Polish Journal of Veterinary Sciences Vol. 19, No. 4 (2016), 865–876

DOI 10.1515/pjvs-2016-0108

Original article

White and red blood cell picture in rabbits experimentally infected with strains of the rabbit haemorrhagic disease (RHD) virus without or with variable haemagglutination capacity

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Abstract

The aim of the study was to establish if haemagglutination of rabbit haemorrhagic disease virus (RHDV) affects haematological picture of peripheral blood in rabbits and the pathogenicity of the virus. The study analyzed white and red blood cell picture in rabbits experimentally infected with two non-haemagglutinating (HA-) RHDV strains (Frankfurt and Asturias) and one strain with variable haemagglutination capacity (HA+/-) (Hagenow). Studies with HA- and HA +/- are rare and relate only to 4 HA- strains (2 RHDV: BLA and Rainham; 2 RHDVa: Pv97 and 9905) and 1 HA+/- RHDV strain: ŻD, where less changes in haematological indices and less pathogenicity were observed. We found that changes caused by HA- Frankfurt strain were related to the number of neutrophils and thrombocytes, while in HA- strain Asturias, in thrombocytes and leukocytes. Changes evoked by HA+/- Hagenow strain pertained to the number of eosinophils, thrombocytes, leukocytes, monocytes, and concentration of hemoglobin. Mortality caused by the Frankfurt strain was 100% between 36 and 48 h post infection (p.i.), while that caused by Asturias strain was 100% between 24 and 36 h p.i., and that observed in case of Hagenow strain was 90% between 36 and 48 h p.i. The changes in haematological picture caused by the HA- and HA+/- RHDV strains were less intensive than those found in case of the HA+ RHDV strains, which cannot be confirmed for pathogenicity, and is not in line with the existing hypothesis suggesting higher pathogenicity in HA+ viruses.

Key words: rabbit haemorrhagic disease virus, haemagglutination, haematology

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Introduction

In the assessment of the organism's health, hematological factors in infections play a major role. As indicated by the studies on the rabbit haemorrhagic disease virus (RHDV), the analysis of haematological factors, including the immunological picture, is an important element in assessing the physiological condition in animals infected with this virus (Piekarski 1994, Deptuła et al. 1997, Tokarz-Deptuła 1998, Hukowska-Szematowicz 2006, Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009, 2011a,b, Tokarz-Deptuła 2009). Haematological analyzes in rabbits infected with RHDV carried out by authors from outside Poland (Table 1) regarding 9 different exclusively haemagglutinating (HA+) virus strains showed a decrease in values of haematological factors dealing with the total number of leukocytes, lymphocytes, and thrombocytes. Polish studies involving 21 HA+ (Table 2) RHDV strains including RHDVa, obtained from various European countries, evidenced that the infection causes changes in haematological parameters for HA+ RHDV strains (Table 2, 2a), represented by both a decrease and increase in the values, although there were more decreases referring mainly to the number of lymphocytes as well as quantities of leukocytes and neutrophils. The same Polish studies involving 4 non-haemagglutinating (HA-) strains (two RHDV and RHDVa) and one RHDV strain with variable haemagglutinating (HA+/-) (Table 2, 2a) revealed fewer changes in haematological factors in HA+ RHDV strains, yet they were also more frequently manifested with a decrease in the values referring to thrombocytes, leukocytes, lymphocytes, neutrophils, and haemoglobin concentration. The study results proved that the animal mortality after the infection with 16 HA+ RHDV and RHDVa strains, four HA- strains, and one HA+/- strain of the RHD virus (Table 1, 2, 2a) is similar, as in the majority of both RHDV and RHDVa strains, mortality ranged from 80% to 100%, while very rarely below 50%, whereas deaths most frequently occurred between 48 and 60 h after rabbit infection with the virus. In clinical sciences, however, it is assumed that haemagglutination capacity of viruses is a decisive element for their pathogenicity (Ruvoén-Clouet et al. 2000), which was also confirmed for RHDV by Capucci et al. (1998); hence, it was assumed that HA+ RHDV strains should cause greater changes in the haematological picture than HA- strains as a result of the macro-organism's response to more pathogenic agents.

In addition, RHDV's haemagglutination capacity is a specific feature of the virus, and its absence is very rare, recorded just in 7 out of approximately 500 RHDV strains known worldwide. The feature is absent in English Rainham strain described in 1993 (Capucci et al. 1996), Polish Blaszki (BLA) recorded in 1994 (Kęsy et al. 1996), German Frankfurt (Fra) (Schirrmeier et al. 1999), and Spanish Asturias strains isolated in 1996 (Prieto et al. 2000), as well as in three Chinese strains (whn-1, whn-2, and whn-3) reported in 2005 (Tian et al. 2007). Moreover, within RHDVa, French 9905 (Le Gall Recule 2003) and Italian Pv97 (Capucci et al. 1998) strains were recorded as non-haemagglutinating. RHDV strains exhibit variable haemagglutination capacity – German Hagenow strain described in 1990 (Schirrmeier et al. 1999), Italian Bg97 from 1997 (Capucci et al. 1998), and Polish ŻD strain isolated in 2000 (Fitzner 2006). Also among RHDV2 strains, which can infect not only rabbits but also hares (Le Gall Recule 2013), strain N11 was recorded, which shows a negative capacity for blood groups O and A while positive for blood groups B and AB (Dalton et al. 2012).

Because previous studies analyzing haematological factors in rabbits infected with non-haemagglutinating (HA-) strains of RHDV and strains revealing variable haemagglutination capacity (HA+/-), involving just two HA- strains (BLA and Rainham) and one HA+/- strain (ŻD), the research was performed to extend the data referring to the white and red blood cell picture of peripheral blood in rabbits infected with other two HA- RHDV strains (Frankfurt and Asturias) and one HA+/- strain (Hagenow), which (together with recorded mortality rate) should provide the answer whether RHDV haemagglutination capacity affects the haematological picture of peripheral blood and conditions its pathogenicity.

Materials and Methods

The studies involved 60 mixed-race rabbits of both sexes weighing from 3.2 to 4.2 kg. The animals originated from a licensed farm, and remained under continuous veterinary-zootechnical supervision (Anon 1987). During the experiment, the animals stayed at the vivarium of the Department of Microbiology and Department of Immunology, Faculty of Biology, University of Szczecin, where zootechnical parameters were conformant to the standards recommended in Poland in respect of temperature, ventilation, lighting, and size of cages for animals (Anon 2010). After transporting to the vivarium, the animals were subjected to a two-week adaptation period and were fed with full-portion rabbit feed (16% Królik z Motycza), ranging in the quantity of 0.15-0.20 kg/day and had unlimited access to water. The studies were accepted

Table 1. Results of haematological studies performed abroad in rabbits infected with 9 different strains of RHDV*.

RHDV strains and references	Chinese (no name) (Chen 1986; Chen and Zeng 1986; Zeng et al. 1987; Xu and Chen 1989; Xu et al. 1985)	Austrian (no name) (Kölbl et al. 1990; Nowotny et al. 1993)	Israeli (no name) (Kuttin et al. 1991)	Italian (no name) (Marcato el at. 1988)	German (no name) (Schluter et al. 1990)	Korean (no name) (Ueda et al. 1992)	Spanish (no name) (Ferreira et al. 2004)	French (no name) (Plassiart et al. 1992)	Bulgarian (Slivnitsa/88) (Alexandrov et al. 2009)
Biological feature of the strain	HA+	HA+	HA+	HA+	HA+	HA+	HA+	HA+	HA+
Haemoglobin	Nb	Nb	Nb	Nb	Nb	Nb	Nb	Nb	Nb
Leucocytes	↓	↓	↓	Nb	↓	↓	↓	Nb	↓
Erythrocytes	↓	Nb	Nb	↓	Nb	Nb	Nb	Nb	Nb
Thrombocytes	↓	Nb	Nb	Nb	Nb	↓	Nb	↓	Nb
Lymphocytes	↓	Nb	↓	Nb	Nb	↓	Nb	↓	Nb
Neutrophils	Nb	Nb	Nb	Nb	Nb	↓	Nb	↓	Nb
Eosinophils	Nb	Nb	Nb	Nb	Nb	↓	Nb	Nb	Nb
Basophils	Nb	Nb	Nb	Nb	Nb	↓	Nb	Nb	Nb
Monocytes	Nb	Nb	Nb	Nb	Nb	↓	Nb	Nb	Nb

Legend: HA+ – haemagglutinating strain; ↑ – statistically significant increase; ↓ – statistically significant decrease; Bz – no changes; Nb– not studied; ( ) – reference; * – the studies do not take mortality of the animals into account.

Table 2. Results of Polish haematological and mortality studies in rabbits infected with 21 different RHDV strains.

RHDV strains and references	French Fr-1 (Tokarz-Deptuła 2009)	French Fr-2 (Tokarz-Deptuła 2009)	Polish SGM (Tokarz-Deptuła 2009)	Polish MAŁ (Tokarz-Deptuła 2009)	Polish KGM (Tokarz-Deptuła 2009)	Polish PD (Tokarz-Deptuła 2009)	Polish GSK (Tokarz-Deptuła 2009)	Polish Kr-1 (Tokarz-Deptuła 2009)
Biological feature of the strain	HA+	HA+	HA+	HA+	HA+	HA+	HA+	HA+
Haemoglobin	Bz	Bz	↓	Bz	↓	Bz	↓	↑
Leucocytes	Bz	↓	↑	↓	↓	Bz	↑	↓
E*rythrocytes*	Bz	Bz	Bz	Bz	Bz	Bz	Bz	Bz
Thrombocytes	Bz	Bz	Bz	Bz	Bz	↑	Bz	Bz
Lymphocytes	Bz	↓	↑	↓	↓	↓	↓	Bz
Neutrophils	↑	↑	Bz	↑	↑	↑	↑	Bz
Eosinophils	↓	↑	↑	Bz	Bz	Bz	Bz	Bz
Basophils	↑	↑	Bz	↓	↑	Bz	Bz	Bz
Monocytes	Bz	↑	Bz	Bz	Bz	Bz	Bz	Bz
Mortality in hour	90% w 60h	100% w 60h	95% w 60h	80% w 60h	65% w 56h	0% do 60h	95% w 36h	90% w 52h

Legend: HA+ – haemagglutinating strain; ↑ – statistically significant increase; ↓ – statistically significant decrease; Bz – no changes; Nb – not studied; ( ) – reference.

Table 2a. Results of Polish hematological and mortality studies in rabbits infected with 21 different RHDV strains (continued).

RHDV strains and references	Italian BS89 (Niedźwiedzka 2008)	Czech CAMP V-351 (Hukowska-Szematowicz 2006)	Czech CAMP V-561 (Hukowska-Szematowicz 2006)	Czech CAMP V-562 (Hukowska-Szematowicz 2006)	Czech CAMP V-558 (Hukowska-Szematowicz 2006)	Italian Vt97 (Niedźwiedzka 2008)	German Triptis (Niedźwiedzka 2008)	German Hartmannsdorf (Niedźwiedzka 2008)	English Rainh-am (Niedźwiedzka 2008)	Polish BLA (Tokarz-Deptuła 2009)	Polish ŻD (Tokarz-Deptuła 2009)	Italian Pv97 (Niedźwiedzka 2008)	French 9905 (Niedźwiedzka 2008)
Biological feature of the strain	HA+	HA+	HA+	HA+	HA+	HA+ RHDVa	HA+ RHDVa	HA+ RHDVa	HA-	HA-	HA+/-	HA- RHDVa	HA- RHDVa
Hemoglobin	Bz	↓	↓	Bz	↑i↓	↓	Bz	Bz	↑i↓	Bz	Bz	Bz	↑
Leucocytes	↓	↓	↑	Bz	Bz	Bz	Bz	↑	↓	↓	Bz	Bz	Bz
Erythrocytes	Bz	↓	↓	Bz	↑	Bz	Bz	Bz	Bz	Bz	↑i↓	Bz	Bz
Thrombocytes	↓	↓	Bz	↓	↓	Bz	Bz	Bz	↓	Bz	Bz	↓	Bz
Lymphocytes	↓	↓	↓	↓	↓	Bz	↑	↓	↑	Bz	Bz	↓	Bz
Neutrophils	↑	↑	↑	Bz	↑	Bz	Bz	Bz	Bz	Bz	↑	↑	Bz
Eosinophils	Bz	Bz	Bz	↑	↓	Bz	Bz	Bz	Bz	Bz	Bz	Bz	Bz
Basophils	Bz	Bz	Bz	↑	Bz	Bz	Bz	Bz	Bz	↓	Bz	Bz	Bz
Monocytes	Bz	Bz	Bz	↑	Bz	Bz	Bz	Bz	Bz	Bz	↓	Bz	Bz
Mortality in hour	100% w 48h	80% w 72h	100% w 48h	85% w 72h	45% w 72h	30% w 48h	100% w 48h	100% w 36h	100% w 48h	60% w 60h	100% w 48h	100% w 48h	90% w 48h

Legend: HA+ – haemagglutinating strain; ↑ – statistically significant increase; ↓ – statistically significant decrease; Bz – no changes; Nb – not studied; ( ) – reference

Table 3. Haematological indices in rabbits experimentally infected with non-haemagglutinating Frankfurt RHDV strain.

Parameters			Values in hours											
			0		4		8		12		24		36	
			Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (8)	K (10)	Z (8)	K (10)
Haemoglobin [mmol/l]		$\bar{x}$	7.09	7.73	8.71	7.11	7.73	6.80	6.62	6.41	6.09	6.47	6.95	7.73
		SD ±	0.78	0.27	0.69	0.38	0.73	0.28	0.57	0.26	0.89	0.36	0.06	0.07
Leucocytes [10^9/l]		$\bar{x}$	3.30	3.84	3.30	3.90	3.40	3.54	4.60	3.74	2.70	3.68	2.50	3.30
		SD ±	0.40	0.27	0.60	0.21	0.60	0.90	0.60	0.36	0.30	0.33	0.10	0.05
Erythrocytes [10^{12}/l]		$\bar{x}$	3.70	3.79	3.70	3.44	3.10	3.51	3.00	3.14	2.50	3.19	2.70	3.83
		SD ±	0.30	0.25	0.30	0.18	0.40	0.16	0.60	0.16	0.50	0.02	0.10	0.13
Thrombocytes [10^9/l]		$\bar{x}$	536.00	493.80	496.00	476.90	642.00*	456.00	585.00*	456.60	520.00	483.60	558.00	483.00
		SD ±	57.00	29.85	59.00	30.61	42.00	35.35	42.00	37.35	35.00	33.06	47.00	31.92
Quality picture of blood – 1	*Lymphocytes*	$\bar{x}$	0.42	0.64	0.35	0.57	0.42	0.59	0.55	0.61	0.44	0.61	0.44	0.66
		SD ±	0.170	0.022	0.070	0.018	0.090	0.025	0.060	0.013	0.200	0.016	0.080	0.011
	Granulocytes *Neutrophils*	$\bar{x}$	0.56	0.34	0.57*	0.38	0.48*	0.38	0.38	0.36	0.46*	0.35	0.46	0.40
		SD ±	0.170	0.032	0.070	0.013	0.080	0.022	0.110	0.010	0.210	0.016	0.060	0.011
	Granulocytes *Eosinophils*	$\bar{x}$	0.03	0.01	0.05	0.02	0.05	0.02	0.06	0.02	0.07	0.01	0.04	0.02
		SD ±	0.010	0.005	0.030	0.006	0.040	0.004	0.030	0.006	0.020	0.006	0.010	0.006
	Granulocytes *Basophils*	$\bar{x}$	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.02	0.00	0.04
		SD ±	0.010	0.005	0.000	0.003	0.010	0.002	0.010	0.002	0.000	0.003	0.000	0.002
	Monocytes	$\bar{x}$	0.03	0.02	0.02	0.02	0.05	0.01	0.02	0.01	0.03	0.01	0.05	0.01
		SD ±	0.020	0.005	0.020	0.003	0.010	0.003	0.000	0.002	0.020	0.003	0.010	0.005

Legend: Z – infected animals; K – control animals; ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation.

Table 4. Haematological indices in rabbits experimentally infected with non-haemagglutinating Asturias RHDV strain.

Parameters				Values in hours									
				0		4		8		12		24	
				Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (9)	K (10)	Z (9)	K (10)
Haemoglobin [mmol/l]			$\bar{x}$	8.37	7.73	8.35	7.11	7.38	6.80	6.85	6.41	6.51	6.47
			SD ±	0.45	0.27	1.25	0.38	0.95	0.28	0.63	0.26	0.92	0.36
Leucocytes [10^9/l]			$\bar{x}$	4.20	3.84	4.60	3.90	3.70	3.54	3.90	3.74	2.00	3.68*
			SD ±	0.30	0.27	0.30	0.21	0.30	0.28	0.60	0.36	0.30	0.33
Erythrocytes [10^{12}/l]			$\bar{x}$	4.30	3.79	4.10	3.44	3.50	3.51	3.40	3.14	3.10	3.19
			SD ±	0.30	0.25	0.30	0.18	0.60	0.16	0.40	0.16	0.50	0.02
Thrombocytes [10^9/l]			$\bar{x}$	517.00	493.80	550.00	476.90	415.00	456.00	336.00	456.60*	291.00	483.60*
			SD ±	73.00	29.85	76.00	30.61	46.00	35.35	28.00	37.35	40.00	33.06
Quality picture of blood – 1	*Lymphocytes*		$\bar{x}$	0.69	0.64	0.53	0.57	0.63	0.59	0.64	0.61	0.70	0.61
			SD ±	0.050	0.022	0.140	0.018	0.070	0.025	0.090	0.013	0.080	0.016
	Granulocytes	*Neutrophils*	$\bar{x}$	0.23	0.34	0.40	0.38	0.28	0.38	0.27	0.36	0.25	0.35
			SD ±	0.040	0.032	0.070	0.013	0.040	0.022	0.040	0.010	0.080	0.016
		Eosinophils	$\bar{x}$	0.03	0.01	0.04	0.02	0.04	0.02	0.04	0.02	0.03	0.01
			SD ±	0.020	0.0005	0.020	0.006	0.010	0.004	0.030	0.006	0.020	0.006
		Basophils	$\bar{x}$	0.01	0.01	0.00	0.01	0.00	0.01	0.02	0.01	0.00	0.02
			SD ±	0.000	0.005	0.000	0.003	0.000	0.002	0.010	0.002	0.000	0.003
	Monocytes		$\bar{x}$	0.06	0.02	0.03	0.02	0.05	0.01	0.05	0.01	0.02	0.01
			SD ±	0.020	0.005	0.010	0.005	0.020	0.003	0.20	0.002	0.020	0.005

Legend: Z – infected animals; K – control animals; ( ) – number of animals;- mean value; SD – standard deviation.

Table 5. Haematological indices in rabbits experimentally infected with RHDV strain Hagenow with variable haemagglutination capacity.

Parameters				Values in hours											
				0		4		8		12		24		36	
				Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (8)	K (10)	Z (8)	K (10)	Z (1)	K (10)
Haemoglobin [mmol/l]			$\bar{x}$	7.83	7.73	8.29	7.11	8.01*	6.80	7.26*	6.41	6.84	6.47	5.48	7.73*
			SD ±	0.46	0.27	0.32	0.38	0.46	0.28	0.42	0.26	0.64	0.36	0.48	0.37
Leucocytes [10^9/l]			$\bar{x}$	4.50	3.84	4.30	3.90	6.00*	3.54	6.20*	3.74	5.20*	3.68	2.30	3.30
			SD ±	0.46	0.27	0.70	0.21	0.58	0.28	0.71	0.36	0.67	0.33	0.80	0.15
Erythrocytes [10^{12}/l]			$\bar{x}$	3.50	3.79	3.90	3.44	3.50	3.51	3.10	3.14	3.00	3.19	3.10	3.83
			SD ±	0.30	0.25	0.10	0.18	0.40	0.16	0.40	0.16	0.40	0.22	0.20	0.13
Thrombocytes [10^9/l]			$\bar{x}$	567.0	493.8	579.0*	476.9	571.0*	456.0	578.0	456.6	622.0*	483.6	616.0*	483.0
			SD ±	79.00	29.85	58.00	30.61	31.00	35.35	32.00	37.35	44.00	33.06	38.00	31.92
Quality picture of blood – 1	*Lymphocytes*		$\bar{x}$	0.56	0.64	0.52	0.57	0.62	0.59	0.68	0.61	0.63	0.61	0.59	0.66
			SD ±	0.100	0.120	0.060	0.018	0.090	0.025	0.050	0.023	0.070	0.016	0.080	0.011
	Granulocytes	*Neutrophils*	$\bar{x}$	0.36	0.34	0.40	0.38	0.31	0.38	0.26	0.36	0.30	0.35	0.35	0.34
			SD ±	0.080	0.032	0.050	0.023	0.070	0.022	0.090	0.010	0.030	0.016	0.040	0.011
		Eosinophils	$\bar{x}$	0.04	0.035	0.05*	0.02	0.05*	0.02	0.06*	0.02	0.05*	0.01	0.04*	0.02
			SD ±	0.020	0.015	0.030	0.016	0.030	0.004	0.010	0.006	0.010	0.006	0.030	0.006
		Basophils	$\bar{x}$	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.03	0.04
			SD ±	0.010	0.005	0.010	0.003	0.001	0.002	0.010	0.002	0.001	0.003	0.008	0.002
	Monocytes		$\bar{x}$	0.03	0.02	0.03	0.02	0.03*	0.01	0.02*	0.01	0.03*	0.01	0.02	0.01
			SD ±	0.010	0.005	0.010	0.003	0.010	0.003	0.010	0.002	0.020	0.003	0.020	0.005

Legend: Z – infected animals; K – control animals; ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation.

by the Ethics Committee in Szczecin (permission number 11/06).

Rabbits (60 animals) were divided into the following two groups: infected animals – 10 rabbits for each of the three analyzed RHDV strains; 10 animals as controls for each strain. The animals from infected groups were intramuscularly (leg muscles) administered the same doze of RHDV: non-haemagglutinating Frankfurt strain from Germany, and Asturias strain from Spain, and strain with variable haemagglutination capacity Hagenow from Germany, which was prepared according to the previously described procedure (Niedźwiedzka-Rystwej and Deptuła 2010). Such prepared viral antigens were collected to infect animals in these groups, while blood samples were drawn from them and control animals from the auricular marginal vein at hour "0", before administering RHDV and glycerol, and next at 4, 8, 12, 24, and 36 h of the experiment, until the occurrence of clinical symptoms or first animal deaths (Table 6), according to the recommendation of the Ethics Committee.

Blood analysis involved haemoglobin concentration, quantity of erythrocytes, leukocytes, thrombocytes, and white cell picture (lymphocytes, neutrophil granulocytes, basophil granulocytes, acidophil granulocytes, and monocytes) according to the commonly known and used standards. The assessment of the pathogenicity of the analyzed RHDV strains was carried out by observing clinical symptoms and mortality among the animals, recorded in rabbits observed at 0, 4, 8, 12, 24, 36, and 48 h after their infection with the virus.

The results of haematological tests were subject to the statistical analysis with t-Student test in Statistica 6.0 software as shown in Tables 3-5.

Results

The changes in HA- Frankfurt and Asturias strains (Tables 3, 4) were only recorded in the number of neutrophils and thrombocytes for HA- Frankfurt strain, while for HA- Asturias strain they were recorded in the volume of thrombocytes and leukocytes. In HA+/- Hagenow strain, the changes referred to the volume of eosinophils, thrombocytes, leukocytes, monocytes, and haemoglobin concentration.

The analysis of changes in haematological parameters in rabbits infected with HA- Frankfurt strain of RHDV showed (Table 3) an increase in the number of neutrophils at 4, 8, and 24 h p.i. and an increase in thrombocytes at 8 h p.i. For HA- Asturias strain of RHDV (Table 4), the changes involved a decrease in the quantity of thrombocytes and leukocytes, falling at 12 and 24 h p.i. for thrombocytes and at 24 h p.i for leukocytes. The changes in the haematological picture for HA+/- RHDV Hagenow strain (Table 5) involved an increase in the volume of eosinophils (4, 8, 12, 24, and 36 h), thrombocytes (4, 8, 24, and 36 h), leukocytes (8, 12, and 24 h), monocytes (8, 12, and 24 h), and haemoglobin concentration (8 and 12 h). For this strain, a decrease in haemoglobin concentration value was also recorded at 36 h p.i.

When analyzing the clinical picture in rabbits infected with three currently analyzed RHDV strains (Frankfurt, Asturias and Hagenow), it was stated that in HA- strains such as Frankfurt and Asturias, the mortality rate totaled 100%, with the deaths occurring in the period between 36 and 48 h p.i. for Frankfurt strain and in the period between 24 and 36 h p.i. for Asturias strain. In HA+/- Hagenow RHDV strain, mortality was 90% and, similarly as with HA- Frankfurt, deaths occurred between 36 and 48 h p.i. Clinical symptoms were only observed in one rabbit infected with HA+/- Hagenow strain, between 36 and 48 h p.i., characterized by apathy, difficulties in breathing, lack of response to external stimuli, and nasal fluids. In other animals infected with Hagenow, Frankfurt, and Asturias strains, no clinical symptoms were recorded.

Discussion

When analyzing the results in haematological parameters in rabbits infected with two non-haemagglutinating Frankfurt and Asturias RHDV strains and one strain with variable haemagglutination capacity Hagenow, it must be stated that the image of changes is slightly different than that previously observed for (HA-) strains, Rainham (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009) and BLA, and for (HA+/-) strain, ŻD (Tokarz-Deptuła 2009). In fact, the changes observed in the present study in the haematological picture for HA- Frankfurt RHDV strain, manifested with the increase in the volume of neutrophils at 4, 8, and 24 h p.i., do not conform to the image of changes obtained for two HA- strains: Rainham and BLA; as in the case of such strains, the changes exclusively referred to the volume of leukocytes (decrease at 12 h for Rainham, and at 48 h for BLA) and thrombocytes (decrease at 12, 24, and 36 h for Rainham, with no changes for BLA) (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009, Tokarz-Deptuła 2009). Similarly, an increase in the volume of thrombocytes at 8 h from infection for the currently analyzed Frankfurt strain does not corroborate the changes recorded for Rainham (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009) and BLA (Tokarz-Deptuła 2009), as

for Rainham, the volume of thrombocytes decreased at 12, 24, and 36 h p.i. (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009), while BLA strain showed no changes (Tokarz-Deptuła 2009). Therefore, it must be assumed that image of changes observed in this study in Frankfurt strain, which only manifested an increase in neutrophils and thrombocytes, does not correspond with changes described for previously analyzed Rainham (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009) and BLA (Tokarz-Deptuła 2009) strains, for which a decrease was referred in leukocytes (at 12 h for Rainham, and at 48 h for BLA) and thrombocytes (at 12, 24, 36 h for Rainham, with no changes for BLA).

For currently analyzed non-haemagglutinating Asturias RHDV strain, it must be stated that currently recorded decrease in leukocytes at 24 h p.i. is analogical to that obtained in Rainham strain (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009) and is similar to the image of changes recorded at 48 h p.i. with BLA strain (Tokarz-Deptuła 2009). The decrease in thrombocytes at 12 and 24 h p.i. with Asturias strain is very similar to the decrease in this factor recorded at 12, 24, and 36 h for Rainham strain (Niedźwiedzka 2008, Niedźwiedzka-Rystwej and Deptuła 2009), although it differs from the image reported for BLA strain (Tokarz-Deptuła 2009), where no changes in thrombocytes were observed. Therefore, for HA- RHDV strains, it can be assumed that the decreases in the values of haematological factors (leukocytes and thrombocytes) for the currently investigated Asturias strain are similar to the image of changes recorded for previously analyzed Rainham and BLA strains, for which observations included a decrease in leukocytes (decrease at 12 h for Rainham, and at 48 h for BLA) and thrombocytes (decrease at 12, 24, and 36 h for Rainham, with no changes for BLA). For currently analyzed Hagenow strain with variable haemagglutination capacity, the recorded increase in eosinophils at 4, 8, 12, 24, and 36 h p.i. does not confirm the results obtained previously for ŻD strain (Tokarz-Deptuła 2009), similar to the differences in the image of monocytes, which increased at 8, 12, 24 h for Hagenow. The image also differed for Hagenow and ŻD in respect of the volume of thrombocytes, as for Hagenow, they increased at 4, 8, 24, and 36 h p.i., while no changes were observed for ŻD (Tokarz-Deptuła 2009). Moreover, Hagenow strain does not show changes in the volume of neutrophils, whereas ŻD strain (Tokarz-Deptuła 2009) caused the long-term increase in this factor in the period from 4 to 36 h p.i. Also, the changes in haemoglobin concentration recorded in the form of an increase at 8 and 12 h and a decrease at 36 h for the Hagenow strain do not find the confirmation in the results for ŻD strain, as no changes in this factor were reported for this strain (Tokarz-Deptuła 2009). A similar image of changes was recorded considering the increase in leukocytes both for the Hagenow strain at 8, 12, and 24 h and for ŻD strain at 8 h, although the latter additionally caused a decrease at 60 h p.i. (Tokarz-Deptuła 2009). Therefore, to conclude on the present results for HA+/- Hagenow and ŻD strains, it can be stated that the changes caused by those strains differ, but regardless of the different image of the changes, these strains show more increases than decreases in the analyzed haematological factors.

When analyzing the changes in the clinical image caused by two currently investigated HA- strains (Frankfurt, Asturias) of RHDV, it must be stated that HA- strains cause 100% mortality of the animals between 24 and 36 h (Asturias) and 36 and 48 h (Frankfurt) p.i. The results confirm the mortality rate obtained for HA- Rainham strain (which also totaled 100%, but deaths occurred at 24 h p.i.) but slightly differ from the image obtained for HA- BLA strain, where just 60% mortality was observed, with deaths occurring 60 h p.i. (Niedźwiedzka 2008). Mortality at the level of 90% between 36 and 48 h p.i. for the currently investigated HA+/- Hagenow RHDV strain is similar to the results obtained for the previously analyzed strain with variable haemagglutination capacity, ŻD strain, for which 100% mortality was recorded with deaths at 48 h p.i.

In conclusion, based on the results obtained, it must be stated that two currently investigated non-haemagglutinating Frankfurt and Asturias strains of RHDV and one strain with variable haemagglutination capacity Hagenow (Tables 3-5) cause different images of the analyzed haematological factors, because Frankfurt strain only causes increases while Asturias exclusively decreases, whereas Hagenow causes both increases and decreases, with the majority of the increases. While assessing the mortality rate of rabbits infected with three currently investigated strains (Frankfurt, Asturias, Hagenow), it must be assumed that they cause high mortality rate because non-haemagglutinating RHDV strains (Frankfurt and Asturias) result in 100% mortality rate, whereas the investigated Hagenow strain with variable haemagglutination capacity causes 90% mortality among rabbits, which is analogical to the results obtained for 16 HA+ RHDV strains (Table 2), where mortality was reported at the level of 80-100%, with deaths occurring later from infection (48-60 h) than for currently investigated strains (Frankfurt, Asturias, Hagenow) (24-36 h).

From the present (Tables 3-5) and previous studies (Tables 2, 2a) regarding the assessment of haematological factors for RHDV strains without or with

variable haemagglutination capacity, it can be stated that these strains, as compared to haemagglutinating strains (Tables 1, 2, 2a), cause fewer changes and are characterized with less clear changes in the haematological picture than that observed for haemagglutinating strains, where more decreases are clearly recorded in haematological parameters. Furthermore, in all HA- and HA+/- RHDV strains analyzed now and previously, the changes principally referred to thrombocytes and leukocytes, although earlier studies on HA- and HA+/- strains also refer to lymphocytes, neutrophils, and haemoglobin concentration, while for haemagglutinating strains, the changes principally referred to lymphocytes. Also, high mortality obtained for presently analyzed HA- and HA+/- strains, amounting to 90-100%, is confirmed in the picture of haematological changes, yet it does not confirm the hypothesis popular in clinical sciences (Capucci et al. 1998, Ruvoén-Clouet et al. 2000) that haemagglutinating strains are more pathogenic.

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