Increased pathogenicity in rabbit haemorrhagic disease virus type 2 (RHDV2)

L. Capucci, P. Cavadini, M. Schiavitto, G. Lombardi, A. Lavazza

RABBIT haemorrhagic disease (RHD) is an acute and lethal form of viral hepatitis in rabbits (Oryctolagus cuniculus) with a mortality rate in adults ranging from 70 per cent to 100 per cent. RHD was first reported in China in 1984, in Europe in 1986, where it caused severe losses to rabbit, and in Australia in 1996 (Abrantes and others 2012). RHD is caused by the lagovirus RHD virus (RHDV) in the family Caliciviridae. In the course of its evolution, RHDV split into six genotypes (Kerr and others 2009), all highly pathogenic and virulent. Genotype 6 is the antigenic subtype (RHDV-a) that became prevalent in certain countries, including the USA (McIntosh and others 2007). In addition, other enteric non-pathogenic rabbit caliciviruses (RCV-a) related to RHDV have been identified in Europe and Australia (Capucci and others 1996, Strive and others 2009, Le Gall-Reculé and others 2011a).

In 2010, a new lagovirus was identified in France. This virus showed a capsid protein sequence identity of about 80 per cent with RHDV and was able to cause RHD in vaccinated and young rabbits (15–25 days old) (Le Gall-Reculé and others 2011a, b). In addition, it showed a distinct antigenic profile and induced an average mortality rate of 20–30 per cent in both experimental infections and natural cases; such a low mortality rate was never observed in the many experimental rabbit infections carried out with other strains of RHDV. The remaining 70–80 per cent of the rabbits survived the infection without showing typical signs of RHD (Le Gall-Reculé and others 2011b). Unexpectedly, in autumn 2011, this new virus also caused fatal cases in cape hares (Lepus capensis var mediterraneus) (Fuggioni and others 2013). All these features strongly suggested that the virus was not derived from RHDV but rather that it had recently emerged from an unknown source; for this reason, we named it RHDV type 2 (RHDV2). Within a couple of years, RHDV2 spread throughout Europe (Le Gall-Reculé and others 2013, Dalton and others 2014), where it caused outbreaks on rabbit farms and in wild populations. Over time, the mortality rate of RHDV2 cases in Italy appeared to increase, as did the proportion of cases resulting in acute RHD.

With the aim of establishing the pathogenicity (ie, the proportion of infected rabbits that develop RHD) of recent field strains, we infected three groups, each consisting of five healthy, seronegative, adult New Zealand rabbits. Each rabbit received, orally, 1 ml of 0.5 per cent w/v liver homogenate from rabbits that died of acute RHD caused by the following strains: RHDVBoS9 (Capucci and others 1996), which is the reference RHDV strain, RHDV2Ta14 from an outbreak in the Puglia region in 2014 and RHDV2Ch15 from an outbreak in the Abruzzo region in 2015. Trials were performed in a Biosafety Level 3-designated area. All animal work was approved by the Ministry of Health and conducted according to the requirements of national (DM 4/3/2014 n. 26) and European (2010/63/EU) laws regarding the care and use of animals.

The three different isolates caused similar RHD symptoms and degrees of pathogenicity (ie, 80 per cent of rabbits developed acute RHD) (Fig 1).

The average time to death postinfection (p.i.) was similar for RHDVBoS9 and RHDV2Ta14, approximately 70 hours, and slightly higher for RHDV2Ch15, 85 hours. RHDV and RHDV2-specific sandwich ELISAs (OIE – Rabbit Haemorrhagic Disease 2012) performed on 10 per cent w/v liver homogenates, revealed viral titres (ie, ELISA endpoints) ranging from 10^-2 to 10^-3, typical of acute RHD. During the experiments, we also recorded the death of three rabbits, one from each group, without signs of RHD. During necropsy, we found relatively severe enteritis with fluid faecal contents, likely due to a bacterial infection; this outcome may have been a consequence of the sudden environmental change from the farm to the experimental area.

The livers of these rabbits were negative for RHDV based on an ELISA but positive based on RT-PCR, indicating that the infection was under way. Since 1990, we have performed several experimental infections of rabbits with RHDV; the present experiments were the first in which some rabbits died from causes other than RHD. Although the unusual deaths were neither desired nor planned, they did permit us to observe a new and interesting phenomenon. In the three rabbits suffering from severe enteric bacterial infection, the replication of highly pathogenic RHDV was strongly inhibited until at least 96 hours p.i., a time point at which all others infected rabbits had already died from RHD. Such acquired ‘resistance’ to RHD in these three rabbits could be due to the prompt activation and action of the innate immune system stimulated by the bacterial infection, which indirectly limited RHDV replication. Studies on innate immunity in young rabbits have previously suggested the direct involvement of the innate immune system in resistance and susceptibility to RHD (Marques and others 2014).

This virulence trial, the first described so far to use RHDV2 strains identified after 2011, showed that the two Italian RHDV2 strains isolated in 2014 and 2015 induced at least 80 per cent mortality, which approaches the usual mortality rate of RHDV and is four times higher than that found in the early RHDV2 isolates (Le Gall-Reculé and others 2013). Considering that we recently observed several natural RHDV2 outbreaks in farmed rabbits characterised by higher mortality rates, we conclude that highly pathogenic RHDV2 strains have emerged during the virus’s evolution and have become prevalent in the field.

The demonstrated significant increase in RHDV2 pathogenicity within a few years supports two hypotheses. The first has already been put forth to explain RHDV evolution in wild rabbits in Australia (Elsworth and others 2014); high pathogenicity and virulence in RHDV are traits that undergo positive selection. Presumably, they allow for RHDV’s rapid spread and sustained presence in rabbit host populations. The second
hypothesis relates to the origin of RHDV2 and its status as a newly emerging virus and not as a direct variant of former RHDVs (Le Gall-Reculé and others 2013). Indeed, our results indicate that the first RHDV2 isolates from 2010 to 2011 are, among the known RHDVs, the only mildly pathogenic ones. Thus, in this respect, they resemble the Michigan rabbit calici-virus (MRCV), identified in 2001 at a USA rabbit farm, which was associated with episodes of RHD-like disease and had a total case-fatality rate of approximately 30 per cent (Bergin and others 2009). Because the MRCV genome is unique among the lagoviruses and no other outbreaks were reported nor was it identified again, MRCV could be considered an ‘attempt’ at emergence by a new RHDV-like virus. The reason for the transmission and evolutionary ‘success’ of RHDV2, compared with the ‘failure’ of MRCV, could be the strong presence in Europe of susceptible hosts (Oryctolagus cuniculus) in the wild and on farms; this may have allowed RHDV2 to complete its transition to a highly pathogenic virus. In contrast, in the USA, European rabbits are present only on a few farms and there are no wild populations, a situation not sufficient to support a lagovirus diffusion and persistence. This is also demonstrated by the fact that the pathogenic RHDVs is not endemic in USA in spite of the occurrence of some outbreaks.

In light of these results and the fact that the immunogenic differences between RHDV and RHDV2 occur at the serotype level, there is an urgent need for homologous RHDV2 vaccines that can fully protect rabbits from RHD and limit the environmental contamination and diffusion of RHDV2 in affected countries. RHDV2 has been present since 2011 in several European countries but, as suggested by this study, it is evolving rapidly, and the distribution of antigenic variants (subtypes) is likely shifting. Therefore, the specific choice of RHDV2 isolate will be important in the production of specific vaccines.

Finally, although RHDV2 has been the dominant virus causing RHD in most European countries in recent years, the fact that RHDV2 still coexists in some areas (ie, Italy) makes it advisable to vaccinate animals against RHDV2 and the ‘original’ RHDV/RHDV2 strains.

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