International Journal of Livestock Research, Vol. 10 (12) Dec' 2020
http://www.ijlr.org
eISSN: 2277-1964
NAAS Rating 2020 – 5.36

Review Article

Rabbit Haemorrhagic Disease: Biological Pest Control Method to Evolve as a Transboundary Disease

Bramhadev Pattnaik[1], Sharanagouda S Patil[2]*, Pinaki Panigrahi[3] and Mahendra Pal Yadav[4]

[1]Director, One Health Center for surveillance and disease dynamics, AIPH University, Bhubaneswar, Odisha & Former Director, ICAR- Directorate of Foot and Mouth Disease, Mukteswar, INDIA
[2]Principal Scientist, Virology Section, ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI), Yelahanka, Bengaluru, Karnataka, INDIA
[3]Professor, Department of Pediatrics, Division of Neonatal-Perinatal Medicine, Georgetown University Medical Center, Washington, D.C. USA 20007
[4]Former Vice-Chancellor, SVP University of Agriculture & Technology, Meerut, INDIA

*Corresponding Author: sharanspin13@gmail.com

How to cite this paper: Pattnaik, B., Patil, S. S., Panigrahi, P., & Yadav, M. P. (2020). Rabbit Haemorrhagic Disease: Biological Pest Control Method to Evolve as a Transboundary Disease. *International Journal of Livestock Research*, 10(12), 1-13. doi: http://dx.doi.org/10.5455/ijlr.20201007045754

Received : Oct 07, 2020
Accepted : Nov 06, 2020
Published : Dec 31, 2020

Abstract

Rabbit haemorrhagic disease (RHD), also known as viral haemorrhagic disease was a highly infectious and contagious disease affecting only European rabbits. It is caused by a host-specific calicivirus, called RHD virus (RHDV). The virus once used to control rabbit population in Australia is currently an important transboundary pathogen causing economic losses to the rabbit industry. RHDV appears to have evolved from a pre-existing avirulent rabbit calicivirus circulating in Europe, Australia and New Zealand. However, there is no conclusive proof. Emergence of RHDV was first detected in 1984 in China and then spread to Asia, Europe, Africa, Americas, Australia and New Zealand. With the evolution process, either by mutation or recombination or otherwise, there was antigenic drift in the original RHDV population (of 1984) leading to the development of antigenic variant RHDV2 (different serotype) that was detected in 2010, and is currently in circulation causing RHD with broader host susceptibility, and has almost replaced the parent serotype (RHDV1) of 1984. The death rate is observed to be 40-100% for RHDV1 and 5-70% for RHDV2. Rabbits vaccinated with RHDV1 are not protected from RHDV2 infection. The current review includes virology, pathology, epidemiology, diagnosis and prevention of RHD.

Keywords: Calicivirus, India, Rabbit Haemorrhagic Disease, RHD, RHDV1, RHDV2, Transboundary Disease

Introduction

The family *Caliciviridae* currently comprises of five genera (*Vesivirus, Lagovirus, Norovirus, Sapovirus, and Nebovirus*), with an additional six genera (*Recovirus, Valovirus, Bavovirus, Nacovirus, Minovirus, and Salovirus*) proposed. All genera include viruses that infect animals/ human beings (Desselberger, 2019). The genus *Vesivirus* consists of feline calicivirus and vesicular exanthema virus of swine, and close relatives that include the numerous San Miguel sea lion viruses, cetacean calicivirus, primate calicivirus, skunk calicivirus, bovine calicivirus, reptile calicivirus, and mink calicivirus. Vesiviruses cause mucosal or cutaneous vesicles/blisters in their respective hosts. The genus *Lagovirus* (from *Lago*morph) includes RHD and European brown hare syndrome (EBHS) viruses. The genus *Norovirus* contains six different genogroups with multiple subgroups within each, and are associated with outbreaks of acute gastroenteritis in humans. Noroviruses also infect cattle, sheep, pigs, dogs, cats, and mice. The genus *Sapovirus* includes viruses that have been linked to outbreaks of human gastroenteritis, besides porcine enteric calicivirus and mink enteric sapovirus. The genus *Nebovirus* comprises of two virus species that infect cattle; the type species being Newbury-1 virus and bovine enteric calicivirus NB (Nebraska). These viruses cause mild enteritis in calves. Of the four proposed genera, *Recovirus* contain Tulane calicivirus, a virus isolated from Rhesus monkeys (*Macaca mulatta*) with gastroenteritis, *Valovirus* which contain St-Valérien-like viruses isolated from pigs, and *Nacovirus* and *Bavovirus* comprising of novel caliciviruses recovered from chickens and turkeys (Virus Taxonomy, 9th Report of ICTV). With the advent of metagenomics, the group of unassigned caliciviruses is growing rapidly. The caliciviruses are genetically heterogeneous.

The virions of the family *Caliciviridae* are non-enveloped with icosahedral symmetry, and of 27–40 nm in diameter. Caliciviruses have single stranded, positive sense genomic RNA with 2 major ORFs. The capsid is composed of 90 dimers of the major structural protein VP1 arranged on a T- 3 icosahedral lattice. A characteristic feature of the calicivirus capsid architecture is the 32 cup-shaped depressions at each of the icosahedral five-fold and three-fold axes. The virus has conserved nucleotide motifs at the 5´-terminus of the RNA genome, and at the junction of the coding sequences for the non-structural/structural proteins. The virions are predominantly composed of one major capsid protein, VP1 (58–60 kDa) and a second minor structural protein named VP2 (8.5–23 kDa) has been found in FCV (feline calicivirus), RHDV, and Norwalk virus-like particles. Some non-structural proteins have sequence homology with those of the family Picornaviridae, viz. 2C (NTPase), 3C (cysteine protease) and 3D (RNA-dependent RNA polymerase; RdRp). Caliciviruses are stable in the environment and usually resistant to inactivation by heat and chemicals like ether, chloroform and mild detergents (Virus Taxonomy, 9th Report of ICTV).

Viruses of the *Lagovirus* genus in the family *Caliciviridae* include RHDV, EBHSV and non-pathogenic rabbit calicivirus (RCV). The RHDV specifically infects and cause disease in *Oryctolagus cuniculus*, the predominant species of domestic rabbits worldwide. EBHSV affects European hares of the *Lepus* genus. RHDV does not cause disease in wild cottontail rabbits, jackrabbits, or hares (Katherine and James,2012). RHD is an acute, lethal disease of European rabbits *(Oryctolagus cuniculus)* caused by RHDV that first emerged in China in 1984 in angora rabbits imported from Europe (Germany) and then spread gradually to all the five continents. Synonyms of the RHD are rabbit calicivirus disease (RCD), viral haemorrhagic disease (VHD) of rabbits, haemorrhagic pneumonia (China), Infectious necrotic hepatitis and Malattia X (in Italy before viral origin was understood) (Terio *et al.*, 2018).

The RHDV was used in Australia as a pest control measure to control large and destructive populations of rabbits. When RHDV was being studied on an island off the south coast of Australia as a possible rabbit control agent, it inadvertently leaked into the mainland. Then the virus spread rapidly in Australia, and later extended up to New Zealand. The virus was only moderately successful in controlling the rabbit populations. When first introduced, it was highly pathogenic, causing high fatality in rabbits. After some time, an increasing proportion of rabbits survived the infection. In New Zealand, the virus appeared to have developed the ability to establish persistent / latent infections which was thought to be because of existing background immunity to the virus (RHDV) caused by the circulation of a similar virus in the rabbit population of New Zealand for many years before introduction of the virulent RHDV (Strauss and Strauss ,2008).

Pathophysiology of the Disease

The pathophysiology of the disease includes acute hepatic necrosis, nephrosis, disseminated intravascular coagulation (DIC), hypoglycaemia, hepatic encephalopathy and Leukopenia (Terio *et al.*, 2018). The virus RHDV1/a can infect rabbits of any age and sex, but very young rabbits (younger than 4 weeks of age) do not usually

develop disease following infection. This age-related resistance, in the absence of maternal antibody, wanes subsequently. Despite resistance to the disease, these rabbits may still shed the virus and can infect in-contact rabbits. The incubation period may be as short as 1-2 days, followed by sudden death with no previous clinical signs (Suckow *et al.*, 2002). The virus is highly infectious by oral, nasal, conjunctival, and parenteral routes. Insect vectors such as flies, fleas, and mosquitoes can spread the virus mechanically. The virus may be transmitted via respiratory and intestinal secretions. Contaminated fomites can spread the infection as the virus is resistant to environmental inactivation. The virus can persist in infected animal carcasses for long periods. The disease (RHD) occurs in four forms, viz. (1) peracute infection: sudden death with no clinical signs, (2) acute: rabbits may appear quiet and may have fever and an increased respiratory rate before death, (3) subacute: jaundice and death over a period of several days to 2 weeks, and (4) subclinical: a small proportion of experimentally infected adult rabbits develop few or no clinical signs and clear the virus. Upon infection, Kits of less than 4 to 8 weeks of age do not develop clinical signs other than fever, but shed the virus. There is lack of availability of appropriate cell culture system for growth of virus. High concentration of the virus occurs in tissues of infected rabbits and can be detected by immunofluorescence/ immunohistochemical staining with specific antibodies. Using viral antigens from infected tissue or capsid protein (vp60) expressed *in vitro, antibodies in surviving animals* can be detected by enzyme-linked immunosorbent assay (ELISA). Some strains of RHDV hemagglutinate human erythrocytes, so antibody detection can be made by hemagglutination inhibition (HI) test. RT-PCR assays for the detection of viral nucleic acid are routinely used in diagnosis (Kovaliski *et al.*, 2014). Specific antibodies can be detected by ELISA and HI test. Rabbit colonies with this disease should be quarantined and depopulated, and the environment thoroughly cleansed and disinfected (Suckow *et al.*, 2002).

The RHDV2/ RHDVb, a genetically distinct rabbit calicivirus related to RHDV and causing similar lesions, has emerged since 2010. Rabbits infected with RHDV2 may survive longer than those infected with RHDV. In Europe, there have been epidemics of RHVD2-related disease in wild rabbit populations since immunity to RHDV is not protective for RHDV2. These outbreaks primarily affected wild rabbits, and also hare species including brown hare, Italian hare, and Cape hare. (Terio *et al.*, 2018). A distinct calicivirus, called Michigan rabbit calicivirus was isolated in 2001 from an outbreak (in rabbits) in Michigan, United States. It caused nearly 33% mortality with clinical signs and lesions similar to RHD (Terio *et al.*, 2018).

Genetic Evolution of RHDV

The direction of genetic variation and evolution of RHDV which has been present worldwide for over 30 years, has led from RHDV, through RHDVa, to RHDV2 (Hukowska-Szematowicz,2020). Bioinformatics/ phylogenetic analysis of vp60 capsid protein of different subtypes of RHDV revealed that the isolates of classic RHDV, RHDVa, and RHDVb form different and distinct clades (Qi *et al.*, 2019). Divergence analysis suggested that the accumulation of amino acid changes might be due to adaptive diversification of vp60 capsid protein during the division between classical RHDV, RHDVa, RHDVb, and RCV. The prediction of N-glycosylation sites in vp60 revealed that RHDVb subtype had two unique N-glycosylation sites at amino acid positions 301 and 362, but lacked three other N-glycosylation sites at amino acid positions 45, 308 and 474 displayed in vp60 of classic RHDV and RHDVa. This divergence in N-glycosylation sites in RHDV might affect virulence of the virus. Analysis of phosphorylation sites also indicated that some phosphorylation sites in RHDVa and RHDVb differed from those in classic RHDV; this could be related to antigenic variation in RHDV1. As of today, the RHDV2/b subtype (since 2010) has almost replaced the classical RHDV1 (since 1984) in causing RHD in several parts of the World, affecting both young and adult European rabbits and not cross-protected by RHDV1 vaccine. The present review includes all aspects of the RHD and the causative virus.

History of the Disease

The first outbreak of RHD was reported in 1984 in the Jiangsu Province of China in Angora rabbits imported from Germany (Liu *et al.*, 1984), followed by Korea (Park *et al.*, 1987). The disease then spread to Europe and was reported in Italy in 1986 (Cancellotti and Renzi, 1991), Spain in 1988 and Portugal in 1989 (Abrantes *et al.*, 2012) causing severe reduction of wild rabbit populations. Then it was reported in North Africa (Morisse *et al.*, 1991) and North America (Abrantes *et al.*, 2012). The disease is currently reported worldwide (Table 1) (Figure 1) (Harcourt-Brown, 2020). The disease has not been reported in India.

Table 1: Details of countries that reported RHD (Harcout-Brown, 2020)

S. No.	Name of the Country	Year of Report
Occurrence of RHDV		
	China	1984
	Italy	1986
	Korea	1987
	Europe	1988
	Scandinavia	1990
	First report in UK	1991
	North Africa	1991
	RHDV was released into Wardang Island, Southern Australia as biological control for wild rabbits	1995
	Illegally introduced into New Zealand	1997
	First confirmed report in United States of America (North)	2000
	RHD became endemic in many regions globally	2000-2010
Occurrence of RHDV2		
	France	2010
	Europe	2014
	Australia	2015
	Vancouver Island, Canada	2018
	New Zealand	2018
	United States of America	2019 continuing

Figure 1: Global distribution of RHD (https://www.cabi.org/isc/datasheet/66455)

In Australia and New Zealand, where the rabbit is considered an important agricultural pest, RHDV (Czech reference strain, Czech V351) was released in the Wardang Island (Wauraltee Island**)** in Spencer Gulf, South Australia to control rabbit population, but in spite of quarantine measures, the virus escaped from the island in 1995 and reached the mainland (Abrantes *et al.*, 2012; Cooke and Fenner, 2002). From there the virus spread to New Zealand (Thompson and Clark,1997; O'Keefe *et al.*, 1998) . Now RHDV is endemic in most parts of Europe, Asia, and parts of Africa, Australia and New Zealand (Abrantes *et al.*, 2012).

The Virus

The origin of RHDV and its emergence as a rabbit pathogen remain unclear. There are two hypotheses; (1) a species jump from a closely related species or (2) the emergence from a pre-existing non-pathogenic lagovirus circulating

in leporids, i.e. rabbit and hare (Esteves *et al.*, 2015; Lopes *et al.*, 2017). The detection of anti-RHDV antibodies prior to documented outbreaks along with the characterization of a weakly pathogenic and several non-pathogenic strains support the emergence of RHDV from circulating non-pathogenic lagoviruses (Lopes *et al.*, 2017). Either of the hypotheses are yet to be confirmed. The RHDV belongs to the genus *Lagovirus* in the family *Caliciviridae*. The virus is non-enveloped with a diameter of 35–40 nm and having T-3 icosahedral symmetry. It contains a linear positive-sense RNA genome of ~ 7.4 kb that is organised into two overlapping open reading frames (Lopes *et al.*, 2017). The RHDV genome also has 5′ and 3′ untranslated regions (5′UTR and 3′UTR). In addition to genomic positive sense single-stranded RNA molecule, the RHD virion also carries a sub-genomic RNA molecule of 2.2Kb that is collinear with the 3' end of the genomic RNA and encodes both the major and minor structural proteins (Meyers *et al.*, 1991a; Meyers *et al.*, 1991b; Meyers *et al.*, 2000). Both the genomic and sub-genomic RNA have 3' poly (A) tail and covalently linked to VPg protein at the 5' end through a Tyrosine residue (Machin *et al.*, 2001). The sub- genomic RNA molecule also codes for non-structural proteins that are required during replication of the virus. The genomic RNA comprises of 7437 nucleotides having two slightly overlapping open reading frames (ORF). The ORF1 spans from nucleotides 10 to 7044 and ORF2 from nucleotides 7025 to 7378 (Meyers *et al.*, 1991a). The ORF1 codes for a large polyprotein of 257 kDa (Meyers *et al.*, 1991a) that is subjected to post-translational cleavage by a virus-encoded trypsin-like cysteine protease into several non-structural proteins such as helicase, RNA-dependent RNA polymerase (RdRp), protease, and the major structural capsid protein vp60 (Konig *et al.*, 1998;Lopes *et al.*, 2017; Meyers *et al.*, 2000; Sibilia *et al.*, 1995). The minor structural protein vp10 is encoded by the 3'end of genomic and sub-genomic RNA in the reading frame ORF2 (Lopes *et al.*, 2017). The biological role of some of the non-structural proteins encoded by the caliciviruses has been elucidated from the previous knowledge generated on the members of *Picornaviridae* family (Konig *et al.*, 1998; Meyers *et al.*, 1991a; Meyers *et al.*, 1991b; Meyers *et al.*, 2000). Viral helicase and RNA-dependent RNA polymerase (RdRp) are involved in the replication of the RNA of RHDV. The RdRp also catalyses uridylation of VPg (Machin *et al.*, 2009). The VPg has a role in translation of viral proteins (Goodfellow *et al.*, 2005). The minor structural protein vp10 enhances virus replication and promotes apoptosis (Liu *et al.*, 2008), and regulates virus replication and release of progeny virion particles from infected cells (Chen *et al.*, 2009).

RHDV2/RHDVb

Lagovirus europaeus GI.2, also known as RHDV2 or RHDVb, is an emerging virus that causes rabbit haemorrhagic disease (RHD) in European rabbits (*Oryctolagus cuniculus*). In contrast to *L. europaeus* GI.1 viruses (RHDV1/RHDVa) that are only pathogenic for adult rabbits, GI.2 (RHDV2/RHDVb) causes clinical disease in both adult rabbits and kittens (Neimanis *et al.*, 2018a). Until 2010, phylogenetic analyses revealed the progressive emergence of distinct but closely related variants of RHDV (Kinnear and Linde, 2010). Pathogenic (G1-G6 or variants GI.1a-GI.1d) and non-pathogenic strains (GI.4) of RHDV have been identified. In 2010, the new variant of RHDV (RHDV2/RHDVb/GI.2) was detected in France with distinct pathogenic, genetic and antigenic profiles (Le Gall-Reculé *et al.*, 2013; Silvério *et al.*, 2018). The disease caused by the new variant has been described as a 'novel' rabbit haemorrhagic disease of the European rabbit (*Oryctolagus cuniculus*) (Rouco *et al.*, 2019). RHDV2 rapidly spread throughout Europe, causing disease in both domestic and wild rabbits (Abrantes *et al.*, 2012; Westcott and Choudhury, 2015). It has been again recently detected in Australia, North America and Africa (Duarte *et al*., 2015a; Hall *et al.*, 2015; Martin-Alonso *et al.*, 2016). In Western Europe, RHDV2 was responsible for almost all cases of RHD in both domestic and wild rabbits, and cases of RHD caused by former RHDV (RHDV1/a) strains became rare (Le Gall-Reculé *et al.*, 2017). RHDV2 quickly evolved and recombination with rabbit lagoviruses that co-circulate in Europe was observed in the Iberian Peninsula (also known as Iberia) (Almeida *et al.*, 2015; Lopes *et al.*, 2015. This recombination occurred between the genomic region coding for the structural vp60 and vp10 proteins of RHDV2 and the upstream region of the genome encoding the non-structural proteins, either from the RHDV genogroup G1 or from non-pathogenic lagoviruses (Le Gall-Reculé *et al.*, 2017). RHDV2 exhibits a broader host range than the classical RHDV and infects not only rabbits but also different hare species (*Lepus capensis mediterraneus, Lepus corsicanus, Lepus europaeus, Lepus timidus*) (Camarda *et al.*, 2014; Puggioni *et al.*, 2013; Velarde *et al.*, 2017). RHDV2-infected hares show clinical signs similar to European brown hare syndrome (EBHS) (Camarda *et al.*, 2014; Puggioni *et al.*, 2013; Velarde *et al.*, 2017), first described in 1980. One study reported RHDV2 in the Madeira archipelago and Portugal and identified five haplotypes (Carvalho *et al.*, 2017a). Genetic characterization of RHDV from Iberian Peninsula before 2015 had revealed the existence of two genogroups, G1 and sporadically G6. However, in 2016 (Martin-Alonso *et al.*, 2016), vp60 sequence analyses revealed the presence of RHDV2 in both farm and wild rabbits from several areas of Tenerife, the largest island of Canary Islands (a Spanish archipelago), and these RHDV2 strains found in Tenerife shared two exclusive SNPs that have not been

observed in the rest of RHDV2 strains. Diagnostic investigation by PCR, revealed that the RHDV2 was widespread in wild populations of European rabbit in Portugal affecting both adults and young ones (Rouco *et al*., 2018). Within 18 months of initial detection in May 2015, RHDV2 spread replacing RHDV1, to all Australian states and territories and rapidly became the dominant circulating strain (Mahar *et al.,* 2018). Four SNPs in terminal 942 nucleotides of the vp60 gene were observed in the RHDV2 Azorean strains (S. Miguel and St. Maria islands of Azores, Portugal) isolated during 2014-15 (Duarte *et al.,* 2015). Azorean RHDV2 strains shared a non-synonymous substitution in one hypervariable region of vp60. The RHDV2/b genotype has also been reported by duplex real-time PCR in species outside the order Lagomorpha in carcasses of one Mediterranean pine vole (*Microtus duodecimcostatus*) and two white-toothed shrews (*Crocidura russula* (Calvete *et al*., 2019).

The first (Polish) RHDV2 isolates (RED 2016 and VMS 2017) obtained from liver samples of RHD-suspected rabbits from Lodzkie and west Pomeranian voivodeships in Poland, tested by rRT-PCR and sequenced in the vp60 and NSP regions/genes, had about 97% nucleotide sequence identity with the reference RHDV2 strains and approximately 18% divergent from classic RHDV1/a and its variants (Fitzner and Niedbalski , 2018). In both the cases, typical clinical symptoms of the disease were observed in spite of previous vaccination against RHD (RHDV1).

Incursion of RHDV into Sweden was documented in 1990 and it is now considered endemic (Neimanis *et al.,* 2018b). Emergence of RHDV2 in Sweden in both wild and domestic rabbits (*Oryctolagus cuniculus*) was identified by application of RT-qPCR, sequencing of the VP60 gene and immunological virus typing. The earliest documented outbreak occurred on 22 May 2013. Phylogenetic analysis of the VP60 gene segregated the Swedish isolates into three separate clusters within two different clades according to geographic location and time that suggested virus evolution, multiple introduction events or both. The cases of RHD examined during 2013-16 suggested that RHDV2 might replace RHDV1 as the predominant cause of RHD in Sweden.

Analysis of the genetic variability of 105 strains of *Lagovirus europaeus* (both RHDV1 and 2) indicated a growing genetic distance between the strains, both in time and location (Hukowska-Szematowicz,2020). Phylogenetic analysis divided the strains into seven groups, dictated by the chronology, geographical location, mutations and recombination. It was observed that the variability within the RdRp gene of RHDV2 is a major factor for its expansion and survival in the environment. The capacity of RHDV2 to overcome immunity derived from natural infections with RHDV1 supports immunologic difference between them (RHDV1 and RHDV2) at the level of serotype (Peacock *et al.,* 2017).

Recombination

Several subtypes of RHDV2 has been reported in Portugal (Lopes *et al.,* 2018), viz., non-recombinant RHDV2 in 2012, NP+RHDV2 in 2013, and G1+RHDV2 in 2014. Single recombination breakpoint was observed in RdRp-VP60 junction. Recombination events are common among different strains of RHDV. The new genotype GI.2 is a result of recombination of pathogenic variants viz., GI.1a &GI.1b and benign GI.4 strain. The recombination events occurred between structural and non-structural parts of virus (Abrantes *et al.,* 2020). There were reports that Chinese strain (first identified) had a common lineage with German strains which was evident in capsid gene and is very important observation that plays a significant role in evolution and epidemiology of RHDV (Forrester *et al.,* 2008).

Pathology

Rabbit haemorrhagic disease can be suspected from a history of sudden death. There is not always macroscopic evidence of the disease but histopathology is useful in diagnosis of RHD (Harcourt-Brown *et al.,* 2020). The authors conducted histopathological examination of heart, lungs, liver, spleen and kidney samples. Hepatocellular necrosis, characteristic of RHD, was observed in 185 of 300 (62%) submissions, often accompanied by glomerular thrombosis and changes in other organs. Evidence of RHD was not apparent on histopathology in 113 of 300 (38%) rabbits. No macroscopic abnormalities were seen in 78/185 (42%) of RHD cases. Ante mortem clinical signs included anorexia, collapse, lethargy, seizures, icterus, bleeding from the mouth, dyspnoea, hypothermia, pyrexia, bradycardia or poor blood clotting.

Using an Australian GI.2 (RHDV2/b) field strain isolated in 2015, (Neimanis *et al.,* 2018a) described the first detailed description of pathology, viral antigen distribution and tissue load of the virus in adult and 5-week old New

Zealand white rabbits using histology, immunohistochemistry and RT-qPCR. Liver was the target organ, but in contrast to GI.1(RHDV1/a) viruses, lesions and inflammatory responses did not differ between adults and kittens. Lymphocytic infiltration, proposed to be protective in kittens infected with RHDV1, was notably absent. There were bone marrow changes including decreased myeloid-to-erythroid ratio. Intracellular viral antigen was demonstrated in hepatocytes and cells of the mononuclear phagocytic system. In terminal stage of the disease, viral load was highest in liver, serum and spleen.

In two pet rabbits positive for RHDV2, the clinicopathologic findings were moderate thrombocytopenia, decreased aspartate aminotransferase and alanine aminotransferase activities and fibrinogen concentrations, prolonged prothrombin and activated partial thromboplastin times, high alkaline phosphatase and gamma-glutamyl transferase activities, and elevated bile acid and bilirubin concentrations. Histopathologic findings included haemorrhagic diathesis, severe centro-acinar and midzonal hepatocellular necrosis, severe necro-suppurative bronchopneumonia, and moderate cardiomyocyte necrosis (Bonvehí *et al.,* 2019).

The rabbit caliciviruses (*Lagovirus europaeus*) RHDV1 and RHDV2 (GI.1 and GI.2) cause acute necrotizing hepatitis in European rabbits (*Oryctolagus cuniculus*). Younger rabbits of < 8 weeks of age are highly resistant to RHDV1, while RHDV2 is highly virulent in both young and adult rabbits. Neave *et al.,* 2018 compared liver transcriptome profile in order to have insight in to the host-pathogen interaction, and found that kittens have stronger innate immunity compared to adult rabbits, in terms of increased expression of major histocompatibility class II (MHC II) molecules and stronger activity of natural killer cells, macrophages, and cholangiocytes that enables younger rabbits to respond more rapidly to RHDV1 infection than adult rabbits and thus limit pathology. They observed that multiple genes associated with innate immunity in kittens are down regulated during RHDV2 infection due to virus mediated immunomodulation, thereby permitting fatal disease to develop.

Diagnosis

The emergence of RHDV2 in Europe has been associated with decline in wild rabbit (*Oryctolagus cuniculus*) populations previously exposed to RHDV. All RHDV1 genotypes of G1–G6 belong to the same antigenic serotype (Le Gall-Reculé *et al.,* 2017). An ELISA employing a RHDV2-specific monoclonal antibody 2D9 for specific, sensitive and reliable detection of RHDVb/RHDV2 was achieved (Dalton *et al.,* 2018a). Application of RT-qPCR and vp60 sequencing are powerful tools in the diagnosis of RHDV1 and 2. Unique single nucleotide polymorphisms were recognised in the Madeira archipelago (in Portugal) RHDV2 strains, two of which resulted in amino acid substitutions at positions 480 and 570 in the vp60 protein (Carvalho *et al.,* 2017a). Interference of RHDV2 vaccination in the results of a RT-qPCR for RHDV2 detection was investigated (Carvalho *et al.*,2017b), and it was found that RHDV2 vaccine-RNA was not detected by the RT-qPCR as early as 15days post-vaccination that helped in diagnosis. RT-PCR and RT-qPCR have been successfully used in differentiation of RHDV subtypes and strains (Dalton *et al*., 2018b; Hall *et al.*,2018).

The RHDV2 exhibits distinctive genetic, antigenic and pathogenic features compared to RHDV1, and RHDV2 kills rabbits previously vaccinated with RHDV vaccines (Bárcena *et al.,* 2015). These authors reported for the first time the generation and characterization of RHDV2-specific virus-like particles (VLPs) and observed that due to the differential antigenic properties between RHDV1 and RHDV2, there is necessity of having RHDV2-specific diagnostic assays to monitor the spread of RHDV2.

A real time RT-PCR assay for the detection of RHDV2, targeting a 127 nucleotide-long region within the vp60 gene, was developed and validated using RHDV1 and RHDV2 RNA preparations from positive field samples (Duarte *et al.,* 2015a). The method was able to detect nine RNA molecules with an efficiency of 99.4%, and proved to be a valuable tool for diagnosis of most of RDVH2 circulating strains, and to monitor viral load, disease progression/ prognosis and vaccination efficacy. Circulation of recombinant viruses containing the RHDV2/b structural protein coding region and the non-structural protein coding regions of either pathogenic RHDV-G1 strains or non-pathogenic rabbit caliciviruses was identified using rRT-PCR (Hall *et al.*,2018). A liver PCR assay was applied to differentiate RHDV 2 from classic RHDV Bonvehí *et al.,* 2019).

The vp60 protein of RHDV is the structural protein that plays important roles in virus replication, assembly, and immunogenicity. A study (DeSheng *et al.,* 2016), using murine monoclonal antibodies (MAbs) raised against recombinant vp60 proteins from different RHDV subtypes, revealed that the epitopes identified by MAbs specific

to RHDV1 and RHDV2 were conserved in respective virus subtypes, and could distinguish both. ELISA and HI tests have been used in seroprevalence studies of RHDV, and results of both were comparable (Fitzner and Niedbalski ,2016). Emergence of New Virulent RHDV strains could be detected by RT-PCR using primers targeting vp60 capsid protein gene (Ismail *et al.*, 2017).

Prophylaxis/ Vaccine/ Epitopes

The unique antigenic nature of RHDV2 is reflected in its capacity to kill rabbits vaccinated against RHDV1, and the capacity of RHDV2 to overcome immunity derived from natural infections with RHDV1 reveals immunogenic difference between RHDV1 and RHDV2(Konig et al., 1998). A baculovirus-based RHDV2-VP1 vaccine protected rabbits against the infection with RHDV2 for at least 14 months (Müller *et al.*,2019). There was limited (50%) cross protection by the RHDV2-VP1 vaccine against classical RHDV1. Immunologic difference between RHDV1 and RHDV2 justifies the need for homologous RHDV2 vaccine to protect rabbits from RHD and limit contamination of the environment and transmission of RHDV2 in affected countries (Capucci *et al.,* 2017). RHDV2 is present in several European countries since 2011, and is evolving rapidly to generate antigenic variants (subtypes). Therefore, it is important to select appropriate RHDV2 strain/ isolate for the production of potent and efficacious vaccines. Although RHDV2 has been the dominant virus in recent years causing RHD in most European countries, co-existence of RHDV1/a in some areas (i.e. Italy) makes it necessary to vaccinate animals against RHDV2 as well as the original RHDV1/a.

Vaccines against viral pathogens are often composed of recombinant proteins expressed in different systems. RHDV2-VP1 VLPs derived from mammalian (RK-13) and insect cells (SF9) were able to induce protective humoral immune response in rabbits against RHDV2 (Müller *et al.*,2019). Immunization of rabbits with RHDV2 VLPs resulted in high production of serum antibodies against vp60, and increased production of cytokines IFN-γ and IL-4 in the immunized rabbits compared to the control animals (Miao *et al*.,2019). The results demonstrated that the recombinant RHDV2 VLPs are highly immunogenic and these VLPs can be of use in serological assays and development of vaccines.

To develop a new subunit vaccine that could protect rabbits against both classic RHDV and RHDV2 infections, Qi *et al.* (2020) constructed a recombinant baculovirus containing the vp60 genes of both classic RHDV and RHDV2 (Bac-classic RHDV vp60-RHDV2 vp60). Both vp60 genes were well expressed simultaneously in Sf9 cells, and the recombinant vp60 self-assembled into VLPs. Both humoral and cellular immune responses were efficiently induced in rabbits by the bivalent VLP vaccine, and the immunized rabbits survived challenge with classic RHDV. The recombinant baculovirus carrying two vp60 genes can be used to develop a bivalent VLP vaccine against both RHDV1 and RHDV2 infections.

Two novel B cell epitopes located at the C-terminus of vp60 were identified in RHDV1, and these were 64 and 53 amino acids longer, respectively than normal B cell epitopes (DeSheng *et al.*, 2015). The capsid protein vp60, is divided into shell (S) and protruding (P) domains, and the more exposed P domain likely contains determinants for cell attachment and antigenic diversity. Linear B-cell epitopes in the P domain of vp60, viz., ^{326}NPISQV^{331}, ^{338}DMSFV^{342} and ^{562}KSTLVFNL^{569} were identified using MAbs (Song *et al.*, 2016). The epitope ^{338}DMSFV^{342} was conserved among all RHDV isolates. The epitopes ^{326}NPISQV^{331}and ^{562}KSTLVFNL^{569} were highly conserved among RHDV G1-G6 and variable in RHDV2 strains. Binding of epitopes in vp60 to histo-blood group antigens (HBGAs) was analysed, and it was found that the epitopes ^{326}NPISQV^{331}, ^{338}DMSFV^{342} in the P2 subdomain could significantly bind to blood group H type 2.

Conclusion

Following the use of Rabbit haemorrhagic disease virus to control European rabbit population in Australia, the disease has become a transboundary infection prevalent in many countries covering all the five continents. Two major antigenic subtypes of the virus, initially RHDV1 in 1984 and RHDV2 since 2010, are responsible for the same clinical disease with different host specificity. The RHDV2 has overtaken the parent virus population of RHDV1 in prevalence, and the rabbits vaccinated with RHDV1 remain susceptible to RHDV2. Detailed analysis of the ecology is required to understand the dominance of RHDV2 over RHDV1 in the nature subsequent to its appearance/ existence. The RHDV is a model to study RNA virus evolution and analysis of quasi species nature of positive sense RNA virus. The precise mechanism of innate immunity imparting resistance in young rabbits

(kittens) to RHDV1 infection, but at the same time maintaining susceptibility to RHDV2 infection, remains to be fully understood. Development of methodology for *in vitro* amplification of the virus is required in order to develop a bi-valent vaccine covering both RHDV1 and 2 antigenic subtypes. Countries where RHD is yet to be reported/ identified need to develop diagnostic preparedness.

Acknowledgements

Information available in the Internet (NCBI-PubMed, WHO, OIE, ICMR, ICAR, Govt of India, Wikipedia, Newspaper and News periodicals etc.) has been incorporated in the present review. We sincerely acknowledge each of these.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Abrantes, J., van der Loo, W., Pendu, J. L. and Esteves, P. J. (2012). Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. *Veterinary Research*, 43,12-31.
2. Abrantes, J., Droillard, C., Lopes, A.M., Lemaitre, E., Lucas, P., Blanchard, Y., Marchandeau, Y., Esteves, P.J. and Le Gall-Reculé, G. (2020). Recombination at the emergence of the pathogenic rabbit haemorrhagic disease virus Lagovirus europaeus/GI.2, *Scientific Reports*, 10, 14502.
3. Almeida, T., Lopes, A.M., Magalhães, M.J., Neves, F., Pinheiro, A., Gonçalves, D., Leitão, M., Esteves, P.J. and Abrantes, J. (2015). Tracking the evolution of the G1/ RHDVb recombinant strains introduced from the Iberian Peninsula to the Azores islands, Portugal. *Infection Genetics and Evolution*, 34, 307–313.
4. Bárcena, J., Guerra, B., Angulo, I., González, J., Valcárcel, F., Mata, C.P., Castón, J. R., Blanco, E. and Alejo, A. (2015). Comparative Analysis of Rabbit Hemorrhagic Disease Virus (RHDV) and New RHDV2 Virus Antigenicity, Using Specific Virus-Like Particles. *Veterinary Research,* 46, 106-112.
5. Bonvehí,C., María, A., Montesinos, A., Juan-Sallés, C., Gómez, A., Teso, B., Barbero, S. and Ferrera, S. E. (2019). Clinicopathologic Findings of Naturally Occurring Rabbit Hemorrhagic Disease Virus 2 Infection in Pet Rabbits. *Veterinary Clinical Pathology*, 48, 89-95.
6. Calvete, C., Mendoza, M., Sarto, M.P., de Bagüés, M.P.J., Luján, L., Molín, J., Calvo, A.J., Monroy, F. and Calvo, J.H. (2019). Detection of Rabbit Hemorrhagic Disease Virus GI.2/RHDV2/b in the Mediterranean Pine Vole (Microtus Duodecimcostatus) and White-Toothed Shrew (*Crocidura russula*). *Journal of Wildlife Disease*, 55, 467-472.
7. Camarda, A., Pugliese, N., Cavadini, P., Circella ,E., Capucci, L., Caroli, A., Legretto, M., Mallia, E. and Lavazza, A. (2014). Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in Sicily from rabbit (Oryctolagus cuniculus) and Italian hare (Lepus corsicanus). *Research in Veterinary Science,* 97,642–645.
8. Cancellotti, F.M. and Renzi, M. (1991). Epidemiology and Current Situation of Viral Haemorrhagic Disease of Rabbits and the European Brown Hare Syndrome in Italy. *Revue Scientifique et Technique,*10, 409-22.
9. Capucci, L., Cavadini, P., Schiavitto, M., Lombardi, G. and Lavazza, A. (2017). Increased pathogenicity in rabbit haemorrhagic disease virus type 2 (RHDV2). *Veterinary Record*, 180, 426-427.
10. Carvalho, C.L., Silva, S., Gouveia, P., Costa, M., Duarte, E.L., Henriques, A.M., Barros, S.S., Luís, T., Ramos., Fagulha, T., Fevereiro, M. and Margarida Dias Duarte, M. D. (2017a). Emergence of rabbit haemorrhagic disease virus 2 in the archipelago of Madeira, Portugal (2016–2017). *Virus Genes,* 53, 922–926.
11. Carvalho, C.L., Duarte, E.L., Monteiro, M., Botelho, A., Albuquerque, T., Fevereiro, M., Henrique, A.M., Barros, S.S. and Duarte, M.D. (2017b). Challenges in the Rabbit Haemorrhagic Disease 2 (RHDV2) Molecular Diagnosis of Vaccinated Rabbits. *Veterinary Microbiology,* 198, 43-50.

12. Chen, L., Liu, G., Ni, Z., Yu, B., Yun, T., Song, Y., Hua, J., Li, S. and Chen, J. (2009). Minor structural protein VP2 in rabbit hemorrhagic disease virus downregulates the expression of the viral capsid protein VP60. *Journal of General Virology,* 90, 2952-55.
13. Cooke, B. D. and Fenner, F. (2002). Rabbit haemorrhagic disease and the biological control of wild rabbits, Oryctolagus cuniculus, in Australia and New Zealand. *Wildlife Research,* 29, 689-706.
14. Dalton, K. P., Arnal, J. L ., Benito, A. A., Chacón, G., Martín Alonso, J.M. and Parra, F. (2018b). Conventional and Real Time RT-PCR Assays for the Detection and Differentiation of Variant Rabbit Hemorrhagic Disease Virus (RHDVb) and Its Recombinants. *Journal of Virological Methods,* 51, 118-122.
15. Dalton, K.P., Podadera, A., Granda, V., Nicieza, I., Del Llano,, González, R., de Los Toyos, J.R., García Ocaña, M., Vázquez, F., Martín Alonso, J.M.M., Prieto, J.M., Parra, F. and Casais, R. (2018a). ELISA for Detection of Variant Rabbit Haemorrhagic Disease Virus RHDV2 Antigen in Liver Extracts. *Journal of Virological Methods,* 251, 38-42.
16. DeSheng, K., Jiasen, L., Qian, J., Zuo, Y., Xiaoliang, H., Dongchun, G., Qianqian, H., Meihui, J. and Liandong, Q. (2016). Production, Characterization, and Epitope Mapping of Monoclonal Antibodies Against Different Subtypes of Rabbit Haemorrhagic Disease Virus (RHDV). *Scientific Reports,* 6, 20857-20869.
17. DeSheng, K., Liu, H., Liu, J., Yu, Z., Jiang, Q., Guo, D., Hu, X., Wang, F., Huang, Q. and Qu, L. (2015). Identification of Two Novel Rabbit Hemorrhagic Disease Virus (RHDV) B Cell Epitopes and Evaluation of Its Immunoprotection Against RHDV. *Applied Microbiology and Biotechnology,* 99, 5951-66.
18. Desselberger, U. (2019). *Caliciviridae* other than *Noroviruses*. *Viruses,*11, 286
19. Duarte, M.D., Carvalho, C., Bernardo, S., Barros, S.V., Benevides, S., Flor, L., Monteiro, M., Marques, I., Henriques, M., Barros, S. C., Fagulha, T., Ramos, F., Luís, T. and Fevereiro, M. (2015). Rabbit haemorrhagic disease virus 2 (RHDV2) outbreak in Azores: Disclosure of common genetic markers and phylogenetic segregation within the European strains. *Infection, Genetics and Evolution,* 35, 163-171.
20. Duarte, M.D., Carvalho, C.L., Barros, S.C., Henriques, A.M., Ramos, F., Fagulha, T., Luís, T., Duarte, E. L. and Fevereiro, M. (2015a). A Real Time Taqman RT-PCR for the Detection of Rabbit Hemorrhagic Disease Virus 2 (RHDV2). *Journal of Virological Methods,* 219, 90-95.
21. Esteves, P. J., Abrantes, J., Bertagnoli, S., Cavadini, P., Gavier-Widén, D. and Guitton, J.S. (2015). Emergence of Pathogenicity in Lagoviruses: Evolution from Pre-existing Nonpathogenic Strains or through a Species Jump? *PLoS Pathogens,* 11-19.
22. Fitzner, A. and Niedbalski, W. (2016). Serological Survey for RHD Antibodies in Rabbits from Two Types of Rabbit Breeding Farms. *Polish Journal of Veterinary Sciences,* 19, 597-607.
23. Fitzner, A. and Niedbalski, W. (2018). Detection of Rabbit Haemorrhagic Disease Virus 2 (GI.2) in Poland. *Polish Journal of Veterinary Sciences,* 21, 451-458.
24. Forrester, N.L., Mossa, S.R., Turner, S.L., Schirrmeier, H and Gould, E.A. (2008). Recombination in rabbit haemorrhagic disease virus: Possible impact on evolution and epidemiology, *Virology,* 376, 390–396.
25. Goodfellow, I., Chaudhry, Y., Gioldasi, I., Gerondopoulos, A., Natoni, A., Labrie, L., Laliberte, J. F. and Roberts, L. (2005). Calicivirus translation initiation requires an interaction between VPg and eIF 4 E. *EMBO Reports,* 6, 968-972.
26. Hall, R,N., Mahar, J.E., Read ,A.J., Mourant, R., Piper, M., Huang, N. and Strive, T. (2018). A Strain-Specific Multiplex RT-PCR for Australian Rabbit Haemorrhagic Disease Viruses Uncovers a New Recombinant Virus Variant in Rabbits and Hares. *Transboundary and Emerging Diseases,* 65(2), 444- 456.
27. Hall, R.N., Mahar, J.E., Haboury, S., Stevens, V., Holmes, E.C. and Strive, T. (2015). Emerging rabbit hemorrhagic disease virus 2 (RHDVb), Australia. *Emerging Infectious Diseases,* 21, 2276–2278.
28. Harcourt-Brown, F. (2020). History of rabbit haemorrhagic disease (RHD). https://www.harcourt-brown.co.uk/articles/infectious-disease/rabbit-haemorrhagic-disease/history-of-rabbit-haemorrhagic-disease).
29. Harcourt-Brown, N., Silkstone, M., Whitbread, T.J. and Harcourt-Brown, F.M. (2020). RHDV2 Epidemic in UK Pet Rabbits. Part 1: Clinical Features, Gross Post Mortem and Histopathological Findings. *Journal of Small Animal Practice,* 61, 419-427.
30. Hukowska-Szematowicz, B. (2020). Genetic variability and phylogenetic analysis of Lagovirus europaeus strains GI.1 (RHDV) and GI.2 (RHDV2) based on the RNA-dependent RNA polymerase (RdRp) coding gene. *Acta Biochemica Polonica,* 67, 111–122.
31. Ismail, M.M., Mahmoud, H.A.M., Ibrahim, M. E. and Mohamed, A.A. (2017). Emergence of New Virulent Rabbit Hemorrhagic Disease Virus Strains in Saudi Arabia. *Tropical Animal Health and Production,* 49(2),295-301.

32. Katherine, E.Q. and James, W.C. (2012). Ferrets, Rabbits, and Rodents-Clinical medicine and surgery. 3rd ed. Elsevier: Saunders, USA.
33. Kinnear, M. and Linde, C.C. (2010). Capsid gene divergence in rabbit hemorrhagic disease virus. *Journal of General Virology,* 91,174–181.
34. Konig, M., Thiel, H.J. and Meyers, G. (1998). Detection of viral proteins after infection of cultured hepatocytes with rabbit hemorrhagic disease virus. *Journal of Virology,* 72, 4492-4497.
35. Kovaliski, J., Sinclair, R., Mutze, G., Peacock, D., Strive, T., Abrantes, J., Esteves, P.J. and Holmes, E.C. (2014). Molecular epidemiology of Rabbit Haemorrhagic Disease Virus in Australia: when one became many. *Molecular Ecology,* 23, 408-420.
36. Le Gall-Reculé, G., Lemaitre, E., Bertagnoli, S., Hubert, C., Top, S., Decors, A., Marchandeau, S. and Guitton, J.S. (2017). Large-scale lagovirus disease outbreaks in European brown hares (Lepus europaeus) in France caused by RHDV2 strains spatially shared with rabbits (Oryctolagus cuniculus). *Veterinary Research,* 48,70-79.
37. Le Gall-Reculé, G., Antonio, L., Stéphane, M., Stéphane, B., Françoise, Z., Patrizia, C., Nicola, M., Guerino, L., Jean-Luc, G., Evelyne, L., Anouk, D., Samuel, B., Bernadette Le, N. and Lorenzo, C. (2013). Emergence of a New Lagovirus Related to Rabbit Haemorrhagic Disease Virus. *Veterinary Research,* 44, 81.
38. Liu, G., Ni, Z., Yun, T., Yu, B., Chen, L., Zhao, W., Hua, J. and Chen, J. (2008). A DNA-launched reverse genetics system for rabbit hemorrhagic disease virus reveals that the VP2 protein is not essential for virus infectivity. *Journal of General Virology,*89, 3080-3085.
39. Liu, S.J., Xue, H.P., Pu, B.Q. and Qian, N.H. (1984). A new viral disease in rabbit. *Animal Husbandry and Veterinary Medicine,* 16, 253-255.
40. Lopes, A. M., Dalton, K.P., Magalhães, M. J., Parra, F., Esteves, P.J., Holmes, E.C. and Abrantes, J. (2015). Full genomic analysis of new variant rabbit hemorrhagic disease virus revealed multiple recombination events. *Journal of General Virology,* 96, 1309–1319.
41. Lopes ,A.M., Blanco-Aguiar, J., Martín-Alonso, A., Leitão, M., Foronda, P., Marco Mendes, M., Gonçalves, D., Abrantes ,J. and Esteves, P.J. (2018). Full Genome Sequences Are Key to Disclose RHDV2 Emergence in the Macaronesian Islands. *Virus Genes,* 54, 1-4.
42. Lopes, A.M., Silvério, D., Magalhães, M.J., Helena, A., Paulo, C., Alves., Pedro, J., Esteves and Joana Abrantes. (2017). Characterization of old RHDV strains by complete genome sequencing identifies a novel genetic group. *Scientific Reports,* 7, 13599-13606.
43. Machin, A., Martin Alonso, J.M, Dalton, K.P. and Parra, F. (2009). Functional differences between precursor and mature forms of the RNA-dependent RNA polymerase from rabbit hemorrhagic disease virus. *Journal of General Virology,* 90, 2114-2118.
44. Machin, A., Martin Alonso, J.M. and Parra, F. (2001). Identification of the amino acid residue involved in rabbit hemorrhagic disease virus VPg uridylylation. *Journal Biological Chemistry,* 276, 27787-92.
45. Mahar , J.E., Hall, R. N., Peacock, D., Kovaliski, J., Piper, M., Mourant, R., Huang, N., Campbell, S., Gu, X., Read, A., Urakova, N., Cox, T., Holme,, E.C. and Strive, T. (2018). Rabbit Hemorrhagic Disease Virus 2 (RHDV2; GI.2) Is Replacing Endemic Strains of RHDV in the Australian Landscape Within 18 Months of Its Arrival. *Journal of Virology,* 92(2), 1374-17.
46. Martin-Alonso, A., Martin-Carrillo, N., Garcia-Livia, K., Valladares, B. and Foronda, P. (2016). Emerging Rabbit Haemorrhagic Disease Virus 2 (RHDV2) at the Gates of the African Continent. *Infection Genetics and Evolution,* 10, 46-50.
47. Meyers, G., Wirblich, C. and Thiel, H. J. (1991a). Rabbit hemorrhagic disease virus–molecular cloning and nucleotide sequencing of a calicivirus genome. *Virology,* 184, 664-676.
48. Meyers, G., Wirblich, C. and Thiel, H.J. (1991b). Genomic and subgenomic RNAs of rabbit hemorrhagic disease virus are both protein-linked and packaged into particles. *Virology,* 184, 677–686.
49. Meyers, G., Wirblich, C., Thiel, H.J. and Thumfart, J.O. (2000). Rabbit hemorrhagic disease virus: genome organization and polyprotein processing of a calicivirus studied after transient expression of cDNA constructs. *Virology,* 276, 349-363.
50. Miao, Q., Ruibing, Q., Luut, V., Jooske, I., Marja, L. K., Jie, Z., Aoxing, T., Dandan, D., Yonghong, S., Monique, M. V., Guangqing, L. and Gorben, P. P. (2019). Immunogenicity in Rabbits of Virus-Like Particles from a Contemporary Rabbit Haemorrhagic Disease Virus Type 2 (GI.2/RHDV2/b) Isolated in The Netherlands. *Viruses,*11, 553-567.
51. Morisse, J.P., Le Gall, G. and Boilletot, E. (1991). Hepatitis of viral origin in Leporidae: introduction and aetiological hypotheses. *Revue Scientifique Technique,*10, 283-295.

52. Müller, C., Ulrich, R., Schinköthe, J., Müller, M. and Köllner, B. (2019).Characterization of Protective Humoral and Cellular Immune Responses Against RHDV2 Induced by a New Vaccine Based on Recombinant Baculovirus. *Vaccine,* 37, 4195-4203.
53. Neave, M.J., Robyn, N. H., Nina, H., Kenneth, A.M., Peter, K., Marion, H., Jennifer, T. and Tanja, S. (2018). Robust Innate Immunity of Young Rabbits Mediates Resistance to Rabbit Hemorrhagic Disease Caused by Lagovirus Europaeus GI.1 But Not GI.2. *Viruses*, 10, 512-534.
54. Neimanis, A., Ulrika, L. P., Nina, H., Dolores, G.W. and Tanja, S. (2018a). Elucidation of the Pathology and Tissue Distribution of Lagovirus Europaeus GI.2/RHDV2 (Rabbit Haemorrhagic Disease Virus 2) in Young and Adult Rabbits (Oryctolagus Cuniculus). *Veterinary Research,* 49, 46-61.
55. Neimanis, A.S., Ahola, H., Zohari, S., Pettersson, U.L., Bröjer, C.L., Capucci, L. and Gavier-Widén, D. (2018b). Arrival of Rabbit Haemorrhagic Disease Virus 2 to Northern Europe: Emergence and Outbreaks in Wild and Domestic Rabbits (Oryctolagus Cuniculus) in Sweden. *Transboundary and Emerging Diseases*, 65, 213-220.
56. O'Keefe, J. S., Tempero, J., Atkinson, P.H., Pacciarini, L., Fallacara, F., Horner, G.W. and Motha, J. (1998). Typing of rabbit haemorrhagic disease virus from New Zealand wild rabbits. *New Zealand Veterinary Journal,* 46, 42-43.
57. Park, N.Y., Chong, C.Y., Kim, J.H., Cho, S.M., Cha, Y.H., Jung, B.T., Kim,D.S. and Yoon, J.B. (1987). An outbreak of viral haemorrhagic pneumonia (tentative name) of rabbits in Korea. *Journal of Korean Veterinary Medical Association,* 23, 603-610.
58. Peacock, D., Kovaliski, J., Sinclair, R., Mutze, G., Iannella, A. and Capucci, L. (2017). RHDV2 overcoming RHDV immunity in wild rabbits (*Oryctolagus cuniculus*) in Australia. *Veterinary Record,* 180, 280-282.
59. Puggioni, G., Cavadini, P., Maestrale, C., Scivoli, R., Botti, G., Ligios, C., Le Gall-Reculé, G., Lavazza, A. and Capucci, L. (2013). The new French 2010 rabbit hemorrhagic disease virus causes an RHD-like disease in the Sardinian Cape hare (Lepus capensis mediterraneus). *Veterinary Research,* 44, 96-103.
60. Qi, R., Miao, Q., Zhu, J., Tang, J., Tang, A., Wang, X., Dong, D., Guo, H. and Liu, G. (2020). Construction and Immunogenicity of Novel Bivalent Virus-Like Particles Bearing VP60 Genes of Classic RHDV(GI.1) and RHDV2(GI.2). *Veterinary Microbiology*, 240, 108529-108540.
61. Qi, R., Zhu, J., Miao, Q., Tang, A., Dong, D., Wang, X. and Liu, G. (2019). Bioinformatics Analysis of Capsid Protein of Different Subtypes Rabbit Hemorrhagic Disease Virus. *BMC Veterinary Research,*15(1), 423-433.
62. Rouco, C., Aguayo-Adán ,J.A., Santoro, S., Abrantes, J. and Delibes-Mateos, M. (2019). Worldwide Rapid Spread of the Novel Rabbit Haemorrhagic Disease Virus (GI.2/RHDV2/b). *Transboundary and Emerging Diseases,* 66, 1762-1764.
63. Rouco, C., Abrantes, J., Serronha, A., Lopes, A.M., Maio, E., Magalhães, M.J., Blanco, E.J., Bárcena, J., Esteves, P.J., Santos, N., Alves, P.C. and Monterroso, P. (2018). Epidemiology of RHDV2 (Lagovirus europaeus/GI.2) in Free-Living Wild European Rabbits in Portugal. *Transboundary and Emerging Diseases*, 65, 373-382.
64. Sibilia, M., Boniotti, M.B., Angoscini, P., Capucci, L. and Rossi, C. (1995). Two independent pathways of expression lead to self-assembly of the rabbit hemorrhagic disease virus capsid protein. *Journal of Virology*,69, 5812-15.
65. Silvério, D., Lopes, A.M., Melo-Ferreira, J., Magalhães, M.J., Monterroso, P., Serronha, A., Maio, E., Alves, P.C., Esteves, P.J. and Abrantes, J. (2018). Insights into the Evolution of the New Variant Rabbit Haemorrhagic Disease Virus (GI.2) and the Identification of Novel Recombinant Strains. *Transboundary and Emerging Diseases*, 65, 983-992.
66. Song, Y., Fang, W., Zhiyu, F., Bo, H., Xing, L., Houjun, W., Jiabin, X., Weizhong, X. and Rulong, Q. (2016). Identification of Novel Rabbit Hemorrhagic Disease Virus B-cell Epitopes and Their Interaction with Host Histo-Blood Group Antigens. *Journal of General Virology,* 97, 356-365.
67. Strauss, J.H. and Strauss, E.G. (2008). Viruses and Human Disease, 2nd ed, Elsevier: Academic Press, USA.
68. Suckow, M. A., Brammer, D.W., Rush, H.G. and Chrisp, C.E. (2002). Biology and Diseases of Rabbits: in Laboratory Animal Medicine, 2nd ed, Elsevier Public Health Emergency Collection.
69. Terio, K.A., McAloose, D. and Leger, J. S. (2018). Pathology of Wildlife and Zoo Animals, 1st ed. Elsevier: Academic Press, USA.
70. Thompson, J. and Clark, G. (1997). Rabbit calicivirus disease now established in New Zealand. *Surveillance,* 24, 5-6.
71. Velarde, R., Cavadini, P., Neimanis, A., Cabezón, O., Chiari, M., Gaffuri, A., Lavín, S., Grilli, G., Gavier-Widén, D., Lavazza, A. and Capucci, L. (2017). Spillover events of infection of brown hares (Lepus

europaeus) with rabbit haemorrhagic disease type 2 virus (RHDV2) caused sporadic cases of a European brown hare syndrome-like disease in Italy and Spain. *Transboundary and Emerging Diseases*, 64, 1750-1761.
72. Virus Taxonomy (2012) Ninth Report of the International Committee on Taxonomy of Viruses, 977-986.
73. Westcott, D.G. and Choudhury, B. (2015). Rabbit haemorrhagic disease virus 2-like variant in Great Britain. *Veterinary Record*, 176,74-76.

****