VIROLOGY

Detection of a new variant of rabbit haemorrhagic disease virus in France

WE wish to report the detection of a new variant of rabbit haemorrhagic disease virus (RHDV) (Lagovirus, Caliciviridae) which is circulating in France and has been causing high mortality in domestic and wild rabbit populations since the end of the summer of 2010.

Rabbit haemorrhagic disease (RHD) is a highly infectious and fatal disease of the European rabbit (*Oryctolagus cuniculus*). Although the development of efficient commercial vaccines has made it possible to control the disease in the rabbit industry, RHD still threatens non-vaccinated wild populations.

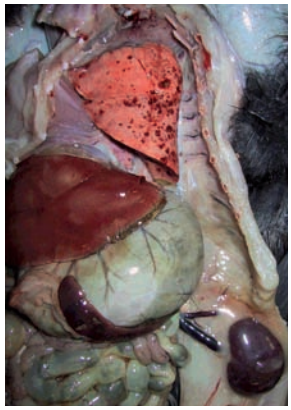

FIG 1: Macroscopic lesions observed in a dead domestic rabbit from a rabbitry. The lesions are specific to rabbit haemorrhagic disease: haemorrhages in lungs, heart and kidneys, pale and friable liver, and congested and enlarged spleen were all seen

Some cases of RHD had been reported in rabbitries in north-western France, but since October 2010 this number has increased. Mortalities occurred in RHDV-vaccinated and non-vaccinated does and fattening rabbits. Emergency vaccination stopped the mortalities but in seven to 15 days, compared with seven to nine days when a 'classical' RHDV is responsible for the disease. Up to the end of January 2011, approximately 60 rabbitries had been affected by this virus in north-western and northern France. During the same period, outbreaks were reported by the SAGIR network (Lamarque and others 2000) in numerous wild rabbit populations in the same areas. Estimated mortalities are unusual, reaching 80 to 90 per cent. They are similar to those observed when RHD emerged and spread in France in native populations at the end of the 1980s.

We analysed three cases of RHD in rabbitries in north-western France, which occurred in mid-October. Postmortem examinations of dead rabbits (Fig 1) and microscopic examinations of organs (lung, liver and kidney) revealed typical RHD lesions (Plassiart and others 1992).

Samples of liver from domestic and wild rabbits were frozen for virological analyses. For the screening of RHDV-positive samples, we amplified and sequenced a region located in the N-terminal of the gene encoding the capsid protein VP60 as previously described by Le Gall-Reculé and others (2011). All the samples were positive. The DNA sequences were closely related (98.5 to 100 per cent homology) and the protein sequences were similar, implying that the same virus was responsible for the clinical cases. This virus is related to, but highly distinct from, RHDV and antigenic variant RHDVa strains currently circulating in France (the average homology is only 85.7 per cent). The virus constitutes a new variant of RHDV.

To estimate its phylogenetic relationship with known rabbit lagoviruses, we performed phylogenetic analyses with all the nucleic sequences available in databases, including the non-pathogenic strains characterised in Italy (RCV) and recently in

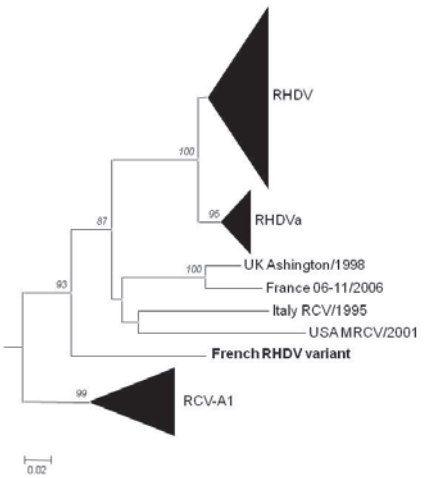

FIG 2: Phylogenetic tree using the minimum evolution method derived for partial VP60 nucleotide (nt) sequences (nt 1188 to 1541) of 93 rabbit haemorrhagic disease virus (RHDV) and 32 antigenic variant RHDVa pathogenic strains, the Ashington strain, the low pathogenic MRCV strain and the non-pathogenic 06-11, RCV and 36 RCV-A1 strains. Bootstrap values greater than 75 per cent (for 1000 replicates) are given in italics before each node. The French reference European brown hare syndrome virus strain EBHSV-GD was used as an outgroup to root the tree. The RHDV, RHDVa and RCV-A1 branches were collapsed to highlight the main genogroups

Australia (RCV-A1) and France (06-11), as well as the assumed pathogenic Ashington strain from the UK and the low pathogenic strain from the USA (MRCV). For this purpose, we amplified and sequenced a region located in the C-terminal of the VP60 gene. Irrespective of the phylogenetic method used, analysis revealed that the RHDV variant formed a new genetic group, relatively distant from pathogenic and non-pathogenic lagoviruses described until now (Fig 2). However, this group is more closely related to RHDV and RHDV-related viruses than to the Australian non-pathogenic strain that constitutes a new member of the genus *Lagovirus* (Strive and others 2009).

An initial experimental study is underway to confirm macroscopic and microscopic lesions of the new variant and to check the protection conferred by current commercial vaccines. Indeed, the mortalities reported in vaccinated rabbits suggest that vaccines may give only partial protection. A modified vaccination schedule or a new RHDV vaccine may be necessary to protect rabbits against this new variant. Other experimental studies will be undertaken specifically to determine the pathogenesis of the virus in very young rabbits. In addition, acquisition of complete molecular data should make it possible to determine the phylogenetic relationships with other lagoviruses and to understand the evolution of RHDV.

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