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Review

Genetic variation and phylogenetic analysis of rabbit haemorrhagic disease virus (RHDV) strains

Beata Hukowska-Szematowicz[1]✉, Beata Tokarz-Deptuła[1] and Wiesław Deptuła[2]

[1]Department of Immunology, [2]Department of Microbiology, Faculty of Biology, University of Szczecin, Szczecin, Poland

Rabbit haemorrhagic disease virus (RHDV) belongs to the family *Caliciviridae* and is the etiological agent of the haemorrhagic disease, also known as rabbit plague. Its genome is a linear single-stranded (ss) RNA of 7437 nucleotides and the capsid is built from a single structural protein VP60. In connection with the discovery of new RHDV strains, there is a constant need to investigate the genetic variation of this virus and perform phylogenetic analyses which may show the evolutionary relationships among the RHDV strains. Studies on the divergence of RHDV have shown that it is genetically quite stable, although recent observations indicate that some new RHDV strains, significantly different from the original RHDV subtype and the new RHDVa subtype, are appearing. These latest findings suggest that a new group of RHDV strains has evolved. The present review summarizes the current knowledge on the genetic variation and the latest achievements in phylogenetic analyses of RHDV strains isolated in various countries.

Key words: RHD virus, genetic variation, phylogenetic analysis, genogroup

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INTRODUCTION

The first epidemic of rabbit haemorrhagic disease (RHD) emerged in China in 1984 (Liu *et al.*, 1984), but nowadays this disease has spread on all continents and is characterized by a very high mortality rate, reaching 100% (Hukowska-Szematowicz, 2006). The RHD virus belongs to the family *Caliciviridae* and has a genome formed by linear single-stranded (ss) RNA composed of 7437 nucleotides (Meyers *et al.*, 1991; Wirblich *et al.*, 1996; Meyers *et al.*, 2000). The genome comprises two open reading frames (ORF), the longer ORF1 (7034 nucleotides) encodes non-structural viral proteins (vp) vp16, vp23, vp37, vp30, virus protein-genome linked (VPg) and a structural capsid protein-viral protein (VP60); the shorter reading frame ORF2 (353 nucleotides) codes for viral protein VP12 with so far unidentified function (Meyers *et al.*, 1991; Wirblich *et al.*, 1996; Meyers *et al.*, 2000).

At present, the GenBank data base contains sequences of the full genome of 35 RHDV strains, structural capsid protein VP60 for 48 RHDV strains, and 200 different genome fragments of 50 RHDV strains (Annon, 2012). Apart from the sequences listed in the Genbank, additional information concerning the sequences of 65 different RHDV strains can be found in many scientific publications (Fitzner & Kęsy, 2003; Chrobocińska & Mizak, 2007; Chrobocińska, 2007; Oem *et al.*, 2009; Pawlikowska *et al.*, 2009; Niedźwiedzka-Rystwej *et al.* 2009; Pawlikowska *et al.* 2010; Fitzner *et al.*, 2012). These data are very valuable in the context of their potential use in studies on genetic variation of the RHDV, which together with phylogentic analyses may provide information on the evolutionary relationships among its different strains. Results of these studies have contributed to distinguishing 55 specific RHDV strains, termed antigenic variants (RHDVa), and isolation of one Chinese strain, described as the new antigenic variant, as well as one French strain, known as the new variant of RHDV (Table 1).

GENETIC VARIATION OF RHDV STRAINS

Until now, sequences of about 240 RHDV strains (including RHDVa) have been analyzed in regard to the genetic variation of the RHD virus. The phylogenetic analyses were based mainly on the alignment of gene sequences (full or fragmentary) coding for the VP60 structural protein and vp30 non-structural one (Milton *et al.*, 1992; Boga et al., 1994; Rasschaert *et al.* 1995; Gould *et al.*, 1997; Nowotny *et al.*, 1997; Le Gall *et al.*, 1998; Asgari *et al.*, 1999; Moss et al., 2002; Fitzner & Kęsy, 2003; Le Gall-Recule *et al.*, 2003; Hukowska-Szematowicz, 2006; Matiz *et al.*, 2006; Chrobocińska, 2007; Chrobocińska & Mizak, 2007; McIntosh *et al.*, 2007; Tian *et al.*, 2007; Abrantes *et al.*, 2008; Forrester *et al.*, 2008; Esteves *et al.*, 2008; Yang *et al.* 2008; Fitzner, 2009; Hukowska-Szematowicz *et al.*, 2009; Muller *et al.*, 2009; Oem *et al.*, 2009; Fitzner *et al.*, 2012; Wang *et al.*, 2012). Among the cited investigations, the studies by Abrantes *et al.* (2008) and Forrester *et al.* (2008) are especially interesting, since they used the whole sequence of the gene encoding VP60 or its fragments and were carried out on 100 RHDV strains collected in Europe, Asia and North America between 1984 and 2005. These studies have revealed that the genetic divergence of RHD virus occurs by recombination, thus it is hypothesized that the haemorrhagic disease outbreak registered in 1984 in China was caused by recombination of the genetic material of RHDV originating from angora rabbits that had been imported from Germany. According to those authors (Abrantes *et al.*, 2008; Forrester *et al.*, 2008) recombination may be the key event in RHD virus evolution. Knowledge of this process, together with identification of positive selection sites, may lead to a better under-

✉e-mail: beatahsz@gmail.com

Abbreviations: HA(+), haemagglutination activity; HA(-), lack of haemagglutination activity; ORF, open reading frame; RCV-like, rabbit calicivirus like; RCV-A1, rabbit calicivirus from Australia; RHDV, rabbit haemorrhagic disease virus; RHD, rabbit haemorrhagic disease; RHDVa, antigenic variant of rabbit haemorrhagic disease virus; VP, viral protein; VPg, virus protein-genome linked.

Table 1. Variants of RHD virus

Variant	RHDV strains	HA	GenBank Accession number	Country of origin/ Year of identification	References
Antigenic variants-RHDVa	Triptis	HA+	Y15442	Germany, 1996	Schirrmeier *et al*., 1999
	Hartsmanndorf	HA+	Y15425	Germany, 1996	Schirrmeier *et al*., 1999
	Viterbo97(Vt97)	HA+	EU250331	Italy, 1997	Capucci *et al*., 1998
	Pavia97 (Pv97)	HA-	EU250330	Italy, 1997	Capucci *et al*., 1998
	9905RHDVa	HA-	AJ302016	France, 1999	Le Gall-Recule *et al*.,2003
	00-Reu	no data	AJ303106	France, 2000	Le Gall-Recule *et al*.,2003
	01-38RHDVa	HA+	not registered	France, 2001	Marchandeau *et al*., 2005
	03-24	no data	AJ969628	France, 2003	Le Gall-Recule *et al*.,2003
	RH29/03	no data	AY935974	Hungary, 2003	Matiz *et al*., 2006
	NL2004-1	no data	DQ296063	Netherlands, 2004	Van de Bildt *et al*., 2006
	NL2004-2	no data	DQ296064	Netherlands, 2004	Van de Bildt *et al*., 2006
	NL2004-3	no data	DQ296065	Netherlands, 2004	Van de Bildt *et al*., 2006
	ROK	HA+	not registered	Poland, 2003/2004	Fitzner *et al*., 2012
	GRZ	HA+	not registered	Poland, 2003/2004	Fitzner *et al*., 2012
	CB	HA+	not registered	Poland, 2003/2004	Fitzner *et al*., 2012
	ZKA (L4)	HA+	not registered	Poland, 2003/2004	Fitzner *et al*., 2012
	KRY	HA+	not registered	Poland, 2003/2004	Fitzner *et al*., 2012
	ZDU (L1-L6)	HA+	not registered	Poland, 2003/2004	Fitzner *et al*., 2012
	L145/04	no data	not registered	Poland, 2004	Chrobocińska & Mizak, 2007
	W147/05	no data	not registered	Poland, 2005	Chrobocińska & Mizak, 2007
	DCE (L1)	HA+	not registered	Poland,2006	Fitzner *et al*., 2012
	NJChina1985	no data	AY269825	China, 1985	McIntosh *et al*., 2007; Tian *et al*., 2007; Wang *et al*., 2012
	JXCHA97	no data	DQ205345	Chiny, 1997	McIntosh *et al*., 2007; Tian *et al*., 2007
	TP	no data	AF453761	China, 2002	McIntosh *et al*., 2007; Tian *et al*., 2007; Wang *et al*., 2012
	HYD	no data	JF412629.1	China 2005	Wang *et al*., 2012
	XJ/China/2002 clone 2	no data	GU339229.1	China 2002	Wang *et al*., 2012
	XJ/China/2002 clone1	no data	GU339228	China 2002	Wang *et al*., 2012
	CD	no data	AY523410	China, 2004	McIntosh *et al*., 2007; Tian *et al*., 2007
	WHNRH	HA+	DQ280493	China, 2005	McIntosh *et al*., 2007; Wang *et al*., 2012
	WHN-1	HA-	DQ069280	China, 2005	McIntosh *et al*., 2007; Tian *et al*., 2007; Wang *et al*., 2012
	WHN-2	HA-	DQ069281	China, 2005	McIntosh *et al*., 2007; Tian *et al*., 2007; Wang *et al*., 2012
	WHN-3	HA-	DQ069281	China, 2005	McIntosh *et al*., 2007; Tian *et al*., 2007
	YL	HA+	DQ530363	China, 2006	McIntosh *et al*., 2007; Tian *et al*., 2007; Wang *et al*., 2012
	SH/China	no data	FJ794179	China, 2006	Wang *et al*., 2012
	TC/China/2007	no data	JN165233	China, 2007	Wang *et al*., 2012
	WF/China	no data	FJ794180.1	China, 2007	Wang *et al*., 2012
	NJ-2009	no data	HM623309.1	China ,2009	Wang *et al*., 2012
	FP/China/2009	no data	JN165235	China, 2009	Wang *et al*., 2012
	BJ/China/2009	no data	JN165236	China, 2009	Wang *et al*., 2012
	09-SD	no data	GU564448	China, 2010	Wang *et al*., 2012
	RHDVHokkaido/2002/ JPN	no data	AB300693.2	Japan, 2002	Wang *et al*., 2012
	06Q48-2	no data	not registered	Korea, 2006	Oem *et al*., 2009
	06D32-1	no data	not registered	Korea, 2006	Oem *et al*., 2009
	06D106-1	no data	not registered	Korea, 2006	Oem *et al*., 2009
	06Q755-1	no data	not registered	Korea, 2006	Oem *et al*., 2009
	07Q92-1	no data	not registered	Korea, 2007	Oem *et al*., 2009
	08Q221	no data	not registered	Korea, 2008	Oem *et al*., 2009
	08Q712	no data	not registered	Korea, 2008	Oem *et al*., 2009

	08Q121	no data	not registered	Korea, 2008	Oem *et al*., 2009
	KV0801	no data	FJ212322	Korea, 2008	Oem *et al*., 2009
	Iowa2000	HA+	AF258618	USA, 2000	McIntosh *et al*., 2007
	NY01	no data	EU003581	USA, 2001	McIntosh *et al*., 2007
	UT01	no data	EU003582	USA, 2001	McIntosh *et al*., 2007
	IN05	no data	EU003578	USA, 2005	McIntosh *et al*., 2007
	CUB5-04	no data	DQ841708	Cuba, 2004	Farnos *et al*., 2007
New RHDVa variant	XA/China/2010	HA+	JN165234	China, 2010	Wang *et al*., 2012
New RHDV variant	French RHD variant	no data	not registered	France, 2010	Le Gall-Recule *et al*., 2011

standing of interactions between the pathogen and its host (Hurst, 2009). Esteves and co-workers (2008), who investigated the positive selection occurring in genes of RHDV strains, have suggested that this process is connected with antigenic regions of the virus. This indicates that the variation among viral strains is caused by the immune response of the host, and is connected with the RHDV pathogenicity and virulence.

Other studies from Australia, China, Czech Republic, France, Italy, Ireland, Korea, Mexico, New Zealand, Poland, Spain and the USA concerning the genetic variation over 100 RHDV strains have shown that it is 14% (Milton *et al*., 1992; Boga *et al*., 1994; Rasschaert *et al*. 1995; Gould *et al*., 1997; Nowotny *et al*., 1997; Le Gall *et al*., 1998; Asgari *et al*., 1999; Moss et al., 2002; Fitzner & Kęsy, 2003; Hukowska-Szematowicz, 2006; Le Gall-Recule *et al*., 2003; Matiz *et al*., 2006; Chrobocińska, 2007; Chrobocińska & Mizak, 2007; McIntosh *et al*., 2007; Tian *et al*., 2007; Yang *et al*. 2008; Fitzner, 2009; Hukowska-Szematowicz *et al*., 2009; Muller *et al*., 2009; Oem *et al*., 2009; Wang *et al*., 2010; Fitzner *et al*., 2012), whereas the divergence within the antigenic variants RHDVa ranges between 9.9% and 10.3% (Le Gall *et al*., 1998; Le Gall-Recule *et al*. 2003; Chrobocińska & Mizak, 2007; McIntosh *et al*., 2007; Fitzner, 2009; Oem *et al*., 2009; Wang *et al*., 2010; Fitzner *et al*., 2012). Another recent estimation done by Le Gall-Recule *et al*. (2011), who investigated 125 RHDV strains, showed that genetic variation amounting to 14.3%. Additionally this study has revealed a new RHDV strain, called French RHDV variant.

PHYLOGENETIC ANALYSES OF RHDV STRAINS

Phylogenetic analyses and phylodynamics of RHDV strains have been extensively conducted by many research groups around the world, resulting is the classing of strains into genetic groups, genogroups, lineages and clades (Table 2). The first phylogenetic study was done by Nowotny and co-workers (1997) and comprised RHDV strains collected in the years 1987-1995 from Europe, Asia and North America. It was based on an analysis of a gene fragment coding for VP60 protein and classified the RHDV strains into three separate groups. The first group consisted of RHDV strains isolated between 1989 and 1995 in Europe and Asia, the second comprised European strains from years 1990-1995, and the third group contained European strains from years 1987–1993. The obtained results indicated that the strains were classified according to the temporal, rather than geographical structure. Further studies of Le Gall and co-workers (Le Gall *et al*., 1998; Le Gall-Recule *et al*., 2003) performed on French RHDV strains (isolated in 1998-1995 and 1993-2000) and other strains from Europe were based on an analysis of gene fragments of VP60 and p30 protein. The earlier investigation determined three genogroups (G1-G3), while the more recent study (Le Gall-Recule *et al*., 2003), in which five RHDVa strains were also included, distinguished six genogroups (G1-G6) classified in accordance to the year of virus collection. Interestingly, all five RHDVa strains (99-05, 00-Reu, Triptis, Hartmannsdorf and Iowa) fell into the G6 genogroup. Even more groups were identified by researchers working on British RHDV strains and samples collected between 1950 and 1980 (Moss *et al*., 2002). That phylogenetic analysis, also based on an alignment of VP60 gene fragment, classified the investigated strains and samples into eight genogroups, however, the distribution was connected with the site of their isolation rather than the time of collection, and the 8th group contained the RHDVa strains.

A phylogenetic study on Polish RHDV strains (SGM, KGM, PD, MAŁ, BLA, GSK, ŻD, ŻD1 and LUB) collected between 1988 and 2000 was performed by Fitzner and Kęsy (2003). The study was based on the nucleotide sequence encoding the N-terminal fragment of VP60, and a fragment of p30 gene sequence. The analysis divided the strains into two genetic groups, showing temporal similarities; however, the Iowa RHDVa strains from the GenBank formed a separate genetic group. A phylogenetic study performed by Forrester *et al*. (2003) concerning New Zealand strains isolated in 1997 and other RHDV strains from Europe and USA. Those strains formed only two groups, of which one was phylogenetically related to the Czech strain V-351, and the second genogroup was significantly distinct from it. Furthermore, the second group included the RHDVa antigentic variants (Hartmannsdorf, Triptis, 99-05, ChinaTP, Iowa and France00), which differed phylogenetically from the other investigated strains.

Next, a study by Matiz and co-workers (2006) concerning Hungarian strains isolated between 1988 and 2003 classified those strains on the basis of the VP60 gene. The strains were grouped according to the time of their isolation and identification, however, the third group included phylogenetically distinct RHDVa strains: Hugarian-RH29/0 as well as 99-05, Triptis and 00-Reu. In the same year another work by Forrester and co-workers (2006b) described results of a phylogenetic analysis of RHDV strains collected between 1984 and 2005, based on the sequence encoding VP60. The authors distinguished seven groups. Interestingly, the second genogroup included Irish strains whose position on the phylogenetic tree indicated that there was a single introduction of the RHD virus to Ireland, after which the virus was able to spread before its virulent form was finally identified in 1995. The seventh group

Table 2. Phylogenetic studies of RHDV strains

Number of strains analysed and their origin	Strain distribution	References
44 from Austria, Germany, France, Spain, Belgium, Hungary, Czech Republic, Israel, Slovakia, Ireland, Sweden, Switzerland, Finland, Mexico, United Kingdom, USA China, Korea	3 genetic groups	Nowotny *et al.,* 1997
61, including 56 from France; others from Czechoslovakia, Italy, Germany, Spain	3 genogroups	Le Gall *et al*., 1998
119, including 104 from France; others from Czechoslovakia, Germany, Italy, Spain, United Kingdom, USA, Mexico	6 genogroups	Le Gall-Recule *et al.*, 2003
61, including 45 from the United Kingdom; others from Germany, Italy, USA	8 groups	Moss *et al*., 2002
14, including 9 from Poland, others from Czechoslovakia, France, Germany, Italy and the USA	2 genetic groups	Fitzner & Kęsy, 2003
78, including 38 from New Zealand; others from Germany, France, Spain, USA	2 groups	Forrester *et al*., 2003
37, including 27 from Hungary; others from France and Germany	3 genogroups	Matiz *et al.,* 2006
71 strains from Germany, France, China, New Zealand, Mexico	7 groups	Forrester *et al*., 2006b
61, including 2 from Saudi Arabia and Bahrain; others from Germany, France, China, Mexico, USA	3 groups	Forrester *et al*., 2006a
24, including 3 from the Netherlands; others from the Czech Republic, Germany, France, Mexico, China, Ireland, USA	6 genogroups	Van de Bildt *et al*., 2006
43, including 18 from Poland; others from Germany, France, Spain, Italy, China and the USA	6 genogroups	Chrobocińska, 2007; Chrobocińska & Mizak, 2007
45 from Germany, France, Spain, Italy, Czechoslovakia, China, USA, Central America	2 groups	McIntosh *et al., 2007*
14 from Czechoslovakia, Germany, USA, Bahrain, Italy, France	4 genetic groups	Hukowska-Szematowicz et al., 2009
4 from the United Kingdom, Germany, Spain, Italy	2 genetic groups	Niedźwiedzka-Rystwej *et al*. 2009
11 from Germany, Hungary, Saudi Arabia, Spain, USA	2 genetic groups	Pawlikowska et al., 2009
66, including 45 from the Iberian Peninsula (Spain and Portugal); others from France, Germany, Czechoslovakia, China, Mexico, Saudi Arabia	3 groups/6 genogroups	Muller *et al*., 2009
145 samples, including 71 from the Iberian Peninsula; others from France, United Kingdom, Germany, China	3 lineages	Alda *et al*., 2010
217 from various continents :Europe, Asia, America, Australia and Oceania	4 groups	Kerr *et al*., 2009
16 from France	4 genetic groups	Hukowska-Szematowicz & Deptuła, 2010
29 from Germany, France, Italy, Spain, USA, China, Australia, Bahrain, Mexico	4 clades	Kinneart & Linde, 2010
46 from Czechoslovakia, Hungary, France, Spain, United Kingdom, Germany, USA, Mexico	6 genogroups	Pawlikowska et al., 2010
34, including 15 from Poland; others from Germany, China, Italy, Czechoslovakia, the USA	3 genetic groups	Fitzner *et al*., 2012
44, including 21 from China; others from Japan, Italy, Germany, France, Spain, USA, Mexico	6 genogrups	Wang *et al*., 2012
14 RHDV from Germany, France, Spain, China, USA ; 4 RCV-like from Italy, United Kingdom; 1 RCV-A1 from Australia	3 genetic groups	Strive *et al*., 2009; Strive *et al*., 2010
93 RHDV strains, 32 RHDVa, 4 RCV strains and 36 RCV-A1	3 genogroups	Le Gall-Recule *et al*., 2011

was formed by RHDVa strains Hartmannsdorf, Triptis, 99-05, ChinaTP, Iowa, France00, China and China 1985, phylogenetically very distinct from other analyzed European, Chinese, New Zealand and Mexican strains. In another study of those authors (Forrester *et al*., 2006a) two strains identified in 2005 from Saudia Arabia and Bahrain were studied. A comparison of those strains with others from the GenBank, showed three groups. The Bahrain strain was located in the second group, indicating its European origin, as this genogroup contained mainly European strains. On the other hand, the Saudi Arabian strain was classified into the third group, which proved its relation with the Chinese strain isolated in 1984, and its phylogenetic relationship with the Czech strain V-351. The third group comprised also the RHDVa strains: Hartmannsdorf, Triptis, 99-05, ChinaTP, Iowa, France00, China and China 1985, proving the genetic distance of these strains from other European lineages examined in this study. Those results were in agreement with other published reports (Moss *et al*., 2002; Forrester *et al*., 2003; Forrester *et al*., 2006b; Muller *et al.,* 2009).

A phylogenetic analysis based on the VP60 gene sequence was also performed on Dutch RHDV strains collected in 2004 (Van de Bildt *et al*., 2006). The analysis divided the strains into six genogroups. The three Dutch RHDV strains localized in group 5 on the phylogenetic tree together with French strain 00–13, indicating their common origin. Once again, in that study the RHDVa strains formed a separate group 6. In 2007, another researches (Chrobocińska, 2007, Chrobocińska & Mizak, 2007) presented results of a phylogenetic analysis of partial capsid protein gene of RHDV strains isolated between 1993 and 2005 in Poland. The Polish RHDV strains (S1/93, S13/94, T20/94, Sz25/94, R45/94, Z73/94, K80/95, Z82/95, B84/95, S86/95, T90/96, Z94/96, S113/97, T116/97, Z118/97, W142/04, L145/04 and W147/05) were localized in four out of six genogroups. It is worth noting that two Polish strains L145/04 and W147/05, were classified into the 6th genogroup, containing other RHDVa strains. A detailed phylogenetic analysis of RHDV strains identified between 1984 and 2005 was done by McIntosh *et al.* (2007). The analysis, also based on the gene sequence of VP60, identified two distinct groups. The first group contained 26 RHDV strains, described as the original RHDV subtype (Italy90, FRG, Eisenhuttenstadt, Frankfurt, Meiningen, Wriezen, Hagenow, SD, Haute88, 95-05, 00-13, 95-10, 00-08, New Zealand, V-351, Mexico89, WX84, Korea90, Saudia Arabia, Bahrain, AST89, Ireland18, Ireland19, Ireland12, Rainham and BS89). The second genogroup consisted of 19 strains of the new RHDVa subtype (99-05, IA00, WHN3, YL, IN05, 03-24, JXCH97, TP, WHN2, WHNRH, CUB5-04, CD, NY01, WHN1, 00-Reu, NJ1985, UT01, Triptis and Hartsmanndorf).

Another phylogenetic study, based on the nucleotide sequence of the gene encoding the p30 non-structural protein, was performed by Hukowska-Szematowicz and co-workers (2009). The cited studies have revealed that these strains can be classified into four genetic groups in regard to the collection period. The nucleotide sequence of an N-terminal fragment of VP60 capsid protein was used by Niedźwiedzka-Rystwej and co-workers (2009), who analyzed four European strains isolated between 1989 and 2000 and showed that they can be grouped into two distinct genetic groups according to the time of their identification. A similar study was presented by Pawlikowska *et al.* (2009), who investigated RHDV strains isolated in 1989–2002 in Europe. The analysis was based on an alignment of nucleotide sequence of N- and C-terminal fragments of VP60 protein. In that case two genetic groups were identified, and the division correlated with the geographical localization, but was independent of the analyzed fragment of VP60 protein.

Another phylogenetic study based on the sequence of the VP60 gene was performed on RHDV strains isolated in the Iberian Peninsula in 1994–2007 (Muller *et al*., 2009) and strains from other regions of Europe and the world. The analyzed strains formed three distinct groups (IB1-3), classified in accordance with the time of collection. The groups IB1 and 2 included strains indentified in the years 1994–1997, while strains isolated in 2000–2007 were found in group IB3. Forty remaining RHDV strains underwent additional classification into six genogroups, also divided according to the time of identification, with an exception of genogroup 6, which contained the RHDVa strains (CUB5-04, 9905 and 03-24) very distinct from the rest of the investigated strains isolated on the Iberian Peninsula. A similar investigation was presented by Alda and co-workers (2010), who analyzed samples from wild rabbits originating from the Iberian Peninsula and other strains from the GenBank, collected between 1984 and 2000. It was noted that the analyzed strains form three distinct lineages (I, II, III), and all strains from the Iberian Peninsula were included in lineage I; however, the exact geographical region of origin could not be identified. Nevertheless, those strains shared a similar temporal structure, except for strains from group IB3, containing the most divergent RHDV strains in regard to their time and place of identification. This study also showed that the 12 RHDVa strains (03France, 05China, 88China, 05China, 85China, 05China, 00Reu, 99France, 97China, 98 China, 00USA and 96German) were grouped into a distinct lineage – lineage III.

The most comprehensive phylodynamic study was performed by Kerr and co-workers (2009). The analysis was based on the complete, as well as fragmentary nucleotide sequence of the VP60 gene and included RHDVa strains collected between 1984 and 2008 on different continents. Four distinct phylogenetic groups (G1-G4) were identified, and the obtained classification divided the strains according to the time and place of identification. The first group G1, included RHDVa strains from Cuba, Korea, China, Japan, Great Britain, and continental Europe. The second group G2 comprised strains isolated on the Iberian Peninsula, in France and Germany. Those RHDV strains were genetically and geographically very distinct from all the other strains identified in years 1989–1997, which localized in groups G1, G3 and G4. The next phylogenetic group G3 was formed by old RHDV strains identified in the year of the first outbreak of haemorrhagic disease in Asia (strain WXChina-1984), Europe and Mexico. This group also contained strains collected more recently in New Zealand (2003) and Australia (2005–2006). Group G4 was the most numerous, containing strains indentified between 1989 and 2004 in central Europe, as well as in France, Belgium, the Netherlands, Great Britain, Ireland and Bahrain. Those groups differed in terms of their phylodynamic patterns. However, groups G1, G3, and G4 showed similarities in the obtained patterns, while G2 represented strains with low genetic variation, especially those originating from Spain and Portugal. A phylogenetic study was then performed on French RHDV strains from years 1992-2005 on the basis of sequence coding for a C-terminal fragment of VP60 protein (Hukowska-Szematowicz & Deptuła, 2010). The analyzed RHDV strains were classified into four genetic groups, formed in regard to their geographical localization and time of virus isolation.

Yet another study (Kinneart & Linde, 2010) based on the VP60 gene sequence of different RHDV strains collected between 1984–2006 divided the investigated strains into four distinct monophyletic clades (A–D) showing little geographical and temporal structure. Clade A contained mainly old RHDV strains from France, Germany, Mexico, the Czech Republic and Slovakia, however, the more recently discovered strains from Australia and Saudi Arabia were also located in this group. Clade B was formed by French, German, English, Italian and Bahrain strains, clade C comprised RHDV strains from Germany, Spain, and France, and clade D was formed by RHDVa strains isolated in Germany, China, France and the USA. On the basis of the obtained results the authors concluded that rapid antigenic selection had played a significant role in the evolution of RHDV, and might have been a factor promoting genetic variation during evolution. A phylogenetic study performed by Pawlikowska and co-workers (2010) concerned RHDV strains from years 1984–2004. That comparison classified the ana-

lyzed strains into six genogroups, localizing the classical RHDV strains into groups 1–5 according to their time of collection, whereas the 6th genogroup comprised RHDVa strains. A recent phylogenetic study was presented by Fitzner and co-workers (2012). The object of the research were Polish RHDV strains of the period 1988-2004 and strains from other countries, and the coding sequence of the VP60 gene was compared. The phylogenetic analysis resulted in three genetic groups. The first group contained old RHDV strains identified in the first decade after the discovery of the rabbit haemorrhagic disease. Among those strains two Polish RHDV strains KGM (1988) and MAŁ (1994) localized together with the classic Chinese strain from 1984, German FRG89, French SD89, Spanish 89, and Czech V351-1987. The second group comprised Polish RHDV strains OPO and LBN (2004), GSK (1998), POZ (1999), ZD0 (2000) and BLA (1994), as well as Italian BS89, German-Frankfurt, and English-Rainham. The third group was formed by RHDVa, including seven Polish antigenic variants CB, DCE, GRZ, KRY, ROK, ZDU and ZKA.

The sequence of the gene encoding VP60 was also used in a phylogenetic analysis of Chinese strains collected between 1984 and 2010 (Wang *et al.*, 2012). All the strains included in the analysis were classified into six genogroups (G1-G6) showing common identification period, with the exception of the G6 group that contained all analyzed RHDVa strains. Furthermore, the Chinese strains were localized in genogroups G2 and G6, among which four subgroups were additionally distinguished (CH1-CH4). This pattern of phylogenetic classification indicates that the Chinese strains underwent independent evolution, resulting in identification of a new Chinese RHDVa variant, XA/China/2010, included in the CH4 subgroup.

Another study worth noting in the context of the latest achievements in the phylogenetic analysis of RHDV was performed by Strive and co-workers (2009, 2010). The analysis involved different lagoviruses RHDV, RHDVa, rabbit caliciviruses-like (RCV-like) and rabbit calicivirus from Australia (RCV-A1), based on a subfragment of the capsid protein nucleotide sequence. The analyzed lagoviruses formed three genetic groups, of which the first contained the RHDV and RHDVa strains, the second was formed by RCV-like and the third grouped an Australian nonpathogenic lagovirus-RCV-A1. These results were confirmed by a recent study by Le Gall-Recule *et al.* (2011), who analyzed the sequence of RHDV strains, RHDVa, RCV and RCV-A1. Their phylogenetic analysis also divided the investigated strains into three genogroups, localizing the RHDV and RHDVa strains in the first group, the RCV strains in the second, and RCV-A1 strains in the third group, which included the nonpathogenic Australian lagovirus.

SUMMARY

Since the time of RHDV identification there has been a constant need to study the genetic variation of this calcivirus and determine its evolutionary pathways. It has been shown that the RHDV is a good model to investigate the divergence and evolution of RNA viruses, as new strains of this virus are constantly appearing in nature. The development of modern methods and bioinformatics techniques allowing transformation of biological observations into mathematical data creates new possibilities of studying the evolutionary relationships among RHDV strains and determining genogroups to identify the lineages and phylogenetic tree topologies of the strains. Studies on the RHD virus divergence have revealed that this virus shows quite high genetic stability, although observation of Le Gall-Recule and co-workers (2011) indicate that new RHDV strains have started to appear. These strains differ significantly from the original RHDV subtype and the new RHDVa subtype, suggesting that a new, distinct group of RHDV strains has been formed. The phylogenetic analyses have shown that the RHDV strains are grouped into lineages according to the time of collection and geographical localization. Furthermore, the strains described as antigenic variants (new RHDVa subtype) are evolutionarily distinct from the original RHDV subtype, which is manifested by an independent localization of their genogroups. It should also be noted that, due to the increasing number of indentified RHDV strains, modern phylogenetic analyses concern not only their genetic origin, but also allow us to indicate the evolutionary relationships among the genogroup thanks to phylodynamic studies. Phylodynamics enables one to recreate changes in the pattern of viral sequence divergence, which allows describing the pattern of virus transfer.

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