgery for colon cancer; the patient also reported tuberculosis in childhood. Chest radiograph revealed a cavitary lesion, a finding confirmed by computed tomography scan (Figure). Cultures in liquid and solid media grew a nonchromogenic mycobacterium from sputum and stool samples; results were negative for urine samples.

The patient was treated with clarithromycin, rifabutin, and ethambutol and showed some improvement. A bronchoscopic investigation was performed, and microscopic examination of bronchoaveolar lavage samples revealed the presence of acid-fast bacilli that subsequently were grown in culture. The patient began improving markedly starting with the second month of treatment, which will be continued for a total of 18 months.

Patient 2 was a 74-year-old woman, living in a community of nuns, who reported cough and dyspnea. Her medical history included renal failure and surgery for breast cancer. A bronchoaveolar lavage was performed; samples yielded in culture Pseudomonas aeruginosa and a nonchromogenic mycobacterium.