Pol. J. Environ. Stud. Vol. 21, No. 4 (2012), 879-892

Original Research

Non-Specific Cellular Immunity in Rabbits Experimentally Infected with Four Czech Strains of the Rabbit Haemorrhagic Disease Virus with Different Pathogenicity

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Received: 29 July 2011
Accepted: 31 January 2012

Abstract

The rabbit haemorrhagic disease (RHD) virus was first described in 1984 in China, where it caused a rabbit plague characterized by an acute course. At present, the disease has spread to rabbits on all continents, and is characterized by morality reaching 100%. Research on the immune response in rabbits after infection by RHD virus strains has so far only been performed by the Deptuła team. In turn, it must be stated that similar research worldwide has been performed in the Chinese centre, yet referring exclusively to rabbits after immunization with inactivated RHD virus. Such research indicates that shortly after immunization, the immunity is coordinated by macrophages and lymphocytes T and B, while further on the protection against the infection is conditioned by humoral immunity. Deptuła's team has investigated 22 strains of RHDV in the aspect of non-specific cellular and humoral immunity, as well as specific cellular and humoral immunity including 3 French strains (FR-1, Fr-2, 9905RHDVa), 10 Polish strains (K-1, Kr-1, KGM, SGM, MAŁ, BLA, PD, GSK, Ż, ŻD), 4 German strains (Hagenow, Frankfurt, Triptis, Hartmannsdorf), 3 Italian strains (BS89-reference strain, Vt97, PV97), 1 English strain (Rainham), and 1 Spanish strain (Asturias). The strains were analyzed in the aspect of such parameters as capacity of adherence and absorption of PMN cells, PMN cell cidal property measured with spontaneous, stimulated, and spectrophotometric NBT test, stimulation index and PMN metabolic activity coefficient; and MPO activity, as well as concentration and activity of LZM. Also, the number was marked of lymphocytes T CD5+, Th with receptor CD4+, Tc/Ts with receptor CD8+, and the number of lymphocytes with receptor CD25+, as well as the percentage of lymphocytes B (IgM). The research indicates the presence of immunogroups within the RHD virus. Assessment of pathogenicity of the RHD virus is actually performed based on the mortality rate in rabbits infected with the virus, which is dictated by the fact that the virus has so far not been obtained in vitro. Niedźwiedzka et al. divided the 10 analyzed strains into strains with high pathogenicity with mortality of 90-100%, up to 36/48 hour of the study (BS89, Hagenow, Rainham, Frankfurt, Asturias, Triptis, Hartmannsdorf, Pv97, 9905RHDVa), and strains with lower pathogenicity with mortality of 30% up to 36/48h (Vt97). In turn, Tokarz-Deptuła divided the 10 analyzed strains of the RHDV (including 8 Polish and 2 French) into strains with mortality of 80-100% (Fr-2, ŻD, GSK, SGM, Fr-1, Kr-1, MAŁ), strains with mortality of 60-65% (KGM, BLA), and strains with mortality below 60% (PD).

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The aim of our study was to record changes to parameters of non-specific cellular immunity (capacity of adherence and absorption of PMN cells, cidal capacity of PMN cells measured with spontaneous NBT test, stimulated and spectrophotometric, and stimulation index and metabolic activity ratio of PMN cells) in rabbits experimentally infected with 4 haemagglutinogenic Czech strains of the RHD virus: CAMPV-351 (reference strain), CAMP-561, CAMPV-562, and CAMPV-558, with different pathogenicity; which strains have not yet been analyzed in this respect. The assessment of pathogenicity of the analyzed strains of the RHDV was performed on the basis of mortality rate among rabbits infected with these strains. On the basis of the number and duration of changes to analyzed parameters of non-specific cellular immunity, the 4 analyzed Czech strains are determined to differentiate in the aspect of immunogenicity into three groups. The first group is formed by the most immunogenic reference strain CAMPV-351, the second – by two medium-immunogenic strains – CAMPV-561 and CAMPV-558, whereas the third one – by the least immunogenic strain CAMPV-562. The results obtained in the area of pathogenicity are not reflected in the division of the analyzed Czech strains according to their immunogenicity.

Keywords: rabbit, RHDV, non-specific cellular immunity, immunogenicity, pathogenicity

Introduction

The RHD virus is an etiological factor in rabbit plague and is the main species in Lagovirus order, the *Caliciviridae* family [1]. Since isolation of the RHD virus in 1984 in China [1], over 400 strains of this virus have been identified, the sequences of which can be found in the Gene Bank [2]. The studies regarding the RHD virus point to its non-homogenity, which is testified to by the isolation of 7 strains of the RHDV defined as reference strains, 9 strains showing no haemagglutination capacity (HA), a specific property of the virus, 37 strains defined as antigen variants- RHDVa (described as both HA+ and HA-), and 1 strain determined as a new variant of the RHD virus -FrenchRHDVvariant (differentiated exclusively on the basis of phylogenetic analysis) (Table 1). Considering the changes caused to the blood of rabbits infected with various strains (SGM, MAŁ, KGM, ŻD, PD, GSK, KR-1, BLA, Fr-1, Fr-2) of the RHD virus, as regards non-specific and specific cellular and humoral immunity parameters, three immunogroups (immunotypes) were identified [1]. The results were confirmed with observations by other authors [22] who studied 10 European strains (Bs89, Hagenow, Rainham, Frankfurt, Asturias, Vt97, Triptis, Hartmannsdorf, Pv97, 9905RHDVa) of the RHD virus and also evidenced their differentiation in the aspect of the analyzed immunological factors.

Another specific phenomenon regarding this virus was the description by Abrantes et al. [23] of recombination within the protein of capsid VP60, which was at the same time the first recombination recorded for the RHDV. The analysis involved VP60 sequences in 43 different RHDV strains (including Hartmannsdorf strains), whereas 3 comparative analyses were performed, comprising: full VP60 nucleotide sequence, hyper-variable region E, and gene encoding VP60 excluding region E. Hartsmannsdorf strain, as the only one, was not included in the first analysis, while it was present in the two remaining ones, which points to its chimeric origin, indicating it was formed from the merger of the genetic material from at least two viruses. The recombination among the RHDV strains was also confirmed with their own research by Forrester et al. [24], considering it may be a key event in the evolution of the virus. The recombination was detected in the gene encoding VP60 (00-08 and China 1984 strains), in the gene encoding RNA-dependent RNA polymerase (Frankfurt5, Frankfurt12, Wika) and in 10 out of 26 analyzed strains of the RHDV with fully known genome, which means that the recombination in RHDV occurs much more frequently than it was originally thought. In turn, Esteves et al. [25] described the presence of positive selection in the protein of VP60 capsid of the RHD virus. Material for the study was formed by 43 strains of the RHD virus with full sequence of the gene encoding VP60. On the basis of the analyses performed in the strains under study, three codons indicated the presence of positive selection in the position of aminoacids 307, 432, and 476, and they were located in hyper-variable regions C and E, and in region F. The presence of positive selection in region E can be explained by the situation in the region of the main antigen determinants of the RHDV, whereas region C can participate in interactions with the host's receptors. The occurrence of positive selection in regions other than C and E can point to the presence of other antigen determinants within VP60 that stimulate the immune response. According to the authors, positive selection is related to antigen regions, which may suggest that the variability of the virus is caused by the host's immune response. Furthermore, according to the authors, identification of positive selection is very important due to the fact that variability of the RHDV is probably connected with pathogenicity and virulence. The study from 2011 [26] indicates that histo-blood group antigens (HBGA) act as additional factors in the infection with the RHD virus in a manner dependent on the viral strain. RHD strains described as the first after the outbreak of the disease did not show capacity to bind to antigen A belonging to the HBGA group, whereas all other strains could bind to it. Moreover, rabbits missing correct HBGA ligands were resistant to mortal infection with RHDV, even at low doses. As indicated by the studies, histo-blood group antigens can act as factors facilitating the infection, whereas polymorphism of the expression of this antigen can contribute to the emergence of genetic immunity to the RHDV at the population level.

A new light onto the division and phylogenetic dependencies among rabbit lagoviruses was cast by the description by Strive et al. [27] of the new representative of this group, namely non-pathogenic lagovirus RCV-A1, obtained from wild rabbits in Australia. The comparison of the virus (RCV-A1) with other rabbit lagoviruses (based on the part of genome encoding fragment of capsid protein) indicated the division of the analyzed sequences of the factor of pathogenic lagovirus into three groups. Group One was formed by strains of the RHDV and RHDVa, Group Two comprised RCV-like strains represented by European non-pathogenic strains, namely Italian RCV, British Ashington and Irish Lambayl, while Group Three was formed by the Australian non-pathogenic lagovirus RCV-A1. The results of the studies, together with the available knowledge on the origin of rabbit lagoviruses, have led to the development of the hypothesis on the part of the authors of the publication [27], according to which the ancestor of RCV-A1 came to Australia together with the first rabbits in the 19th century, while 150 years of geographic separation have caused the difference between non-pathogenic European lagoviruses and the Australian one, which is manifested by the creation of a separate system in the phylogenetic tree. Furthermore, Abrantes and Esteves [28] report the occurrence of a new strain, referred to as MRCV (Michigan Rabbit calicivirus), which turned out to be a new variant belonging to the group of non-pathogenic RCV-like viruses.

The purpose of the study was to record changes to parameters of non-specific cellular immunity (capacity of adherence and absorption of PMN cells, cidal capacity of PMN cells measured with spontaneous NBT test, stimulated and spectrophotometric, stimulation index and metabolic activity ratio of PMN cells) in rabbits experimentally infected with 4 haemagglutinogenic (HA+) Czech strains of the RHD virus: CAMPV-351 (reference strain), CAMP-561, CAMPV-562, and CAMPV-558, with different pathogenicity; whose strains have not yet been analyzed in this respect.

Experimental Procedures

Experimental Animals

The study involved 120 mixed-breed rabbits weighing 2.5-3.5 kg, marked as conventional animals [29]. During the study, the rabbits remained at the vivarium of the Department of Microbiology and Immunology, where zoohygienic parameters in the area of temperature and humidity corresponded to standards applicable in Poland [30].

Experimental Scheme and Substances Administered to Experimental Animals

The study involved 4 haemagglutinogenic (HA+) Czech strains of the RHD virus, which are not antigen variants of the RHD virus: CAMPV-351 (reference strain), CAMPV-561, CAMPV-562, and CAMPV-558. The strains were obtained from Microbial Bank, Veterinary Institut in Brno (Czech Republic). Experimental animals designated for infection were divided into 4 groups of 20 rabbits. Animals in Group 1 were administered intramuscularly (leg muscles) with lyophilizate of reference strain CAMPV-351 diluted in 1ml sterile physiological salt, animals in Group 2 were administered in the same manner the strain CAMPV-561, in Group 3 – CAMPV-562, while in Group 4 – CAMPV-558. Each group of infected animals had a corresponding group of 10 control animals, whereas each animal was intramuscularly administered (leg muscles) 1 ml of sterile physiological salt. Each of the viral strains: CAMPV-351 (obtained in 1987), CAMPV-561 (1996), CAMPV-562 (1992), and CAMPV-558 (1988) came from a naturally dead animal. These strains, in the form of liver homogenisate, were used for infection of rabbits from whom liver was sampled after death, which was then used for infection of animals studied, by administering the liver tissue to them in the form of 20% homogenisate cleared by centrifugation at 3000 rpm, 10% chloroforming for 60 minutes and centrifugation again, and then lyophilization in the 24-hour procedure [1]. All the antigens of the RHD virus prepared had the same number of particles determined with density per 1.310 -1.340 g/dm^3, with titre in the HA test from 5120 to 10240.

Schedule of the Study

Blood from infected and control animals was drawn through a port from the marginal vein of the ear. For all groups of the studied experimental animals, blood was drawn at hour "0" and then at hours 4, 8, 12, 24, 36, 48, 52, 56, 60, and 72. The study was performed according to the recommendations of the Ethics Committee for Animal Experiments, until the first symptoms of the disease or until the death of experimental animals.

Methods

In peripheral blood of rabbits, parameters for non-specific cellular immunity were marked. PMN cell adherence capacity in peripheral blood was determined with the Lorente et al. [31] method. The PMN cell absorption capacity (expressed with absoption index and percentage of absorbing cells) against the model strain of Staphylococcus aureus bacteria, strain 209P, was marked using the Brzuchowska and Ładosz method, as modified by Deptuła [32]. The capacity for reduction of the nitroblue tetrazolium (NBT) in PMN cells of peripheral blood was determined using the cytochemical method in a spontaneous and stimulated test and with the spectrophotometric method. Spontaneous and stimulated test of NBT reduction was performed according to the modified cytochemical method by Park et al. [33], while the spectrophotometric test – according to the modified Raman and Poland method [34]. Also, metabolic activity coefficient of neutrophilic granulocytes (WAMG) was marked according to Grządzielska [35], as well as stimulation index (IS) according to Lechowski [36].

In animals infected with the RHD, mortality was recorded at particular times of the study, on the basis of

Table 1. Reference strains, non-hemagglutinating strains, antigenic variants and new variant of RHDV.

Strains	RHDV strains/ Hemaglutinatination ability		GenBank Accession number	Country of origin/ Year of idenification	References
References	CAMPV-351	HA+	U54983	Czech Republic, 1987	3
	RHDV-FRG	HA+	M67473	Germany, 1989	4
	BS89	HA+	X87607	Italy, 1989	5
	SD	HA+	Z29514	France, 1989	6
	AST89	HA+	Z49271	Spain, 1989	7
	Iowa2000	HA+	AF258618	USA, 2000	8
	WHNRH	HA+	DQ280493	China, 2002	8
Non-hemagglutinating	Rainham	HA-	AJ006019	UK, 1993	9
	Blaszki (BLA)	HA-	not registered	Poland, 1994	10
	Asturias	HA-	not registered	Spain, 1996	11
	Frankfurt	HA-	Y15424	Germany, 1996	12
	Pavia97 (Pv97)	HA-	EU250330	Italy, 1997	5
	9905RHDVa	HA-	AJ302016	France, 1999	13
	WHN-1	HA-	DQ069280	China, 2005	8, 14
	WHN-2	HA-	DQ069281	China, 2005	8, 14
	WHN-3	HA-	DQ069282	China, 2005	8, 14
Antigenic variants-RHDVa	Viterbo97(Vt97)	HA+	EU250331	Italy, 1997	5
	Triptis	HA+	EF558583	Germany, 1996	12
	Hartsmanndorf	HA+	EF558586	Germany, 1996	12
	01-38RHDVa	HA+	not registered	France, 2001	15
	WHNRH	HA+	DQ280493	China, 2002	8
	Iowa2000	HA+	AF258618	USA, 2000	8
	WHN-1	HA-	DQ069280	China, 2005	8, 14
	WHN-2	HA-	DQ069281	China, 2005	8, 14
	WHN-3	HA-	DQ069281	China, 2005	8, 14
	9905RHDVa	HA-	AJ302016	France, 1999	13
	Pavia97 (Pv97)	HA-	EU250330	Italy, 1997	5
	CD	no data	AY523410	China, b.d	8, 14
	YL	no data	DQ530363	China, b.d	8, 14
	TP	no data	AF453761	China, b.d	8, 14
	NJ1985	no data	AY269825	China, 1985	8, 14
	JXCHA97	no data	DQ205345	Chiny, 1997	8, 14
	RH29/03	no data	AY935974	Hungary, 2003	16
	CUB5-04	no data	DQ841708	Cuba, 2004	17
	IN05	no data	EU003578	USA, 2005	8
	NY01	no data	EU003581	USA, 2001	8
	UT01	no data	EU003582	USA, 2001	8
	NL2004-1	no data	DQ296063	Netherlands, 2004	18
	NL2004-2	no data	DQ296064	Netherlands, 2004	18
	NL2004-3	no data	DQ296065	Netherlands, 2004	18

Table 1. Continued.

Strains	RHDV strains/ Hemaglutinatination ability		GenBank Accession number	Country of origin/ Year of idenification	References
Antigenic variants-RHDVa	08Q221	no data	not registered	Korea, 2008	19
	08Q712	no data	not registered	Korea, 2008	19
	08Q121	no data	not registered	Korea, 2008	19
	KV0801	no data	FJ212322	Korea, 2008	19
	06Q48-2	no data	not registered	Korea, 2006	19
	07Q92-1	no data	not registered	Korea, 2007	19
	06D32-1	no data	not registered	Korea, 2006	19
	06D106-1	no data	not registered	Korea, 2006	19
	06Q755-1	no data	not registered	Korea, 2006	19
	L145/04	no data	not registered	Poland, 2004	20
	W147/05	no data	not registered	Poland, 2005	20
	00-Reu	no data	AJ303106	France, 2000	13
	3-24	no data	AJ969628	France, 2003	13
New variant	FrenchRHDVvariant	no data	not registered	France, 2010	21

which the mortality index was calculated and evaluated on the basis of the pathogenicity of strains.

Statystical Analysis

The results of immunological studies were statistically analyzed using t-Student test with p=0.05, in Statistica version 6.0. software (StatSoft, Poland), comparing the obtained values of immunity parameters analyzed in groups of infected animals with the ones obtained in rabbits in control groups. The results of immunological studies are presented in Tables 2-5.

Results

Results of the studies indicated that among the analyzed parameters of non-specific cellular immunity in rabbits experimentally infected with four haemagglutinogenic Czech strains of the RHDV (Tables 2-5), changes in the form of increase and/or decrease occurred for all parameters with variable frequency, whereas they occurred with high frequency at hours 8, 12, 24, 36, 48, 52, and slightly less frequently at the end of the experiment, namely at hours 56, 60, and 72 of the experiment. In the case of CAMPV-561 strain, changes were only recorded until 36 h of the study, as after that time all the animals died.

In the area of adherence capacity, changes in the form of decrease were recorded in rabbits after infection with reference strain CAMPV-351 (12, 48, 56, 72h) and CAMPV-562 (12h), while after infection with CAMPV-561 and CAMPV-558 strains, no statistically significant changes were observed.

In the case of the absorption index, it must be stated that in rabbits infected with reference strain CAMPV-351, both short-lasting increase (52h) and decrease (4 and 8h) to this parameter were recorded. Increases to this parameter were observed for CAMPV-562 strain (60h) and CAMPV-558 strain (12, 24h). In turn, in rabbits infected with CAMPV-561 strain, no statistically significant changes were recorded in the area of absorption index.

In the case of percentages of absorbing cells, for each of the analyzed Czech strains, increase in this parameter was recorded, yet only at single hours: CAMPV-351 (60h), CAMPV-561 (24 h), CAMPV-562 (48 h), and CAMPV-558 (48h).

In the case of spectrophotometric NBT test, changes to this parameter were observed after rabbit infection with three among four of the strains analyzed. In the case of reference strain CAMPV-351, a decrease (12h) and increase (36h) of this parameter was recorded, for CAMPV-561 – just decrease (12, 24, 36h), similarly as in the case of CAMPV-562 (52h). In turn, after infection with CAMPV-558 strain, no statistically significant changes were observed.

In the case of spontaneous NBT test, after infection with reference Czech strain CAMPV-351, both increase (4 h) and decrease (8, 24, 48, 56, 60 h) were observed to the cidal capacity of PMN cells. In turn, decreases to this parameter were observed after infection with CAMPV-561 (12 h) and CAMPV-558 (12, 52 h) strains. CAMPV-562 did not cause changes to the spontaneous NBT test.

Similarly small changes, as in the case of spontaneous NBT test, were obtained for stimulated NBT test. Change in the form of decrease to this parameter was recorded for reference CAMPV-351 strain (12, 48 h) and CAMPV-558

Table 2. Non-specific cellular immunity parameters in rabbits infected with references CAMPV-351 strain of RHDV.

Parameters				Values of parameters in hours																			
				0		4		8		12		24		36		48		52		56		60		72	
				Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (14)	K (10)	Z (6)	K (10)	Z (5)	K (10)	Z (4)	K (10)	Z (4)	K (10)	Z (4)	K (10)
Non-specific cellular immunity	pmn cell adherence capacity (%)		$\bar{x}$	11.5	10.4	13.9	10.7	14.5	13.9	15.2	19.9*	17.6	14.0	16.0	15.6	12.0	21.6*	19.4	21.0	15.0	24.1*	13.8	18.2	12.6	19.5*
			SD ±	3.7	3.5	5.2	4.4	4.6	3.6	4.8	4.4	5.8	4.3	7.6	6.8	6.2	3.1	5.1	4.3	3.5	5.0	3.4	4.4	3.2	3.8
	absorbing capacity	absorption index (l.b)	$\bar{x}$	6.7	6.0	5.9	7.5*	5.6	6.7*	7.0	6.4	7.4	8.3	7.2	8.2	11.3	7.5	11.4*	5.7	5.7	4.9	5.7	5.0	5.8	5.4
			SD ±	1.4	1.2	1.3	1.3	1.2	1.2	1.8	0.8	1.9	2.6	1.9	1.7	4.4	3.2	3.1	2.3	0.4	2.3	0.4	1.5	0.5	0.8
		% of absorbing cells (%)	$\bar{x}$	63.9	66.0	62.1	59.6	60.3	64.4	63.2	68.9	65.9	71.6	72.0	70.2	77.4	70.0	64.0	74.0	78.0	67.3	80.6*	50.5	78.0	71.0
			SD ±	11.2	6.1	11.9	15.3	10.2	14.0	11.1	13.8	12.3	8.6	12.4	11.0	8.3	7.5	11.6	8.0	7.9	9.7	8.2	8.4	8.5	7.1
	test for reduction of the NBT	spectrophotometric (10^9/l)	$\bar{x}$	5.0	5.8	3.9	4.2	3.2	3.4	2.9	4.4*	4.9	2.7	4.1*	2.6	2.4	4.0	2.0	3.4	2.2	2.1	2.6	2.1	3.0	2.1
			SD ±	2.0	2.5	2.1	1.6	1.6	1.5	0.8	2.3	3.8	0.7	2.3	1.1	0.2	1.5	0.4	1.6	1.1	0.6	1.0	0.7	0.9	0.8
		spontaneous (l.b)	$\bar{x}$	9.2	7.2	11.3*	7.0	8.4	14.0	9.5	14.0	8.0	11.4*	10.3	13.1	7.4	15.3*	11.7	14.8	11.7	25.8*	11.0	23.1*	10.3	20.5
			SD ±	3.9	3.1	3.8	3.6	4.0	5.4	5.4	6.3	3.7	6.2	6.0	6.4	3.5	5.1	4.9	3.7	2.9	10.5	3.4	6.3	4.0	2.1
		stimulated (l.b)	$\bar{x}$	18.5	19.5	20.6	21.4	21.6	20.8	20.6	28.4*	15.1	19.6	18.6	19.9	16.8	30.3*	21.0	30.4	22.8	31.3	20.9	25.7	19.0	25.0
			SD ±	7.8	8.0	7.6	11.4	7.9	9.9	8.7	11.9	6.9	7.7	9.5	10.2	9.4	10.7	8.5	8.0	6.5	14.3	8.7	10.7	3.5	7.1
		stimulation index (l.b)	$\bar{x}$	1.8	2.3	2.3	2.2	2.0	2.2	3.1*	1.9	2.6*	1.8	2.7*	2.0	2.1	1.8	1.4	1.9*	2.7	1.5	2.1	1.3	1.6	1.2
			SD ±	0.7	1.0	1.1	1.1	0.7	0.8	1.2	0.8	0.9	0.8	0.6	0.5	0.9	0.8	0.5	0.3	1.8	0.3	1.3	0.2	0.9	0.1
	WAMG	spontaneous (l.b)	$\bar{x}$	0.32	0.32	0.32	0.21	0.27	0.50*	0.24	0.58*	0.36	0.53	0.35	0.47	0.24	0.70*	0.32	0.60	0.19	0.95*	0.19	0.51*	0.20	0.60*
			SD ±	0.06	0.06	0.05	0.06	0.04	0.07	0.04	0.10	0.07	0.08	0.09	0.10	0.04	0.17	0.06	0.09	0.06	0.06	0.08	0.14	0.03	0.04
		stimulated (l.b)	$\bar{x}$	0.75	1.02	0.56	0.64	0.51	0.70*	0.55	0.60	0.40	0.75*	0.60	0.76	0.47	0.75	0.61	0.87	0.33	0.76*	0.24	0.77*	0.30	0.75*
			SD ±	0.08	0.15	0.09	0.14	0.07	0.12	0.07	0.09	0.07	0.10	0.11	0.11	0.06	0.07	0.07	0.08	0.03	0.04	0.09	0.05	0.07	0.07

Z – infected animals, K – control animals, () – number of animals, *– statistically significant change, WAMG – metabolic activity coefficient of neutrophilic granulocytes

Table 3. Non-specific cellular immunity parameters in rabbits infected with references CAMPV-561 strain of RHDV.

Parameters				Values of parameters in hours											
				0		4		8		12		24		36	
				Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (19)	K (10)	Z (5)	K (10)
Non-specific cellular immunity	pmn cell adherence capacity (%)		$\bar{x}$	16.9	16.6	18.2	18.1	22.6	20.3	23.3	19.8	21.5	16.4	14.8	19.1
			SD ±	5.8	4.9	3.3	4.5	5.3	5.3	8.9	6.6	5.9	3.9	1.2	5.0
	absorbing capacity	absorption index (l.b)	$\bar{x}$	5.5	5.4	4.8	5.5	5.2	5.3	5.9	5.7	5.3	4.7	4.3	5.3
			SD ±	1.0	1.7	1.0	0.7	1.0	0.6	1.2	1.9	1.1	1.0	0.8	0.7
		% of absorbing cells (%)	$\bar{x}$	71.3	71.7	75.2	75.7	76.6	75.7	79.4	76.3	81.2*	76.6	77.5	77.7
			SD ±	4.9	7.2	4.0	2.5	4.4	4.1	3.8	3.4	3.9	2.5	1.9	4.1
	test for reduction of the NBT	spectrophotometric (10^9/l)	$\bar{x}$	3.1	3.9	4.5	4.3	3.2	4.6	3.4	6.3*	3.2	5.4*	1.1	6.0*
			SD ±	1.1	1.5	1.9	2.7	1.4	2.2	1.2	1.6	1.0	1.9	0.5	2.3
		spontaneous (l.b)	$\bar{x}$	7.5	8.5	6.9	9.3	9.1	8.8	7.6	11.4*	10.6	11.5	10.0	12.0
			SD ±	1.8	1.7	2.3	3.3	1.9	2.9	1.9	3.3	2.4	2.7	3.4	3.2
		stimulated (l.b)	$\bar{x}$	19.6	21.1	20.5	19.9	18.1	19.1	19.5	21.0	22.0	22.3	24.0	20.5
			SD ±	2.4	3.4	3.5	3.5	2.7	3.8	4.4	3.2	3.4	3.7	3.6	3.9
		stimulation index (l.b)	$\bar{x}$	2.7*	2.0	3.1*	2.2	2.3	1.9	2.7	2.1	2.1	1.9	2.1	1.9
			SD ±	0.6	0.7	1.2	0.8	0.7	0.6	0.7	0.7	0.4	0.4	0.3	0.5
	WAMG	spontaneous (l.b)	$\bar{x}$	0.29	0.25	0.26	0.30	0.19	0.23	0.21	0.65*	0.39	0.79*	0.23	0.73*
			SD ±	0.04	0.05	0.03	0.02	0.06	0.05	0.09	0.10	0.04	0.14	0.05	0.12
		stimulated (l.b)	$\bar{x}$	0.91	1.07	0.77	0.64	0.41	0.84*	0.44	1.11*	0.73	1.39*	0.38	1.58*
			SD ±	0.11	0.12	0.07	0.06	0.05	0.12	0.12	0.10	0.08	0.25	0.06	0.22

Z – infected animals, K – control animals, () – number of animals, *– statistically significant change, WAMG – metabolic activity coefficient of neutrophilic granulocytes

Table 4. Non-specific cellular immunity parameters in rabbits infected with references CAMPV-562 strain of RHDV.

Parameters				Values of parameters in hours																			
				0		4		8		12		24		36		48		52		56		60		72	
				Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (19)	K (10)	Z (7)	K (10)	Z (4)	K (10)	Z (3)	K (10)	Z (3)	K (10)	Z (3)	K (10)	Z (3)	K (10)
Non-specific cellular immunity	pmn cell adherence capacity (%)		$\bar{x}$	17.1	16.8	18.7	16.2	14.8	18.6	18.4	24.8*	20.1	16.5	22.7	18.8	16.5	17.4	23.7	15.6	22.5	15.1	19.0	12.0	17.1	12.7
			SD ±	5.7	9.3	4.6	7.8	6.3	5.4	6.4	5.7	6.5	6.5	9.8	4.4	2.4	4.3	4.8	4.6	5.8	1.5	4.6	0.4	2.0	1.7
	absorbing capacity	absorption index (l.b)	$\bar{x}$	3.9	4.0	4.1	4.0	4.2	4.2	4.2	4.5	3.8	4.4	3.7	4.4	3.9	4.0	4.3	3.6	3.9	3.8	4.5*	3.8	4.1	3.9
			SD ±	1.0	1.2	1.0	1.2	0.9	1.0	1.1	0.9	0.9	1.5	0.4	1.0	0.8	0.4	0.5	0.3	1.0	0.3	0.3	0.2	0.5	0.4
		% of absorbing cells (%)	$\bar{x}$	70.8	72.4	70.8	71.2	73.9	78.0	77.1	78.0	74.0	73.6	76.3	72.4	76.8*	68.0	72.0	74.0	71.3	72.5	74.7	73.0	74.7	75.0
			SD ±	3.9	3.6	5.0	8.1	5.9	6.2	5.7	6.3	7.0	9.7	2.7	3.8	3.0	5.3	2.0	2.8	1.2	4.9	4.6	7.1	5.0	4.2
	test for reduction of the NBT	spectrophotometric (10^9/l)	$\bar{x}$	4.8	4.1	3.6	4.1	3.3	4.3	3.5	4.1	5.8	4.7	5.7	4.4	1.8	4.5	1.6	5.0*	1.6	3.3	2.4	3.5	3.2	4.5
			SD ±	2.2	2.0	1.9	2.8	1.8	2.2	1.8	2.8	3.9	1.0	2.9	0.6	0.6	0.7	0.3	1.0	1.1	1.0	1.1	0.7	1.2	1.4
		spontaneous (l.b)	$\bar{x}$	6.2	6.6	5.8	6.6	6.4	7.8	6.9	7.0	7.7	7.4	8.0	8.4	9.6	7.7	10.0	8.0	8.3	6.2	10.0	9.5	8.7	7.0
			SD ±	2.2	3.2	2.2	1.5	2.1	3.6	2.7	1.6	2.1	3.4	3.9	3.6	2.3	2.1	3.0	1.4	4.2	1.0	3.6	2.1	3.5	1.4
		stimulateD (l.b)	$\bar{x}$	18.4	17.0	15.2	17.0	17.1	20.6	17.7	16.6	19.0	20.2	19.5	20.6	21.4	18.7	21.3*	19.5	21.7	20.0	21.7	19.5	19.0	16.0
			SD ±	4.5	1.4	4.4	2.7	3.7	4.7	3.6	4.0	4.5	3.3	3.2	3.1	2.8	2.1	0.6	0.7	3.8	1.4	2.1	0.7	1.0	4.2
		stimulation index (l.b)	$\bar{x}$	3.0	2.9	2.5	3.0	2.8	2.8	3.3	2.4	2.6	3.1	2.9	2.0	2.4	2.6	2.2	2.3	2.9	2.4	2.4	2.2	2.4	2.7
			SD ±	1.0	1.1	1.0	0.7	0.7	0.6	1.7	0.3	0.7	1.1	1.1	0.3	0.6	0.4	0.7	0.3	0.9	0.4	1.0	0.5	1.0	0.4
	WAMG	spontaneous (l.b)	$\bar{x}$	0.23	0.26	0.22	0.29	0.20	0.24	0.24	0.24	0.27	0.24	0.24	0.39	0.14	0.24	0.13	0.31*	0.16	0.35	0.18	0.28	0.41	0.42
			SD ±	0.08	0.09	0.08	0.08	0.04	0.07	0.09	0.04	0.10	0.07	0.11	0.11	0.03	0.13	0.03	0.02	0.01	0.13	0.04	0.04	0.13	0.04
		stimulated (l.b)	$\bar{x}$	0.66	0.57	0.47	0.88*	0.43	0.57	0.70	0.57	0.68	0.57	0.85	0.99	0.56	0.45	0.31	0.66*	0.69	0.60	0.77	0.62	0.61	0.58
			SD ±	0.10	0.09	0.10	0.11	0.06	0.06	0.06	0.04	0.13	0.13	0.18	0.18	0.08	0.07	0.02	0.03	0.16	0.03	0.09	0.09	0.08	0.08

Z – infected animals, K – control animals, () – number of animals, * – statistically significant change, WAMG – metabolic activity coefficient of neutrophilic granulocytes

Table 5. Non-specific cellular immunity parameters in rabbits infected with references CAMPV-558 strain of RHDV.

Parameters				Values of parameters in hours																			
				0		4		8		12		24		36		48		52		56		60		72	
				Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (20)	K (10)	Z (19)	K (10)	Z (19)	K (10)	Z (14)	K (10)	Z (13)	K (10)	Z (13)	K (10)	Z (13)	K (10)	Z (11)	K (10)
Non-specific cellular immunity	pmn cell adherence capacity (%)		$\bar{x}$	22.2	21.7	18.2	22.3	20.2	17.7	16.8	20.0	17.2	17.5	17.8	19.4	17.0	16.7	16.1	17.3	17.5	16.2	20.9	19.1	19.7	13.2
			SD ±	6.4	6.3	8.0	6.1	6.3	7.3	4.9	4.7	6.0	5.9	6.3	5.0	6.7	3.3	5.6	5.1	4.5	3.0	8.3	7.1	6.6	3.8
	absorbing capacity	absorption index (l.b)	$\bar{x}$	4.2	4.3	4.9	4.8	5.3	4.9	5.4*	4.3	7.2*	4.7	5.0	4.8	4.5	4.7	4.1	4.5	4.3	4.6	4.4	4.8	4.5	4.7
			SD ±	0.7	0.9	0.7	1.2	0.9	0.7	0.8	0.7	0.8	0.3	0.6	0.9	0.6	0.6	0.7	0.4	0.9	0.2	0.8	0.2	0.9	0.7
		% of absorbing cells (%)	$\bar{x}$	70.0	68.8	68.4	73.2	69.7	70.4	72.7	71.2	75.5	76.0	76.2	73.6	76.9*	72.8	77.3	76.0	78.8	76.0	80.7	78.0	77.5	76.4
			SD ±	3.8	6.4	4.8	6.9	4.7	4.3	3.3	3.9	4.0	2.0	3.3	4.1	3.5	3.6	5.0	2.4	4.3	2.4	3.8	2.0	3.0	2.2
	test for reduction of the NBT	spectrophoto-metric (10^9/l)	$\bar{x}$	3.9	3.3	4.5	4.5	5.0	5.3	6.1	4.1	6.7	4.5	6.2	5.6	4.4	4.9	6.1	4.3	5.5	3.7	9.5	4.9	9.5	5.9
			SD ±	1.3	1.3	1.7	2.5	2.0	2.3	2.0	1.3	2.0	1.4	2.0	0.9	2.8	2.5	2.7	0.3	3.3	0.6	3.6	1.4	2.2	2.7
		spontaneous (l.b)	$\bar{x}$	10.1	14.6	12.5	12.2	10.8	12.8	9.5	14.8*	12.5	12.2	13.7	12.6	11.6	12.8	10.7	15.6*	10.3	13.8	12.3	12.8	12.5	11.6
			SD ±	4.5	4.4	4.3	3.1	3.7	3.7	4.2	1.1	3.8	3.1	3.1	3.6	3.8	2.2	4.1	1.1	3.7	2.3	2.7	1.9	2.7	3.3
		stimulated (l.b)	$\bar{x}$	20.6	21.6	19.9	19.8	16.4	20.8*	18.5	23.4*	23.6	22.6	23.3	22.2	17.8	21.0	19.2	22.8*	19.6	23.6	20.0	21.6	21.5	22.2
			SD ±	5.0	4.2	3.8	3.4	3.1	2.9	4.2	2.7	2.9	4.0	2.8	3.3	5.2	2.5	2.1	2.3	2.1	3.5	1.7	3.0	2.0	5.8
		stimulation index (l.b)	$\bar{x}$	1.8	1.5	1.7	1.8	1.7	1.8	2.5	1.7	2.1	2.0	1.8	1.9	1.6	1.7	1.9*	1.4	2.1	1.8	1.7	1.6	1.8	2.2
			SD ±	0.7	0.3	0.5	0.9	0.8	0.7	1.6	0.3	0.5	0.6	0.4	0.4	0.2	0.3	0.5	0.2	0.6	0.3	0.3	0.3	0.4	0.6
	WAMG	spontaneous (l.b)	$\bar{x}$	0.39	0.41	0.36	0.31	0.28	0.72*	0.44	0.51	0.65	0.72	0.59	0.54	0.35	0.64*	0.52	0.65	0.51	0.44	0.80	0.52	1.01	0.63
			SD ±	0.04	0.01	0.06	0.01	0.03	0.06	0.06	0.06	0.07	0.08	0.10	0.08	0.06	0.05	0.13	0.08	0.11	0.08	0.06	0.09	0.18	0.05
		stimulated (l.b)	$\bar{x}$	0.67	0.61	0.54	0.46	0.50	0.66	0.72	0.78	0.94	0.70	1.05	1.23	0.56	1.02*	0.91	0.92	0.82	0.75	0.60	0.81	1.24	0.88
			SD ±	0.05	0.08	0.10	0.05	0.06	0.10	0.07	0.05	0.15	0.14	0.16	0.13	0.08	0.13	0.09	0.10	0.10	0.04	0.08	0.06	0.22	0.06

Z – infected animals, K – control animals, () – number of animals, *– statistically significant change, WAMG – metabolic activity coefficient of neutrophilic granulocytes

(8, 12, 52 h), while an increase was observed in rabbits infected with CAMPV-562 (52 h). CAMPV-561 strain did not cause statistically significant changes to this parameter.

In the area of stimulation index, changes in the form of increase (12, 24, 36) and decrease (52 h) were recorded for reference strain CAMPV-351, and for CAMPV-561 (4 h) and CAMPV-558 (52 h) strains. In turn, in the case of the CAMPV-562 strain, no changes were recorded.

As regards WAMG index for spontaneous test, the greatest number of changes in the form of decrease was observed after infection of rabbits with all four analyzed Czech strains. The most numerous changes were recorded, respectively, for reference strain CAMPV-351 (8, 12, 48, 56, 60, 72 h), CAMPV-561 (12, 24, 36 h), CAMPV-558 (8, 48 h), and CAMPV-562 (52 h).

Many changes in the form of decrease were also recorded for stimulated WAMG, which covered all the analyzed strains, namely reference strain CAMPV-351 (8, 24, 56, 60, and 72 h), CAMPV-561 (8, 12, 24, 36 h), CAMPV-562 (4, 52 h), and CAMPV-558 (48 h).

In the case of Czech strain CAMPV-351, the first symptoms of the disease were observed at 36 h of the experiment. The first deaths were recorded between 24/36 h of the study (6 rabbits). Mortality for this strain in the course of the entire experiment, namely up to 72 h, amounted to 80.0%. In the case of the second of the analyzed strains, CAMPV-561, the first symptoms were recorded as early as at 24h of the experiment, and they preceded numerous deaths. Between 12/24 h, one death took place, whereas between 24 and 36h of the experiment, as many as 14 animals died, while between 36/48 h – 5 animals. The mortality ratio amounted to 100.0%. Experimental infections of rabbits with CAMPV-562 strain caused the occurrence of symptoms in animals starting from 36 h of the experiment, whereas a single death without symptoms was recorded between 12/24 hour. Between 24/36 h, 12 deaths were observed, while in other hours of the experiment, single deaths were recorded. Mortality rate in the case of this strain amounted to 85.0%. The Czech strain CAMPV-558 caused excitability of the infected animals, and increased blood coagulability already starting from 24 h of the experiment. The first deaths were recorded between 24/36 h of the experiment (1 rabbit), and then between 36/48 h (5 rabbits), between 48/52 h (1 rabbit), and between 60/72 h (2 rabbits). At 72 h of the experiment, mortality rate in the case of this strain amounted to 45.0%.

Discussion of Results

When analyzing the results of adherence capacity obtained for reference Czech CAMPV-351 strain, one may state that they differ from the results obtained by Niedźwiedzka [22] and Niedźwiedzka-Rystwej et al. [37, 38] for reference HA+ Italian strain BS89, which caused an increase in adherence capacity at 4 and decrease at 24 h of the experiment. The results of our own studies regarding adherence capacity for two HA+ Czech strains (CAMPV-351 and CAMPV-562) are similar to the results obtained for other haemagglutinogenic strains: Polish PD (increase – absence, decrease at 8, 12, 36, 56 h) [1, 39] and GSK (increase – absence, decrease at 8-56 h) [1, 39], Italian Vt97 (increase – absence, decrease at 12, 36 h) [37, 40], German Triptis (increase – absence, decrease at 24, 36 h) and Hartmannsdorf (increase – absence, decrease at 8, 24 h) [37]. In turn, the results of own studies for strains CAMPV-561 and CAMPV-558 conform to the results obtained for Polish strain Kr-1 [1, 39, 41, 42] and ŻD [1, 39, 42], because no statistical differences were recorded for such strains. It must be stated that the results obtained in our own studies for HA+ Czech strains also differ from the results obtained for other so far analyzed HA+ strains of the RHDV, which caused increase or decrease to the adherence capacity, which included Fr-1 [1], Fr-2 [1], SGM [1, 39], MAŁ [1, 39], and KGM [1, 39, 41]. It must also be observed that the results of our own studies obtained after infection with HA+ strains CAMPV-351 and CAMPV-562 conform to the results obtained for HA- British Rainham strain (increase – absence, decrease at 8, 24, 36h) [37, 38], French 9905RHDVa (increase – absence, decrease at 8, 36 h) [37] and German Hagenow (HA+/-) (increase – absence, decrease at 8, 12 h) [37]. It can also be noticed that the results of our own studies differ from the results for other HA- strains, namely Frankfurt (increase at 36 h, decrease – absence) [37, 38], Asturias (increase at 12, 24 h, decrease – absence) [37, 38], Pv97 (increase at 12 h, decrease at 8, 36 h) [37] and BLA (increase at 52 h, decrease at 4, 8, 48h) [1, 39, 42].

When analyzing the results in the area of absorption index after rabbit infection with reference strain CAMPV-351, it can be concluded that they partially conform to the results obtained for reference strain BS89 [37, 38, 40]. Both reference strains (CAMPV-351 and BS89) decreased the absorption index at 4 and 8 h, while Italian strain additionally at 12 and 24 h [37, 38, 40]. Moreover, differently than in the case of BS89 strain, CAMPV-351 strain caused increase to the absorption index at 52 h. The obtained results in our own study on HA+ strains CAMPV-351, CAMPV-562, and CAMPV-558 partially conform to the results obtained after rabbit infection with other strains with HA+ properties, such as Fr-2 (increase 8, 24 h, decrease 56 h) [1, 40, 43], MAŁ (increase 4, 48 h, decrease – absence) [43], Kr-1 (increase 48 h, decrease – absence) [1, 40, 43], PD (increase 8, 52-60h, decrease – absence) [1, 39], SGM (increase 56, 60h, decrease – absence) [43], KGM (56, 60 h, increase – absence) [1, 43], Vt97RHDVA (increase 12, 24, 36 h, decrease – absence) [37, 40], Triptis (increase 4, 8 h, decrease – absence) [37, 40], GSK (increase 8-60 h, decrease – absence) [1, 39], and ŻD (increase 48h, decrease – absence) [1, 39]. They also partly conform to the results obtained for HA- strains, namely Hagenow (increase 4-36 h, decrease – absence) [37], Asturias (increase 4-24 h, decrease – absence) [37, 38], and Pv97 (increase – absence, decrease at 4-36 h) [37]. The absence of changes to the absorption index recorded in our own study for CAMPV-561 conforms to the results obtained for strains Fr-1 [1, 43], K-1 [44] and Hartmannsdorf [37], and for HA- strains such as BLA [1, 39, 42], Rainham [37, 38], Frankfurt [37, 38], and 99-05RHDVa [37].

Results of our study regarding the percentage of absorbing cells obtained for reference strain CAMPV-351 differ from the results obtained for reference Italian strain BS89 [37, 38, 40]. BS89 caused a decrease to the percentage of absorbing cells at 4 h, whereas in the case of the Czech strain, no decrease to this parameter was recorded. In turn, the Czech strain caused an increase to the percentage of absorbing cells at 60 h of the experiment, whereas the BS89 strain did not cause changes in the form of increase throughout the course of the experiment [37, 38, 40]. The increase to the percentage of absorbing cells recorded in our study at 24 and 48 h of the experiment for HA+ strains CAMPV-351 CAMP-561, CAMPV-562, CAMPV-558 only partially conforms to the results obtained for other HA+ strains, such as Fr-1 (increase 36, 52 h, decrease – absence) [1, 43], MAŁ (increase 4, 60 h, decrease – absence) [1], K-1 (increase 2, 4, 8, 10, 24, 48 h, decrease – absence) [44], Vt97 (increase 4-36 h, decrease – absence) [37], and Triptis (increase 4, 8 h, decrease – absence) [37], and is also similar to the results obtained for H– Hagenow strain (increase 4-24 h) and Frankfurt (increase 8, 12 h, decrease – absence) [37]. In turn, the results of our study differ from the ones obtained for HA+ SGM strain (increase 36h, decrease 56,60 h) [1, 39, 43], KGM (increase 36 h, decrease 8,56,60h) [1, 39, 41, 43], Kr-1 (no changes) [1, 39, 41, 43], PD (no changes) [1, 39], Fr-2 (no changes % absorbing cells) [1,43], Hartmannsdorf (no changes) [37], GSK (no changes) [1, 39], ŻD (no changes) [1, 39] and HA- Asturias (increase – absence, decrease at 8-24 h) [37, 38], Pv97 (increase – absence, decrease at 12-36 h) [37], BLA (no changes) [1, 39], Rainham (no changes) [37, 38], and 9905RHDVa (no changes) [37].

The changes recorded in the form of increase and decreases in spectrophotometric NBT test for reference strain CAMPV-351 only partly conform to the results obtained after rabbit infection with reference strain BS89 [37, 38]. This is because both strains caused a decrease to this parameter, yet at different times, whereas in the case of CAMPV-351, also an increase to this parameter was recorded (36 h). Furthermore, the result obtained for reference strain CAMPV-351 partly conforms to the results obtained after rabbit infection with other HA+ strains, which are not, however, reference strains. Such changes were observed after rabbit infection with strains Fr-1 (increase 4-24, 48-52 h, decrease – absence) [1], Fr-2 (increase 4-48, 56 h) [1], SGM (increase 8-60 h, decrease – absence) [1, 39], MAŁ (increase 52-60 h, decrease – absence) [1, 39], Kr-1 (increase 8, 36-56 h, decrease – absence) [1, 39, 42], KGM (increase 36, 56, 60 h, decrease – absence) [1, 39, 41], and ŻD (increase 8, 36-56 h, decrease – absence) [1, 39]. In turn, the decrease to this parameter, recorded for strains CAMPV-561 and CAMPV-562, partly conforms to the results obtained for HA+ strain PD (increase – absence, decrease at 4, 36, 52, 56, 60 h) [1], BS89 (increase – absence, decrease at 4-12h) [37,38], Vt97 (increase – absence, decrease at 4 h) [37], GSK (increase – absence, decrease at 8, 24-36, 56 h) [1,39] and HA- strains: Hagenow (increase – absence, decrease at 4-36 h) [37], Rainham (increase – absence, decrease at 12 h) [37, 38], Frankfurt (increase – absence, decrease at 12-24 h) [37, 38], and Asturias (increase – absence, decrease at 4-12h) [37, 38]. In turn, the lack of changes recorded for CAMPV-558 strain in the spectrophotometric NBT test is confirmed with the results obtained for HA+ strains: Triptis [37], Hartmnnsdorf [37], and HA- strain 9905RHDVa [37].

When analyzing the results in the area of spontaneous NBT test, it must be noticed that the results obtained for reference strain CAMPV-351 differs very much from the results obtained for reference strain BS89 [37, 38]. This results from the fact that after infection with BS89 strain, no changes to this parameter were recorded, contrary to CAMPV-351 strain, which caused both increases and decreases to this parameter. It must be noted that the changes recorded after rabbit infection with the CAMPV-351 strain are most similar to the ones recorded for Hartmannsdorf (increase 4 h, decrease 24 h) [37] and PD strain (increase 56, decrease 4, 24 h) [1,39]. Changes obtained (in the form of decrease) in spontaneous NBT test for HA+ strains CAMPV-561 and CAMPV-558 are only partly similar to the results obtained for HA+ ŻD strain (increase – absence, decrease at 36 h) [1,39], Kr-1 (increase – absence, decrease at 36 h) [1, 39, 42], and Vt97 (increase – absence, decrease at 4-36 h) [37]. They are more similar to the results obtained after rabbit infection with HA- strains, namely Hagenow (increase – absence, decrease at 12, 36) [37], Rainham (increase – absence, decrease at 24, 36 h) [37, 38], Frankfurt (increase – absence, decrease at 4-36 h) [37, 38], Asturias (increase –absence, decrease at 4-24 h) [37, 38], and Pv97 (increase – absence, decrease at 36 h) [37]. In turn, the lack of changes to this parameter recorded in our study after administration of CAMPV-562 strain is confirmed by the results for HA+ strains: Fr-2 [1], BS89 [37, 38], GSK [1, 39], and HA- strain 9905RHDVa [37]. It must also be pointed out that the results of our study on four Czech strains completely differ from the results obtained for other strains, where only an increase to the cidal capacity of PMN cells was recorded in the spontaneous NBT test. Among HA+ strains, these included: Fr-1 (12, 24 h) [1], SGM (8, 24 h) [1, 39], MAŁ (52 h) [1, 39], K-1 (4-24, 48 h) [44], KGM (56 h) [1, 39, 41], and Triptis (12, 36 h) [37], while among HA- strains: BLA (8-60 h) [1, 39, 42].

The decrease to the cidal capacity of PMN cells in our own study (in stimulated NBT test) for reference strain CAMPV-351 completely differs from the results obtained for reference Italian strain BS89, which did not cause changes to this parameter [37, 38]. The results obtained for HA+ strains (for which only decrease was recorded in the stimulated NBT test): CAMPV-351 (12, 48 h) and CAMPV-558 (8-12, 52 h), are only partly similar to the results obtained for HA+ strains, namely Vt97 (decrease 24-36 h) [37] and HA- strain Frankfurt (4-36 h) [37, 38], 9905RHDVa (decrease 36 h) [37] and Hagenow (HA+/-) (decrease 24-36 h) [37]. The increase to the cidal capacity of PMN cells in our study (in stimulated NBT test) after infection with CAMPV-562 is partly confirmed with the results obtained after infection with HA+ strains: Fr-1 (increase 12, 24, 48 h) [1], Fr-2 (increase 4-24 h) [1, 39], Kr-1 (increase 8

h) [1, 39, 42], K-1 (increase 8, 10, 24, 48 h) [44], KGM (24, 36 h) [1, 39, 41], ŻD (increase 8h) [1, 39], and HA- strain BLA (8-36, 52 h) [1, 39, 42]. Lack of changes recorded for CAMPV-561 conforms to the results obtained for HA+ strains: SGM [1, 39], MAŁ [1, 39], PD [1, 39], GSK [1, 39], Triptis [37], Hartmannsdorf [37], and HA- strains: Rainham [37, 38], Asturias [37, 38], Pv97 [37].

In the case of stimulation index, changes in the form of increase and decrease recorded for reference strain CAMPV-351 differ from the results obtained for BS89 strain [37, 38], in the case of which only a decrease to this parameter was recorded at hour 24 of the experiment [37]. In turn, partially corresponding changes were observed after infection with CAMPV-351 to the ones recorded for SGM (increase 48, decrease 52, 56, 60 h) [1,39]. Increase in the stimulation index after infection with HA+ strains CAMPV-561 and CAMPV-558 was also observed for MAŁ (36, 60 h) [1, 39], Kr-1 (52, 56 h) [1, 39, 42], K-1 (4, 12, 24, 48 h) [44], Vt97 (24, 36 h) [37], and for HA- strains: BLA (24, 36 h) [1, 39, 42], Rainham (24 h) [37, 38], Asturias (24 h) [37, 38], Pv97 (24, 36 h) [37] and ŻD (52, 56 h) [1, 39]. In turn, they completely differed from the results obtained for strains Fr-1 (decrease 8, 24 h) [1], KGM (decrease 56 h) [1,39,41], and BS89 (decrease 24 h) [37, 38]. Lack of changes to the stimulation index in rabbits infected with CAMPV-562 is confirmed by the results for HA+ strains, namely PD [1, 39], GSK [1, 39], Triptis [37], Hartmannsdorf [37], and HA- strains Hagenow [37], Frankfurt [37, 38], and 9905RHDVa [37].

The recorded decrease in spontaneous WAMG tests after rabbit infection with reference strain CAMPV-351 proved partly conformant to the results for reference strain BS89 (decrease 24 h) [37, 38]. Changes recorded in this test after infection with Czech strains conform to the changes caused by strains Fr-2 (decrease 12, 48, 56 h) [1], Hagenow (decrease 36 h) [37], Frankfurt (4-36 h) [37,38], Asturias (4 h) [37, 38], Pv97 (decrease 36 h) [37], GSK (decrease 24-60 h) [1, 39], and PD (decrease 4, 12, 24, 56 h) [1, 39]. In turn, they completely differ from the results recorded for strains: Fr-1 (no changes) [1], SGM (increase 56, 60 h) [1, 39], MAŁ (increase 56 h) [1,39], Kr-1 (increase 52 h) [1, 39, 42], K-1 (increase 8, 24, 48 h) [44], KGM (increase 56, 60, decrease 8 h) [1,39,41], BLA (increase 12, 24 h) [1, 39, 42], Rainham (no changes) [37, 38], Vt97 (no changes) [37], Triptis (no changes) [37], Hartmannsdorf (no changes) [37], 9905RHDVa (no changes) [37], and ŻD (increase 52 h) [1, 39].

Results in the form of decrease obtained in stimulated WAMG test for reference strain CAMPV-351 are similar to the results obtained for Italian BS89 [37, 38]. This is because both strains caused a decrease to this parameter, whereas BS89 exclusively at 4 h of the experiment, while CAMPV-351 at 8, 24, 56, 60, 72 h. The results recorded after rabbit infection with strains CAMPV-351, CAMPV-561, CAMPV-562, and CAMPV-558 very much correlate with the results by other authors studying strains: Hagenow (decrease 36 h) [37], Frankfurt (decrease 8, 24-36 h) [37, 38], Asturias (decrease 4-8, 24 h) [37, 38], Vt97 (decrease 12-36h) [37], GSK (24-60 h) [1, 39]. In turn, the results obtained in our study are not confirmed by the results obtained after infection with strains: Fr-1 (increase 12, 48 h) [1], Fr-2 (increase 4-52 h) [1], SGM (52, 56 h) [1,39], MAŁ (increase 56 h) [1, 39], Kr-1 (4-8, 56 h) [1, 39, 42], K-1 (increase 24, 48 h) [44], BLA (12-36 h) [1, 39, 42], Rainham (36 h) [37, 38], Triptis (12 h) [37], Hartmannsdorf (no changes) [37], 9905RHDVa (no changes) [37], and ŻD (increase 4, 8, 56 h) [1, 39].

The time and number of deaths of rabbits infected with four Czech strains of the RHDV point to differences in their pathogenicity. The highest 100% mortality was recorded in the group of animals infected with CAMPV-561, while the lowest (45%) – in the group of animals infected with CAMPV-558 strain. The recorded mortality was similar to the one recorded by other authors for Polish and foreign strains [1, 8, 22, 37, 44]. Mortality of 100% (CAMPV-561) was also observed for strains ŻD, BS89, Pv97, Frankfurt, Triptis, Hartsmanndorf, Rainham, Asturias [37] and Fr-2 [1]. In turn, it was slightly lower for Kr-1 (90%), SGM (95%), GSK (95%), Hagenow (90%), 9905RHDVa (90%), and Fr-1 (90%). However, mortality for reference strain CAMPV-351 (80%) and CAMPV-562 (85%) was the same as in the case of MAŁ (80%) and American strains (70-95%) [8]. Mortality recorded for CAMPV-558 (45%) was so far the lowest recorded mortality among Czech strains of the RHDV. Lower mortality was only recorded in the case of infection with Italian strain Vt97 (30%) [37]. In the case of French strains - Fr-1, Fr-2 – 90% mortality was recorded for Polish strains: MAŁ, SGM, K-1- 80-95%, PD- 25%, BLA- 60%, GSK- 93%, Ż- 87%, ŻD- 100% and 90-100% mortality for foreign strains. The time of occurrence of clinical symptoms and their type did not differ from clinical symptoms recorded by other authors [1, 37, 44].

Conclusion

After rabbit infection with 4 haemagglutinogenic Czech strains – CAMPV-351, CAMPV-561, CAMPV-562, CAMPV-558 of the RHD virus, a varied image of changes was observed in the non-specific cellular immunity parameters. The most numerous changes to the parameters analyzed were caused by the reference Czech strain CAMPV-351, yielding a total of 33 changes, including as many as 26 in the form of decrease, and only 7 changes in the form of increase. The changes commenced as early as at 8 h from infection and lasted until 60-72 h of the experiment. In turn, the least numerous changes to the parameters analyzed were caused by CAMPV-562, for which only 8 changes were recorded, namely 3 increases and 5 decreases, whereas the changes commenced at 12 h and lasted until 52-60 h of the experiment. In turn, strains CAMPV-561 and CAMPV-558 caused a similar image of changes to the parameters analyzed, yielding 13 and 12 changes, respectively. CAMPV-561 caused 2 changes in the form of increase and 11 in the form of decrease, where the changes commenced at 8 h and lasted until 36 h of the experiment, while CAMPV-558 caused 4 increases and 8 decreases, falling at 8 h and ending at 52 h from infection. Therefore,

on the basis of the number and duration of changes to analyzed parameters of non-specific cellular immunity, it is determined that the 4 analyzed Czech strains can be differentiated in the aspect of immunogenicity into three groups. The first group is formed by the most immunogenic reference strain CAMPV-351, the second – by two medium-immunogenic strains – CAMPV-561 and CAMPV-558, whereas the third one – by the least immunogenic strain CAMPV-562.

In turn, the analysis of mortality after rabbit infection with Czech strains revealed that the most pathogenic strain, causing 100% mortality within up to 48 h of the experiment, is the CAMPV-561 strain, the strains that are less pathogenic and cause 80%-85% mortality within up to 72 h are CAMP-351 and CAMPV-562, whereas the least pathogenic strain causing just 45% mortality within up to 72 h of the study is CAMPV-558. The results obtained in the area of pathogenicity are not reflected in the division of the strain due to their immunogenicity, which contradicts the view generally so far adopted in infection epidemiology that pathogenicity of viruses corresponds to their immunogenicity.

Acknowledgements

Our study was financed by a research grant from the Ministry of Science and Upper Education, No. 2 P06K 02927.

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