3.7. Rabbit haemorrhagic disease (RHD)

Antonio Lavazza, Lorenzo Capucci

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna - OIE Reference Laboratory for Rabbit haemorrhagic disease, Via Bianchi 7 – 25124 Brescia, Italy

1. Introduction

Rabbit haemorrhagic disease (RHD) is a highly contagious and fatal acute hepatitis, of wild and domestic European rabbits (*Oryctolagus cuniculus*).

RHD was first reported in 1984 in the People's Republic of China [Liu *et al.*, 1984] and in Europe two years later enormous damages was caused to the rabbit industry, at least till the development of an inactivated vaccine and introduction of its use in prophylactic programs. RHD has a high rate of diffusion; in fact, outbreaks of RHD have been reported in over 40 countries in North and South America, Africa, Asia and Europe. As a rule, the presence of RHD as an endemic disease in several areas is the consequence of the presence of steady European rabbit population (i.e the contemporary presence of wild rabbits, familiar rabbitries and industrial farms). Of course, in spite of the availability of an effective vaccine, the goal of the eradication of RHD in these areas is very difficult to accomplish. Finally, RHD has been intentionally introduced in Australia and New Zealand (Cooke et Saunders, 2002), where rabbits cause serious ecological and economic problems and are considered a pest, in order to keep the level of rabbits reproduction as low as possible. From a pure scientific point of view, it will be very interesting to follow the evolution of the relationship between rabbits and the virus that cause RHD (RHDV) in Oceania, in comparison with the previous experience of the deliberate introduction of the Myxomavirus. One of the main questions is: will RHDV, a small round RNA virus, evolve in less virulent strains and in resistant populations of rabbits as occurred with the Myxomatosis virus, a large DNA virus?

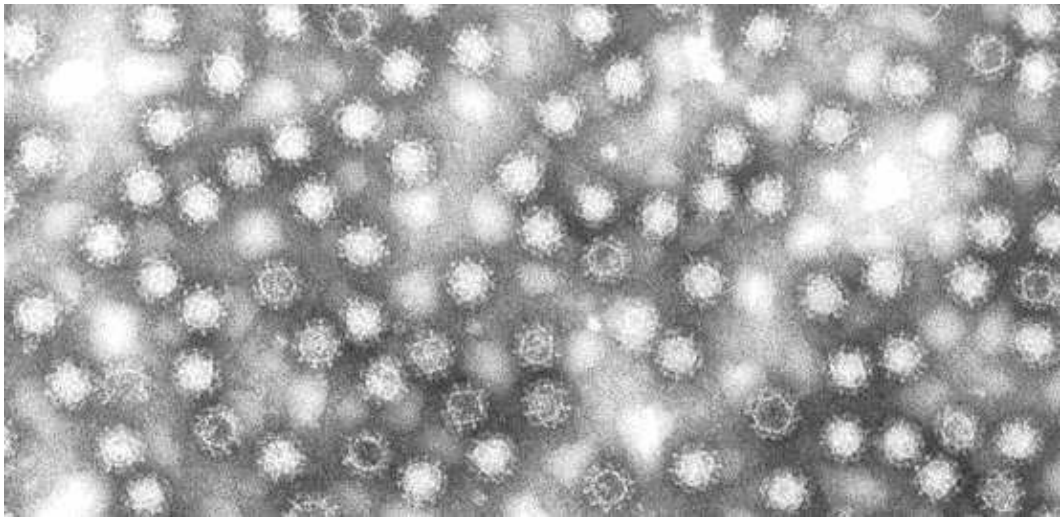

Figure 1. *Electron micrograph of RHD purified virions*

2. Characteristics of the causative agent

The causative agent of RHD (Figure 1) is a Calicivirus classified, together with the European brow hare syndrome virus (EBHSV), in the genus Lagovirus. It is 32–35 nm in diameter and has a single major capsid polypeptide (60 kDa), a positively stranded RNA genome of 7437 kb and a sub-genomic RNA of 2.2 [Meyers et al., 1991a, b; Parra et al., 1990; Ohlinger *et al.*, 1990]. The RHD virus (RHDV) capsid protein (VP60) folds in two

N-terminal 1 – 234 residues constitute the inner domain and the C-terminal residues beyond 235–579 constitute the protruding domain. In the overall picture of the capsid, these domains form the inner shell and the outer shell respectively, which are characterised by arch-like structures [Barcena *et al.*, 2004] (Figure 2). This structure also correlates with the antigenic characteristics of RHDV, in fact the main antigenic determinants are located on the C-terminal end of the VP60 [Capucci *et al.*, 1995; Capucci *et al.*, 1998; Schirrmaier *et al.*, 1999; Wirblich *et al.*, 1994] (Figure 3).

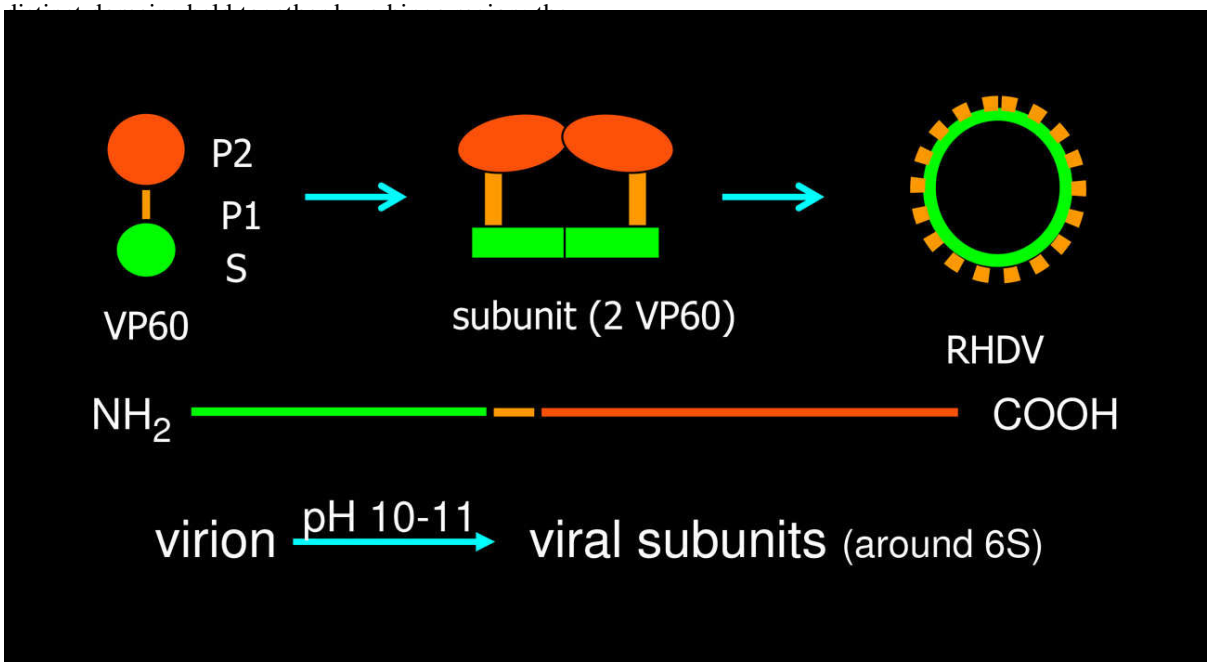

Figure 2. *Schematic representation of the folding of the (VP60) capsid protein of RHDV*

Table 1. Main characteristics of smooth RHDV (sRHDV) in comparison with "full" mature RHD virions

	RHDV	sRHDV
DIAMETER (nm)	32-35	25-30
SEDIMENTATION (S)	170	145
STRUCT. PROTEIN (Kd)	60	28-30
HA (extract 10%)	4-8x103	NEG
INFECTIVITY (LD_{50}) (1 ml extract 10%)	105-107	? NEG ?
ANTIGENICITY		
- RHDV MAbs (ext. epitopes)	pos	neg
- RHDV MAbs (int. epitopes)	pos	pos
- EBHS MAbs (ext. epitopes)	neg	pos
- □RHDV serum	pos	pos
- □EBHSV serum	neg	pos

A second type of virus particle is commonly found as the main component in approximately 5% of the RHDV-positive specimens, i.e. those taken from rabbits showing a protracted course of the disease [Barbieri *et al.*, 1997; Capucci *et al.*, 1991; Granzow *et al.*, 1996]. The main characteristics of this particle, called "smooth RHDV" (s-RHDV) are reported in Table 1. It corresponds to the inner shell of RHDV and large amounts of it could be detected especially from 3–4 days post-infection, when specific anti-RHDV IgM are appearing, only in the liver and spleen and not in the

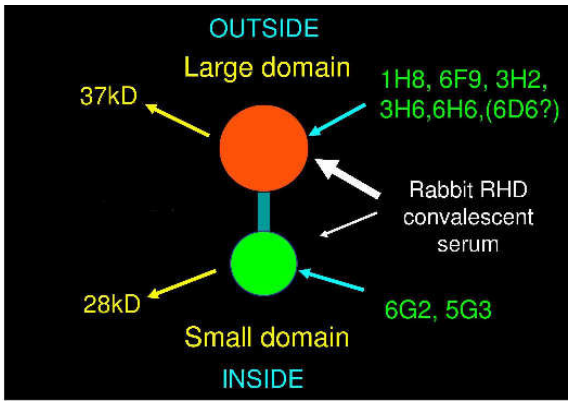

Figure 3. *Schematic representation of the VP60 structure and antigenicity according to the study of Capucci et al., (1995).*

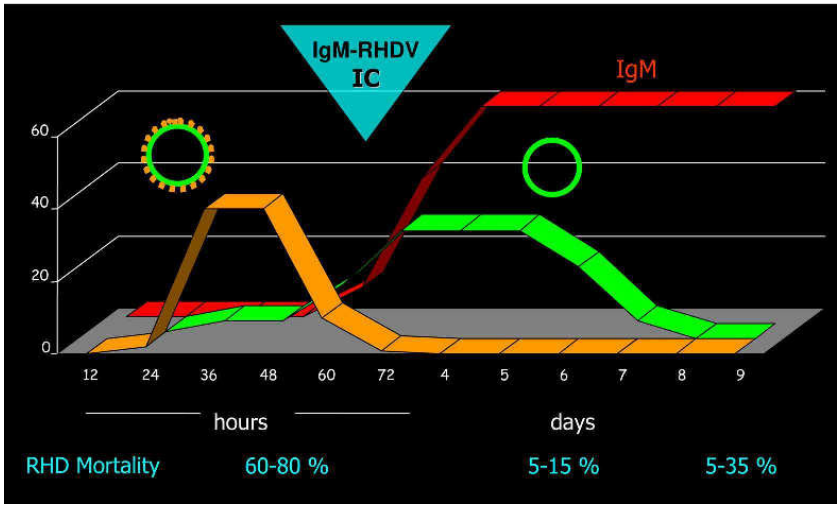

Figure 4. *Timing and evolution of RHD infection following i.m. inoculation of rabbits with a virulent RHDV strain.*

bloodstream These data, in association with the finding of fragments of the VP60 having different molecular weight (41–30 kDa), during transition from RHDV to s-RHDV led Barbieri *et al.* [1997] to conclude that the genesis of the particle is due to a degradative process that is probably the consequence of the physiological clearance of the RHDV-IgM immuno-complex formed in large amounts at the beginning of the humeral response (Figure 4). Therefore the identification of this second particle in the liver of a rabbit can be considered to be a marker of the sub acute/chronic form of RHD that usually evolves between 4 and 8 days post-infection and is followed either by the death of the rabbit or, more often, by its recovery [Barbieri *et al.*, 1997].

RHDV is very stable and resistant in the environment; the viral infectivity is not reduced by treatment with ether or chloroform and trypsin, by exposure to pH 3.0, or by heating to 50°C for 1 hour (Capucci, unpublished data). RHDV in rabbit carcasses can survive for at least 3 month in the field, while virus exposed directly to environmental conditions is viable for a period less than a month [Henning *et al.,* 2005]. Indeed, according to Smid *et al.* [1991] the virus survives at least 225 days in an organ suspension kept at 4°C, at least 105 days in the dried state on cloth at room temperature, and at least 2 days at 60°C, both in organ suspension and in the dried state.

Treatment of RHD virions at pH 11 induces the breakdown of the virions and the production of 6S VP60 subunits (Capucci, unpublished data). RHDV is inactivated by 10% sodium hydroxide, by 1.0–1.4% formaldehyde and by 0.2–0.5% beta-propiolactone at 4°C. Such treatments do not alter the immunogenicity of the virus.

3. Virus variability

All known RHD viral isolates belong to one serotype. The complete sequence of geographically different RHD strains has been reported and their comparison reveals close overall homology in terms of genome sequence with few or no predicted changes in amino acid composition (differences between 2% and 5%) [Le Gall *et al.*, 1998; Nowotny *et al.*, 1997]. Nevertheless, isolates that exhibit temperature-dependant differences in haemoagglutinating characteristics [Capucci *et al.*, 1996a] have been described, and a consistent genetic and antigenic RHDV variant has been identified simultaneously in Italy [Capucci *et al.*, 1998] and Germany [Schirrmaier *et al.*, 1999]. This RHDV variant, named RHDVa, presents amino acid changes in the surface-exposed E region (aa 344–434) that contains the main antigenic epitopes of calicivirus, three times higher than in all previously sequenced RHDV isolates (Figure 5). However, rabbits experimentally vaccinated with the currently available RHDV vaccine were protected from the challenge with RHDVa, even with a lower efficiency.

An epidemiological study carried out to compare the rate of diffusion of RHDV and RHDVa in Italy during the last years [Lavazza *et al.*, 2004] has shown that RHDVa is present in most parts of Italy and that it is rapidly replacing the RHDV "classical" strain (Table 2). Outside Italy, RHDVa was identified almost contemporaneously in Germany but it also caused the first outbreaks of RHD in USA in spring 2000, in Uruguay in winter 2004 and again in USA on 2005. It has also been detected in France (2000) and Malta (2004), which suggests that RHDVa could be diffused in other European countries that have been experiencing the disease for many years. Finally, looking at the

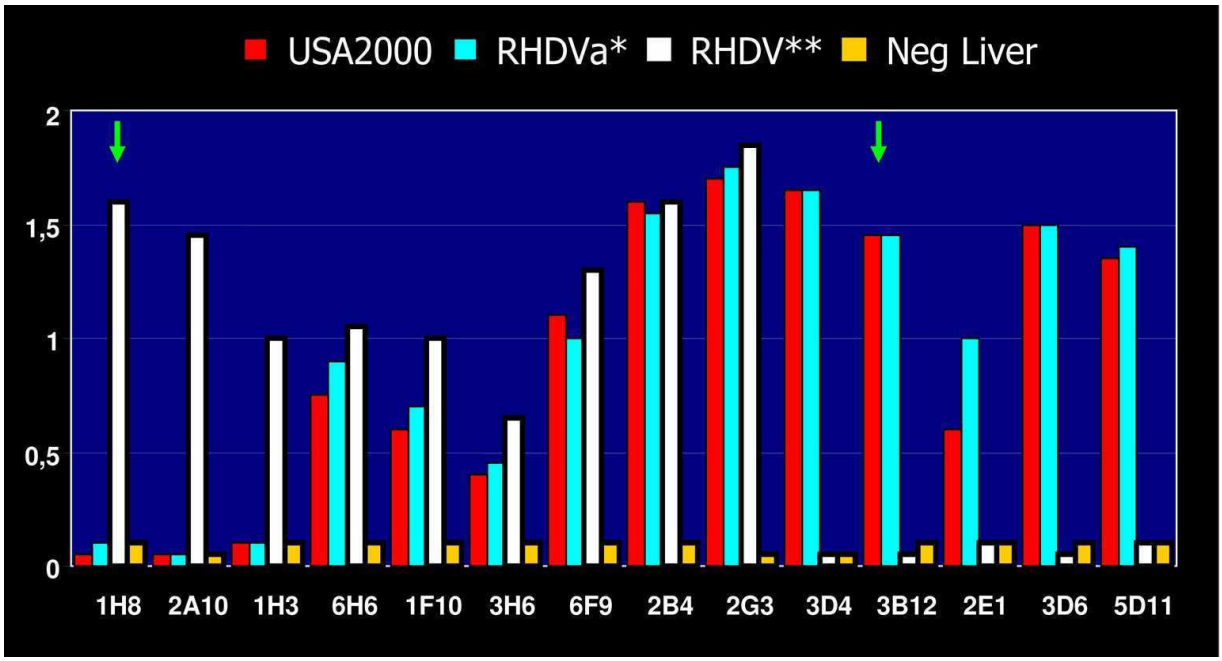

Figure 5. *RHDV antigenic typing using MAbs: the first strain of RHDVa identified at Pavia on 1997 (*) is compared with the RHDV classical strain BS89 (**) and one RHDVa strain isolated in USA on 2000. The arrow indicates the two most relevant MAbs used for differentiating the "classical" RHD from the "variant" RHDVa strain..*

Table 2. Total number of RHD cases observed in Italy during the last four years and relative frequency of classical (RHDV) and Variant (RHDVa) strains.

Year	Total samples examined.	Total RHD positive (%)	(%)RHDV-Positives	(%)RHDVa. Positives
2000	252	134 (53,2%)	89 (66,4%)	45 (33,6%)
2001	136	69 (50,6%)	25 (36,2%)	44 (63,8%)
2002	203	138 (67,9%)	61 (44,2%)	77 (55,8%)
2003	226	63 (25,9%)	12 (19,0%)	51 (81,0%)
2004	209	124 (59,3%)	32 (25,8%)	92 (74,2%)

RHDV genetic sequences deposited at the NCBI databank, the presence of RHDVa in China is evident too.

Another virus, provisionally called rabbit calicivirus (RCV) and related to the RHDV, has been identified in healthy rabbits [Capucci *et al.*, 1996b; 1997]. It is significantly different from the previously characterised RHDV isolates in terms of pathogenicity, viral titre and tissue tropism. RCV is avirulent, replicates in the intestine at a very low titre and has about a 92% genomic similarity to RHDV from which follows a high degree of antigenic correlations.

Recent studies conducted in Italy have shown that such virus is quite widespread in industrial rabbit farms [Capucci *et al.*, 2004b]. In fact, in order to check the diffusion of RCV in Italian rabbit farms we conducted, along a 5 years period: (1999-2004), a survey respectively in 39 farms in North Italy, 23 farms in Central Italy and 21 farms in South Italy, by testing non-vaccinated 80 day old growing rabbits at the slaughterhouse. The results indicate the presence of "natural antibodies" presumably induced by RCV, i.e. over 75% of animals showing titres $\geq$1/20, in almost 30% of farms controlled in North and South Italy, and in 52.2% of the farms controlled in Central Italy (Table 3).

As result of the extensive use of serological test on different rabbits populations, further evidence exist that, in addition to RCV, one or more RHDV-like non-pathogenic viruses are present in wild rabbit populations in a large part of south-eastern Australia as well as in New Zealand [Cooke *et al.*, 2002; Nagesha *et al.*, 2000; O'Keefe *et al.*, 1999; Robinson *et al.*, 2002]. The serological data indicate that the putative RHDV-like virus suspected to be present in Oceania is characterized, differently than

Table 3. Results of seroepidemiological surveys for detecting anti-RCV antibodies in non-vaccinated grow slaughterhouse.

Serological result	Criteria applied	N. groups tested (%)		
		North Italy 1999	Central- South Italy 2002-03	Central Italy 2004
Positive	> 75% of positive sera	13 (33,3%)	4 (19,1%)	12 (52,2%)
Doubtful	5-10% of positive sera	2 (5,2%)	0 (0%)	2 (8,7%)
	20-60% of positive sera	0 (0%)	5 (23,8%)	2 (8,7%)
Seroconversion	from 0% to >75% of positive sera	0 (0%)	1 (4,7%)	0 (0%)
Negative	> 95% of positive sera	24 (61,5%)	11 (52,4%)	7 (30,4%)
Total		39	21	23

RCV, by a consistent genetic and antigenic difference from RHDV, estimable in more than 40% of amino acid substitution in the outer part of the VP60 [Capucci personal observations].
Antibodies against RHD were detected in sera collected in Europe between 1975 and 1987, showing that RHDV-like viruses were already present, but simply had not been detected before the first evidence of the disease [Rodak *et al.*, 1990]. More recent serological data suggest that non-pathogenic strains may usually be present in wild European rabbit populations, because high antibody levels have been detected even where RHD had never been recorded or suspected [Marchendeau *et al.*, 2005].

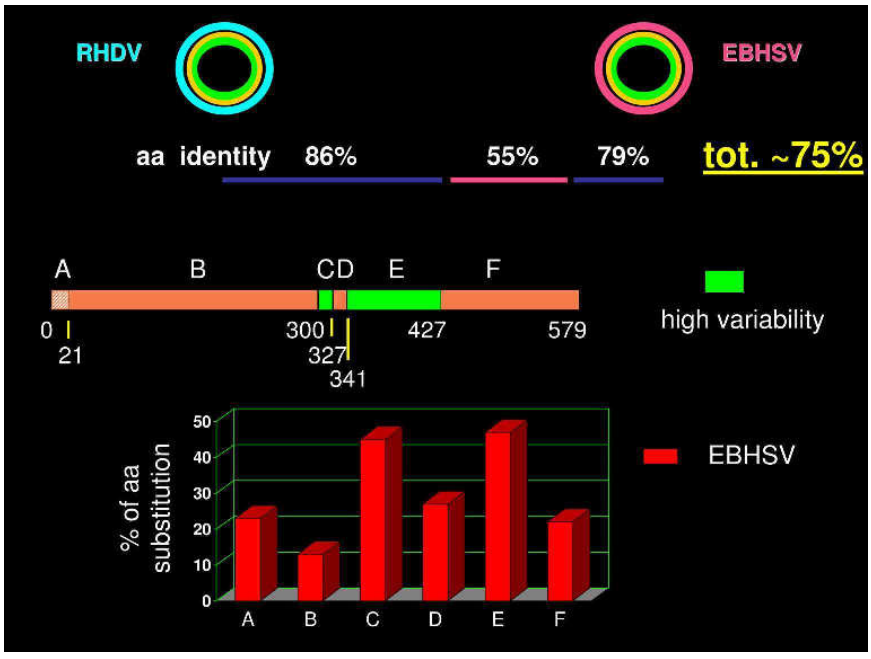

Figure 6. *Schematic representation of the structural differences between RHDV and EBHSV. The subdivision of the structural protein of RHDV in relation to the degree of variability in Calicivirus was done according to Neill (1992).*

4. The disease

The European rabbit (*Oryctolagus cuniculus*) is the only species affected by RHD and no other lagomorphs of the genus *Romerolagus, Lepus* and *Sylvilagus* (including the cottontail) normally present in North Central and South America have been shown to be susceptible [Gould *et al.*, 1997]. A similar disease, termed European brown hare syndrome (EBHS), has been described in the hare (*Lepus europaeus*), but the causative calicivirus is different from RHDV, although it is related antigenically [Capucci *et al.*, 1991] (Figure 6). Cross infection does not occur by experimental infection of rabbits with EBHSV and hares with RHDV [Lavazza *et al.*, 1996]. Recent studies aimed at finding the susceptibility of cottontail to EBHSV revealed a diffuse seroprevalence of the virus in a wild population of cottontail rabbits and the possibility of inducing clinical disease and mortality in a low number of experimentally infected cottontails [Tizzani *et al.*, 2002]. RHD is characterised by high morbidity and a mortality rate between 40%

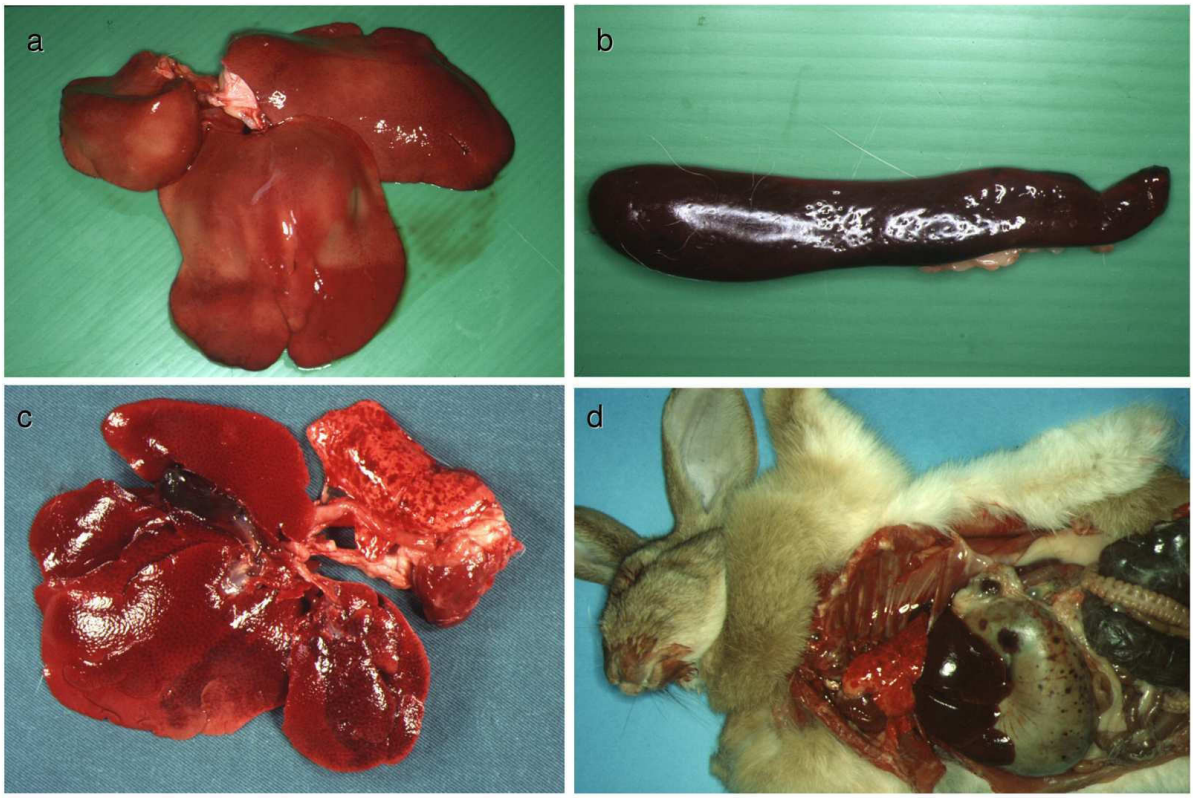

Figure 7. *RHDV macroscopic lesions: a) liver degeneration: the liver is enlarged, discoloured and friable. b) spleen enlargement and congestion. c) liver congestion and lung haemorrhages. d) rabbit die due to acute acute disease shows diffuse haemorrhages and a sero-heamorrhagic liquid from the nostrils*

.

and 90%. Infection occurs in rabbits of all ages, but clinical disease is observed only in adults and young animals older than 40–50 days. The pathogenic mechanism of resistance in young animals is still unclear [Cooke, 2002]. A difference in the cellular inflammatory response of the liver following an RHDV infection of susceptible adult rabbits and resistant young ones was observed, and the persistence, following RHDV infection in young rabbits, of increased value of liver transaminases determines a chronic course of the disease and the possible role of these animals as a source of virus transmission [Ferreira *et al.*, 2004; 2005].

The clinical evolution of the disease [Marcato *et al.*, 1991] can be peracute/acute and subacute/chronic. The acute disease is characterized by few signs and sudden mortality (nervous signs in agonic phase, dyspnoea and even mortality within 48-96 hrs The incubation period varies between 1 and 3 days; death may occur 12–36 hours after the onset of fever (>40°C). During an outbreak, a limited number of rabbits (5–10%) may show a subacute/chronic or even a subclinical evolution of the disease. These animals often die 1 or 2 weeks later, probably due to a liver dysfunction (Figure 4).

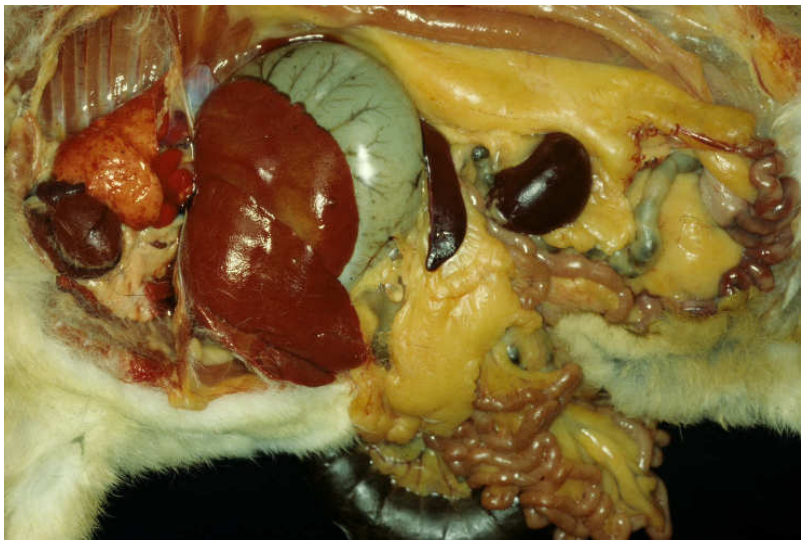

Figure 8. *Rabbit die do to subacute-chronic disease shows liver degeneration and an icteric discoloration of the visceral fat and subcutis.*

The gross pathological lesions [Marcato *et al.*, 1991] are variable and may be subtle. Liver necrosis and splenomegaly are the primary lesions (Figure 7a, b,) However, a

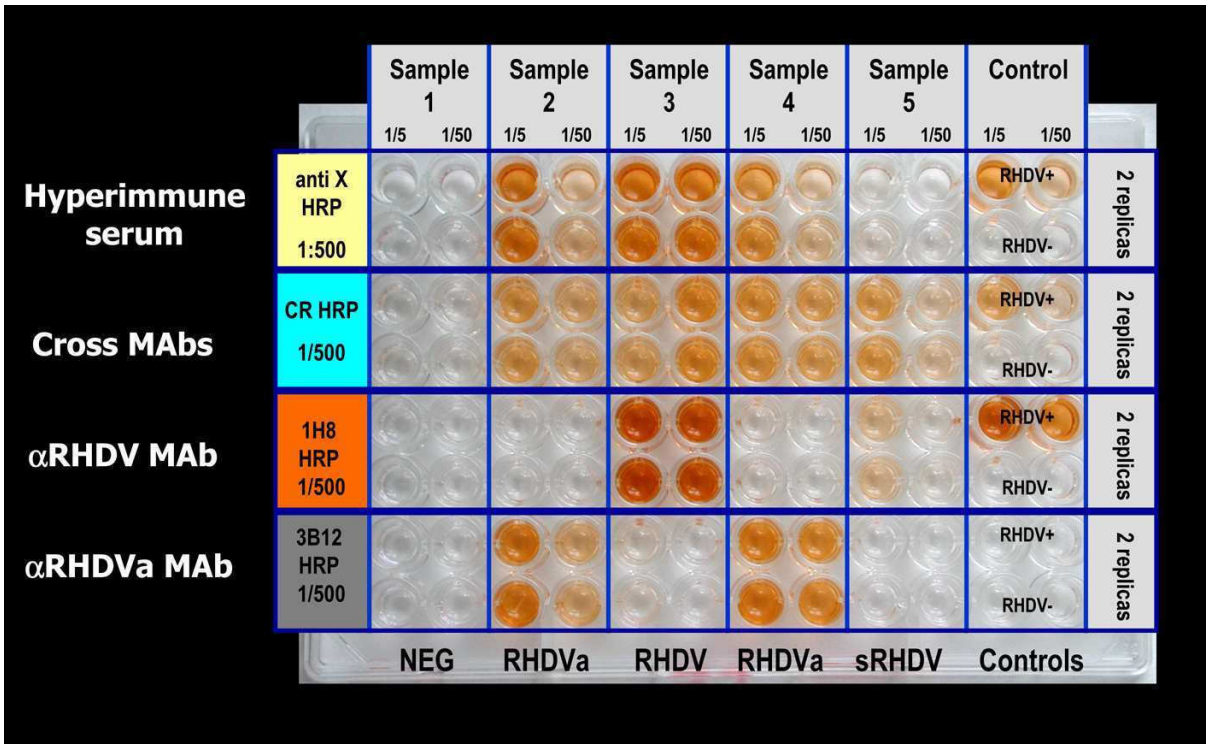

Figure 9. *ELISA test for RHDV routine diagnosis using RHDV and RHDVa specific MAbs: sample 1 is negative, samples 2 and 4 are RHDVa variant, sample 3 is a "classical" RHDV and sample 5 is a smooth "degraded" RHDV.*

massive coagulopathy is usually the cause of haemorrhages in a variety of organs and sudden death (Figure 7c,d). In subacute and chronic disease, an icteric discoloration on the ears, conjunctiva and subcutis is clearly evident (Figure. 8).

5. Diagnosis

Presumptive diagnosis is based on clinical signs, lesions and epidemiology (respiratory distress, high mortality and rapid spread); diagnosis of confirmation as well as strain characterization is achieved by laboratory tests.

The liver contains the highest viral titer and is the organ of choice to submit to viral identification. The amount of virus present in other parts of the body is directly proportional to vascularization; thus spleen, lungs and serum are quite rich in virus and can serve as alternative diagnostic material. Tissue suspensions of organs (5-20% w/v) can be directly examined by hemagglutination (HA) test using human type O erythrocytes, electron microscopy or enzyme-linked immunosorbent assay (ELISA).

The test commonly used for routine examinations are:

1) Sandwich ELISA using RHDV specific Monoclonal Antibody (MAb) [Capucci *et al.*, 1995; Capucci and Lavazza, 2004] (Figure 9).

2) Sandwich ELISA test using a panel of RHDV specific MAbs. This test permits the identification of RHDV variants and particularly to distinguish between the original RHD virus and its first consistent antigenic variant RHDVa [Capucci *et al.*, 1998].

3) Western Blot analysis using RHDV-MAbs that recognize internal epitopes and also cross-reactive with EBHSV [Capucci *et al.*, 1991]. It is usually performed on the few samples, which give doubtful results in Elisa test, and in animals died due to the "chronic" form of the disease.

Other diagnostic methods have been developed including plate agglutination test, immunostaining of paraffin embedded sections, fluorescent antibody test on tissue cryosections, western blot, *in situ* hybridization. Reverse transcription Polymerase Chain Reaction (RT-PCR) [Guitrre *et al.*, 1995; Gould *et al.*, 1997] is an extremely sensitive method for the detection of RHDV and it is 10^4-fold more sensitive than ELISA. However RT-PCR is not strictly necessary for routine diagnosis but it is more appropriate for investigations on molecular epidemiology, to study the pathogenesis of the infection and to detect virions in young animals at the time they get infected and are not diseased (less than 40-50 days of age), in non-specific hosts (other vertebrates) and in vectors (mosquitoes and fleas).

As no satisfactory growth condition and sensitive cell substrates have been established, *in vitro* isolation of RHD virus cannot be included among the virological methods. Therefore, to date viral isolation *in vivo* by experimental reproduction of RHD retains paramount importance. In fact large quantities of viral antigen are needed to prepare diagnostic reagents and produce inactivated tissue-

derived vaccines. Experimental infection is not practical as a routine diagnostic method although it is still desirable in the case of unusual samples (HA negative / ELISA positive) or not clearly positive.

To succeed in reproducing the disease, the inoculated rabbits must be fully susceptible to the virus. Susceptibility depends both on the age of animals, which should be more than two months old, and on the absence of specific antibodies, even at low titres.

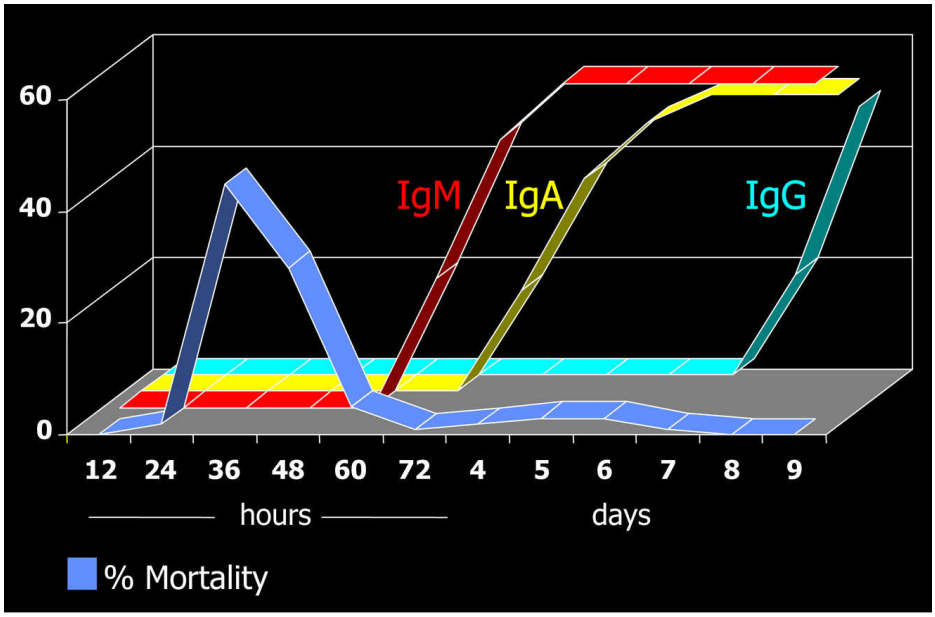

Figure 10. *Schematic representation of the humoral response in rabbits following i.m. inoculation of a virulent RHDV strain, compared with mortality rate.*

Infection by RHDV can be diagnosed through detection of a specific antibody response. Animals that overcome the disease present a striking seroconversion, which can be easily detected 4-6 days p.i. (Figure 10). Indeed, as the humoral response is of great importance in protecting animals from RHD, determination of the specific antibody titer after vaccination or in convalescent animals is predictive of the ability of rabbits to resist RHD virus infection.

Three basic techniques are applied to the serological diagnosis of RHDV: haemoagglutination inhibition (HI) [Gregg, 1992], indirect ELISA and competitive ELISA [Capucci *et al.*, 1991; Capucci *et al.*, 2004a]. With respect to the availability of reagents and technical complexity HI is certainly the most convenient method. On the other hand ELISA reactions are more easily and quickly performed, particularly when a high number of samples are tested. The sensitivity and specificity of competition ELISA (cELISA) using MAbs is markedly higher than those achievable with other methods [Capucci *et al.*, 1991] since it mainly measures antibodies directed against antigenic determinants on the external surface of the virus, usually the most specific and functionally important. Therefore it is considered the standard and reference test for RHD.

Three additional sandwich ELISA tests were developed using antisotype MAbs (isoELISAs) to test the sera for the presence of specific anti-RHDV IgM, IgA and IgG. The isotype titres could be critical for the interpretation of field serology and for correctly classifying the immunological status of rabbits [Cooke *et al.*, 2000]. Some other tests could be used for specific investigations and particularly when a higher level of sensitivity is needed in order to detect antibodies in non-target species (including humans) or antibodies induced by cross reacting RHDV-like agents. They include: 1) Indirect ELISA (inELISA); it has a slight higher sensitivity in respect to cELISA, making possible to measure highly cross-reactive antibodies, and it can detect antibodies with low avidity. 2) Solid phase ELISA (spELISA); the purified antigen is directly adsorbed to the solid phase and due to virus deformation internal epitopes are exposed. Therefore it detects a wider spectrum of antibodies with high sensitivity and low specificity. 3) Sandwich Elisa to detect IgM and IgG in liver or spleen samples already examined with the virological test. Such test is particularly useful in those animals which die due to the "chronic" form of the disease, when the detection of the virus could be difficult. In this case, a high level of RHDV specific IgM and a low level, if any, of IgG are the unambiguous marker of RHD positive samples.

Technical details and full references on the different virological and serological tests are reported in the RHDV dedicated chapter in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [Capucci and Lavazza, 2004a].

6. Epidemiology. Exposure factors. High and Low risk assessment

Incidence of RHDV in industrial units is low since the disease can be easily controlled by vaccination. In the recent year the spreading of the new variant strain (RHDVa) has determined an increase of outbreaks due to vaccination failures [Lavazza *et al.*, 2004]

Currently RHD is endemic in East Asia, Europe and in Australia and New Zealand. Outbreaks have also been recorded in Central America (Mexico and

Cuba), Saudi Arabia and West and North Africa. In 2000 and 2001 three independent outbreaks were recorded in the United States of America. The endemic persistence of RHD in a country is usually guaranteed by the spreading of the disease in rural units and wild animals.

RHD spreads very rapidly and infection can occur by nasal, conjunctival or oral routes. The disease is commonly observed throughout the year and could be transmitted directly or indirectly by equipments, cages, instruments, humans, birds, insects etc. [Allegranza, *et al.*, 1990; Asgari *et al.*, 1998; Cooke, 2002]. Indirect transmission by means of animated vectors, including man, or unanimated tools is favored by the high stability and resistance of the virus in the environment. Wild rabbit population can act as reservoir. Among the risk factors that should be considered for explaining the occurrence of outbreaks in industrial farms are: 1) the introduction of breeders of unknown origin and/or without application of quarantine period; 2) the transport of animals when trucks visit farms to pick up animals to go to the abattoir.

7. Prophylaxis - Good agricultural practices

Where RHD is endemic, an indirect control of the disease in industrial rabbitries is mainly achieved by vaccination. Indeed, the application of strict biosecurity measures is suggested to prevent the introduction of the infection in industrial farms. Some sanitary and environmental arrangements are very helpful, including: 1) the application of biosecurity programs; 2) the culling and removal of ill or dead animals; 3) the cleaning and disinfection of equipment, cages, instruments etc.; 4) the use of single use instruments for AI and therapies; 5) visitor controls: restriction to visits of humans and other animals such as dogs and cats; 6) insect traps at the windows and ventilation intakes; 7) avoiding wild rabbits entering the farm.

Vaccination is a routine practice in industrial rabbit farm. Vaccines are usually prepared by using clarified liver suspension of experimentally infected rabbits, subsequently inactivated and adjuvated (see more details in the RHDV chapter in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [Capucci and Lavazza, 2004a]). Vaccinated breeders quickly produce stray humeral immunity i.e. within 10-15 days post vaccination. The usual program is to administer the inactivated vaccine twice with an interval of at least two weeks.

Normally, a 1 ml dose is inoculated subcutaneously in the neck region. In those units where the anamnesis for RHD is negative, it is advisable to vaccinate only the breeding stocks; the first injection should be done at three months of age. Annual re-vaccination is strongly recommended to ensure a good level of protection, although experimental data indicate that protection usually lasts for a long time (more than one year) [Arguello-Villares, 1991].

Growing rabbits are usually not vaccinated if the sanitary situation of the farm is normal, since their susceptibility period is quite narrow i.e. between 35-40 days of age to slaughtering age around 80 days. Nevertheless in area at risk or after major outbreak, even if strict hygienic and sanitary measures are adopted, it is strongly recommended to vaccinate growing rabbits at the age of 40 days because the incidence of infection/re-infection is very high. Only after a certain number of production cycles it is advisable to stop vaccination and to do so a variable number of growing rabbits, starting with a small group, should not be vaccinated in order to verify the persistence of infective RHD inside the unit.

Vaccination could also be considered a quite effective post-exposure treatment to be included in the emergency strategies applied when RHD occurs in rabbitries. Indeed, better results in limiting the diffusion of the disease and reducing the economic losses could be obtained by using seroterapy through the parenteral administration of anti-RHDV hyperimmune sera.

Other types of vaccines based on biotechnologies have been prepared and experimented with, with some equally good results but none of them is presently commercially available [Capucci and Lavazza, 2004a].

8. Conclusions

Due to the broad antigenic and genomic variability of rabbit caliciviruses the importance of a continuous epidemiological and antigenic surveillance on RHD must be stressed, also considering that an efficacious vaccine is the main, if not the only, tool to protect rabbits. Indeed, the combination of the available different serological and virological methods of diagnosis provides novel and highly sensitive means for the identification and characterisation of such viruses, with special regard to genome composition, evolution, features of pathogenicity and molecular epizootiology.

Nevertheless, the complex epidemiological pattern of RHD should consider the potential role of non-pathogenic strains of RHDV-like viruses, also potentially derived by the attenuation of the original RHDV strains, and, therefore it is particularly important that serological surveys are made using methods able to distinguish between antibodies that are protective against RHD and antibodies that are not. At the same it must be a priority for future research to isolate and characterize the RHDV-like strains in order to determine the level of protection that each of them can induce and to better understand the epidemiology of RHD in wild as well as domestic and industrial populations.

References

Allegranza G., Vanzetti T., Lavazza A., Capucci L., Scicluna M.T.,1990. Malattia emorragica virale del coniglio: indagine epidemiologica nel Canton Ticino, Svizzera. Sel.Vet., 31 (7), 847-858.

Arguello Villares J.L., 1991. Viral haemorrhagic disease of rabbits: vaccination and immune response. Rev. Sci. Tech. OIE, 10 (2), 471- 480.

Asgari S., Hardy J.R.E., Sinclair R.G., Cooke B.D., 1998. Field evidence for mechanical transmission of rabbit haemorrhagic disease virus (RHDV) by flies (Diptera: Calliphoridae) among wild rabbits in Australia. Virus Res., 54, 123-132.

Barbieri I., Lavazza A., Brocchi E., Konig M., Capucci L., 1997. Morphological, structural and antigenic modifications of rabbit haemorrhagic disease virus in the course of the disease. Proc.1st Symp. on Calicivirus of the Europ. Society of Vet. Virology (ESVV), Reading, UK, 15–17 September 1996, 182–193.

Barcena J., Verdaguer N., Roca R., Morales M., Angulo I., Risco C., Carrascosa JL., Torres JM, Caston JR., 1994. The coat protein of Rabbit hemorrhagic disease virus contains a molecular switch at the N-terminal region facing the inner surface of the capsid. Virology, 322, 118-34.

Capucci L., Cerrone A., Botti G., Mariani F., Bartoli M., Lavazza A., 2004b. Results of seroepidemiological surveys for the detection of natural anti-RHD antibodies induced by the nonpathogenic rabbit calicivirus (RCV) in meat rabbits. Proc. 8th Congr. World Rabbit Science, Puebla, Mexixo. 7-11 September 2004, 477-483.

Capucci L., Chasey D., Lavazza A. & Westcott D., 1996a. Preliminary characterisation of a non-haemagglutinating strain of rabbit haemorrhagic disease virus from the United Kingdom. J. Vet. Med. [B], 43, 245–250.

Capucci L., Fallacara F., Grazioli S., Lavazza A., Pacciarini M.L., Brocchi E., 1998. A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. Virus Res., 58, 115–126.

Capucci L., Frigoli G., Ronsholt L., Lavazza A., Brocchi E., Rossi C., 1995. Antigenicity of the rabbit hemorrhagic disease virus studied by its reactivity with monoclonal antibodies. Virus Res., 37, 221–238.

Capucci L., Fusi P., Lavazza A., Pacciarini M.L., Rossi C., 1996b. Detection and preliminary characterization of a new rabbit calicivirus related to rabbit hemorrhagic disease virus but nonpathogenic. J. Virol., 70, 8614–8623.

Capucci L., Lavazza A., 2004a. Chapter 2.8.3."Rabbit Haemorrhagic Disease", in "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals". 5° ed., OIE, Paris, 950-962.

Capucci L., Nardin A., Lavazza A., 1997. Seroconversion in an industrial unit of rabbits infected with a non-pathogenic rabbit haemorrhagic disease-like virus. Vet. Rec., 140, 647–650.

Capucci L., Scicluna M.T., Lavazza A., 1991. Diagnosis of viral haemorrhagic disease of rabbits and European brown hare syndrome. Rev. Sci. Tech. Off. int. Epiz., 10, 347–370.

Capucci L., Scicluna M.T., Lavazza A., Brocchi E., 1990. Purificazione e caratterizzazione dell'agente eziologico della malattia emorragica virale del coniglio. Sel. Vet., 31, 301–312.

Cooke B.D. and Saunders G., 2002. Rabbit haemorrhagic disease in Australia and New Zealand. Wildlife Research, 29 (6), 1.

Cooke B.D., 2002. Rabbit haemorrhagic Disease: field epidemiology and the management of wild rabbit populations. Rev. Sci. Tech. OIE, 21 (2), 347-358.

Cooke B.D., McPhee S., Robinson A.J., Capucci L., 2002. RHDV: does a pre-existing RHDV-like virus reduce the effectiveness of RDH as a biological control in Australia? Wildlife Res., 29, 673-682.

Cooke B.D., Robinson A.J., Merchant J.C., Nardin A., Capucci L., 2000. Use of ELISAs in field studies of rabbit haemorrhagic disease (RHD) in Australia. Epidemiol. Infect., 124, 563–576.

Ferreira P.G., Costa-e-Silva A., Monteiro E., Oliveira M.J., Aguas A.P., 2004. Transient decrease in blood heterophils and sustained liver damage caused by calicivirus infection of young rabbits that are naturally resistant to rabbit haemorrhagic disease. Res. Vet. Sci., 76, 83-94.

Ferreira P.G., Costa-E-Silva A., Oliveira M.J., Monteiro E., Aguas A.P., 2005. Leukocyte-hepatocyte interaction in calicivirus infection: differences between rabbits that are resistant or susceptible to rabbit haemorrhagic disease (RHD). Vet. Immunol. Immunopathol., 103, 217-221.

Gould A.R., Kattenbelt J.A., Lenghaus C., Morrissy C., Chamberlain T., Collins B.J., Westbury H.A., 1997. 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., 47, 7–17.

Granzow H., Weiland F., Strebelow H.-G., Lu C.M., Schirrmeier H., 1996. Rabbit hemorrhagic disease virus (RHDV): ultrastructure and biochemical studies of typical and core-like particles present in liver homogenates. Virus Res., 41, 163–172.

Gregg D., 1992. Viral haemorrhagic disease of rabbits. OIE Manual Standards for Diagnostic Tests and Vaccines, 2nd ed, OIE, Paris, 736-741.

Guittre C., Baginski I., Le Gall G., Prave M., Trepo O., Cova L., 1995. Detection of rabbit haemorrhagic disease virus isolates and sequence comparison of the N-terminus of the capsid protein gene by the polymerise chain reaction. Res. Vet. Sci., 58, 128–132.

Henning J., Meers J., Davies Pr., Morris R., 2005. Survival of rabbit haemorrhagic disease virus (RHDV) in the environment. Epidemiol Infect., 133, 719-730.

Lavazza A., Cerrone A., Agnoletti F., Perugini G., Fioretti A., Botti G., Bozzoni G., Cerioli M., Capucci L., 2004. An update on the presence and spreading in Italy of rabbit haemorrhagic disease virus and of its antigenic variant RHDVa. Proc. 8th of World Rabbit Sci. Congress, Puebla, Mexico. 7-11 September 2004, 562-568.

Lavazza A., Scicluna M.T., Capucci L., 1996. Susceptibility of hares and rabbits to the European Brown Hare Syndrome Virus (EBHSV) and Rabbit Hemorrhagic Disease Virus (RHDV) under experimental conditions. J. Vet. Med. [B], 43, 401–410.

Le Gall G., Arnaud C., Boilletot E., Morisse J.P., Rasschaert D., 1998. Molecular epidemiology of rabbit hemorrhagic disease virus outbreaks in France during 1988 to 1995. J. Gen. Virol. 79, 11-16.

Liu S.J., Xue H.P., Pu B.Q., Quian N.H., 1984. A new viral disease in rabbits. Anim. Hus. Vet. Med., 16, 253–255.

Marcato P.S., Benazzi C., Vecchi G., Galeotti M., Della Salda L., Sarli G., Lucidi P., 1991. Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Rev. Sci. Tech. OIE, 10 (2), 371-392.

Marchandeau S., Le Gall-Recule G., Bertagnoli S., Aubineau J., Botti G., Lavazza A., 2005. Serological evidence for a non-protective RHDV-like virus. Vet. Research, 36, 53-62.

Meyers G., Wirblich C., Thiel H.J., 1991a. Rabbit haemorrhagic disease virus – molecular cloning and nucleotide sequencing of a calicivirus genome. Virology, 184, 664–676.

Meyers G., Wirblich C., Thiel H.J., 1991b. Genomic and subgenomic RNAs of rabbit haemorrhagic disease virus are both protein-linked and packaged into particles. Virology, 184, 677–686.

Nagesha H,S., McColl K.A., Collins B.J., Morrissy C.J., Wang L.F., Westbury, 2000. The presence of croos-reactive antibodies to RHDV in Australian wild rabbits prior to the escape of the virus from the quarantine. Arch. Virol., 145, 749-757.

Neill J.D., 1992. Nucleotide sequence of the capsid protein gene of two serotypes of San Miguel sea lion virus: identification of cones

rved and non-conserved amino acid sequences among calicivirus capsid proteins. Virus Res., 24, 211–222.

Nowotny N., Ros Bascunana C., Ballagi-Pordany A., Gavier-Widen D., Uhlen D., Belak S., 1997. Phylogenetic analysis of rabbit hemorrhagic disease and European brown hare syndrome viruses by comparison of sequence from the capsid protein gene. Arch. Virol. 142, 657-673.

Ohlinger R.F., Haas B., Meyers G., Weiland F., Thiel H.J., 1990. Identification and characterization of the virus causing rabbit haemorrhagic disease. J. Virol., 64, 3331–3336.

O'keefe J.S., Tempero J.E., Motha M.X.J., Hansen M.F., Atkinson P.H., 1999. Serology of rabbit haemorrhagic disease virus in wild rabbits before and after the release of the virus in New Zealand. Vet. Microbiol. 66, 29-40.

Parra F., Prieto M., 1990. Purification and characterization of a calicivirus as the causative agent of a lethal hemorrhagic disease in rabbits. J. Virol., 64, 4013-4015.

Robinson A.J., Lirkland P.D., Forrester R.I., Capucci L., Cooke B.D., 2002. Serological evidence for the presence of a calicivirus in Australian wild rabbits, Oryctolagus cuniculis, before the introduction of RHDV: its potential influence on the specificity of a competitive ELISA for RHDV. Wildlife Res., 29, 655-662.

Rodak L., Smid B., Valicek L., Vesely T., Stepanek J., Hampl J., Jurak E., 1990. Enzyme-linked immunosorbent assay of antibodies to rabbit haemorrhagic disease virus and determination of its major structural proteins, J. Gen. Virol., 71, 1075-1080.

Schirrmaier H., Reimann I., Kollner B., Granzow H, 1999. Pathogenic, antigenic and molecular properties of rabbit haemorrhagic disease virus (RHDV) isolated from vaccinated rabbits: detection and characterization of antigenic variants. Arch. Virol., 144, 719–735.

Smid B., Valicek L., Rodak L., Stepanek J., Jurak E., 1991. Rabbit haemorrhagic disease: an investigation of some properties of the virus and evaluation of an inactivated vaccine. Vet. Microbiol., 26, 77–85.

Tizzani P., Lavazza A., Capucci L., Meneguz P.G., 2002. Presence of infectious agents and parasites in wild population of cottontail (Sylvilagus floridanus) and consideration on its role in the diffusion of pathogens infecting hares. Proc. 4th Scientific Meeting European Association of Zoo- and Wildlife Veterinarians and of the European Wildlife Disease Association, Heildelberg (Germany) 8-12 May 2002, 245-248.

Wirblich C., Meyers G., Ohlinger V.F., Capucci L., Eskens U., Haas B., H.-J. Thiel, 1994. European brown hare syndrome virus: relationship to rabbit hemorrhagic disease virus and other caliciviruses. J. Virol., 68, 5164–5173.