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Original article

Phagocytosis of neutrophils in rabbits infected with antigenic variants of RHD (rabbit haemorrhagic disease) virus

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Abstract

The present study was aimed at determining changes in chosen elements of phagocytosis in rabbits infected with 3 antigenic variants of RHD – Hartmannsdorf, Pv97 and 9905, which differed in haemagglutination ability. The animals were tested for phagocytosis parameters, and the results revealed that the examined strains showed the differences. These variations regarded mainly Pv97 strain, as the intensity of the changes were 5 times stronger in comparison to strain Hartmannsdorf and 9905. As all of the strains examined are signified as antigenic variants, we have stated that this feature does not determine their immunological picture. The results suggest the existence of immunological dissimilarities among strains of the RHD virus, which was revealed for the first time in antigenic variants.

Key words: RHDV, antigenic variant, haemagglutination

Introduction

Viral infections of free-living, and farm animals constitute a significant epidemiological problem. One of main causes of sudden changes in the population size of wild and domestic rabbits is rabbit haemorrhagic disease virus (RHDV), leading to death of these animals. Therefore there is a growing need for understanding the pathogenic activity of RHD virus, and pathogenesis of the disease caused by it. In recent years, evaluation of immunological profile in terms of specific and non-specific immunity indexes, as well as humoral immunity index, have been included to the methods of RHDV pathogenesis evaluation (Piekarski 1994, Hukowska-Szematowicz 2006, Tokarz-Deptuła et al. 2007, Niedźwiedzka et al. 2008, Tokarz-Deptuła 2009). So far studies on the aspects of phagocytosis – an element of natural immunity described by the ability of adherence, absorption, and killing of PMN cells, was carried out on 14 haemagglutinating RHDV strains (Fr-1, Fr-2, CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558, SGM, MAŁ, Kr-1, K-1, KGM, PD, GSK, ŻD) and one non-haemagglutinating BLA strain (Piekarski 1994, Hukowska-Szematowicz 2006, Tokarz-Deptuła et al. 2007, Niedźwiedzka et al. 2008, Tokarz-Deptuła 2009). These investigations were based on spectrophotometric, spontaneous, and stimulated NBT

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tests, stimulation index, and coefficient of metabolic activity (CMAC) of PMN cells. It was shown that in most cases RHDV infection caused an increase in measured indexes and coefficients. Changes, including increases (in most cases), or decreases of all immunological indexes tested began between the 4th and 8th hour after infection, and lasted for a different period of time, depending on the strain of the RHD virus, i.e.: 4, 8, 52-60 h, although in many cases the changes were also observed in the 12th, 24th, 36th and 48th hour. In each of the study mentioned, these changes were noted shortly before the death of animals, which occurred 52-60 h after RHDV infection (Piekarski 1994, Hukowska-Szematowicz 2006, Tokarz-Deptuła et al. 2007, Niedźwiedzka et al. 2008, Tokarz-Deptuła 2009), or 24-36 h after administration of the virus in case of experiments of Niedźwiedzka-Rystwej and Deptuła (2010).

Among RHDV strains, antigenic variants were described in 1998 (Capucci et al. 1998) and until now there are 37 of them (Niedźwiedzka-Rystwej and Deptuła 2010), among which one may find two Polish strains L145/04 and W147/05 (Chrobocińska 2007). Nevertheless, no immunological studies parallel to present research have been conducted in regards to the antigenic variants of RHD. According to Capucci et al. (1998) the strains described as antigenic variants of RHDV are characterized by the distinct binding ability to specific antibodies (Capucci et al. 1998, Grazioli et al. 2000), which is connected with a specific structure of the C and E region of VP60 polyprotein (Capucci et al. 1998, Schirrmeier et al. 1999). The mortality of animals, caused by the antigenic variants is also higher in comparison to the infection with RHDV strains which do not belong to the antigenic variants, and amounts to 90-100% (Capucci et al. 1998, Schirrmeier et al. 1999). Moreover, it is worth underlining that these strains show different haemagglutination abilities (Tokarz-Deptuła 2009). Since this property is directly connected with the virulence of the virus (Tokarz-Deptuła 2009, Niedźwiedzka-Rystwej and Deptuła 2010) the characteristics of the antigenic variants constitute a very interesting subject of studies.

So far there is no evidence showing differential immunogenicity among antigenic variants of the RHD virus. Therefore the aim of the present study was to determine changes in chosen elements of phagocytosis in rabbits infected with a haemagglutinating RHDV antigenic variant – Hartmannsdorf, isolated in 1996 in Germany (Schirrmeier et al. 1999), and two non-haemagglutinating RHDV antigenic variants – Italian Pv97, described in 1997 (Capucci et al. 1998), and French 9905, described in 1999 (Le Gall Recule et al. 2003). Additionally, due to the high mortality of rabbits infected with RHDV antigenic variants, this study also aimed at determining the mortality rate of the animals infected.

Materials and Methods

The study was performed on 60 mixed breed rabbits of both sexes, weighting in the range of 3.2-4.2 kg. The rabbits were marked as conventional animals, coming from a licensed breeding farm, under a constant veterinary and zootechnique supervision (Annon 1987). During the experiment, the animals were kept at vivarium, where zootechnical parameters such as: temperature, humidity, lighting, and size of cages, were adjusted to the standards recommended in Poland (Annon 2006). After transportation to the vivarium, the rabbits were subjected to a two-week adaptation period. The animals were fed full-portion rabbit feed (brand: 16% Królik z Motycza) and had an unlimited access to water.

For the purpose of this study, the rabbits were divided into 3 infected groups, each consisting of 10 animals, and 3 corresponding control groups, also containing 10 animals. The rabbits from the experimental groups were infected with appropriate RHDV antigenic variant (Hartmannsdorf, Pv97, or 9905) suspended in 1 ml glycerol, administered in a form of intramuscular injection (lower limb muscle), while rabbits from the corresponding control groups were injected with placebo – 1 ml glycerol. RHDV antigenic variants originated from naturally dead animals from the area of Italy, Germany and France. The strains were obtained from the Instituto Zooprofilattico Sperimentale Della Lombardia E Dell'Emilia Romagna, Bresci (Italy), Friedrich – Loeffler Institut Greiswald, Insel Riems (Germany), and Agence Francaise de Securite Sanitaire des Aliments, Ploufragan (France), respectively.

First, three rabbits were infected with doses of antigens in the form of liver homogenates (strain Hartmannsdorf HA 2560, strains Pv97 and 9905 HA-), for the purpose of multiplying the infectious material kindly obtained from the above mentioned institutes. After naturally deceased animals, livers were collected and used to prepare a 20% homogenates, which served as a source of virus for the experimental infection of the rabbits in infected and control groups. Twenty percent liver homogenates were prepared and purified by centrifugation at 3000 rpm, 10% chloroforming for 60 minutes, and another centrifugation step. The obtained liver homogenate samples were suspended in glycerol in the 1:1 ratio (Niedźwiedzka-Rystwej and Deptuła 2010). All prepared antigens showed the same

Table 1. Chosen parameters of phagocytosis in rabbits infected with haemagglutinating antigenic variant Hartmannsdorf of RHD virus.

Parameters			Values of parameters in hours									
			0		4		8		12		24	
			Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (10)	K (10)	Z (9)	K (10)
Adherence ability (%)		$\bar{x}$	44.45	39.19	46.51	40.26	34.40	46.03*	39.85	39.01	32.39	43.10*
		SD±	2.63	2.55	4.3	4.25	3.16	4.17	4.14	2.74	2.05	2.98
Adsorption ability	Absorption index (l.b.)	$\bar{x}$	4.42	4.63	4.30	4.55	4.60	4.98	4.20	4.67	4.49	4.41
		SD±	0.30	0.45	0.35	0.43	0.58	0.48	0.67	0.43	0.11	0.43
	% of absorbing cells (%)	$\bar{x}$	68.80	67.89	67.20	70.20	68.00	66.26	67.60	68.70	67.50	68.09
		SD±	50.2	4.86	3.35	3.89	4.24	3.91	3.28	4.00	1.00	3.09
NBT reduction test	Spectrophotometric (10^9/l)	$\bar{x}$	4.05	4.13	4.34	4.42	4.87	4.99	5.03	5.60	5.77	4.95
		SD±	1.62	0.88	1.40	0.77	1.14	1.31	0.54	1.16	1.35	0.75
	Spontaneous (l.b.)	$\bar{x}$	10.60	10.11	13.00*	10.81	10.80	9.93	10.40	9.81	7.75	8.81*
		SD±	1.52	0.96	1.00	1.81	1.30	1.00	1.67	0.89	0.96	0.86
	Stimulated (l.b.)	$\bar{x}$	21.00	21.11	20.04	20.20	19.60	19.87	19.20	18.85	17.00	18.09
		SD±	0.71	1.78	1.14	1.72	1.67	1.69	0.92	1.97	0.81	2.16
	Index of stimulation (l.b.)	$\bar{x}$	2.06	2.07	1.60	2.05	1.84	2.09	1.85	1.88	2.24	2.02
		SD±	0.39	0.30	0.19	0.47	0.23	0.33	0.21	0.20	0.38	0.25
Coefficient of the metabolic activity of PMN	Spontaneous (l.b.)	$\bar{x}$	0.30	0.33	0.32	0.29	0.22	0.27	0.24	0.28	0.14	0.25
		SD±	0.04	0.06	0.00	0.07	0.02	0.06	0.06	0.08	0.03	0.06
	Stimulated (l.b.)	$\bar{x}$	0.61	0.63	0.50	0.52	0.40	0.53	0.45	0.53	0.30	0.50
		SD±	0.04	0.06	0.05	0.04	0.06	0.05	0.13	0.05	0.15	0.06

Legend: $\bar{x}$ – mean value; SD± – standard deviation; Z – infected animals, K – control animals; () – number of animals, * – statistically significant change

number of virus particles, estimated to be 1.31-1.34 g/dm^3.

Blood samples were collected from all groups of rabbits (control, and experimental), from the marginal vein of ear at a time point 0 – before RHD virus, or glycerol injections, and after 4, 8, 12, 24, 36 hours of the experiment. The polymorphonuclear (PMN) cells' adherence ability, and absorption ability, measured by the absorption index and the percentage of absorbing cells, were determined in the blood samples. Additionally the killing ability of PMN cells was measured using: the Nitroblue-Tetrazolium (NBT) reduction test: spectrophotometric, and cytochemical (spontaneous and stimulated), coefficient of the metabolic activity of granulocytes (spontaneous and stimulated), and the index of stimulation. The PMN cells' adherence ability was assessed using a method described by Lorente et al. (Lorente et al. 1973), while the absorption ability was determined by Brzuchowska and Ładosz method with own modifications (Deptuła 1991); it was expressed as absorption index and percentage of absorbing cells. The ability to reduce nitroblue-tetrazolium in PMN cells of peripheral blood was determined by cytochemical method, using spontaneous and stimulated test according to Park et al. (Park et al. 1968), and a spectrophotometric method described by Raman and Poland (Raman and Poland 1975). The coefficient of the metabolic activity of neutrophils was assessed using Grządzielska method (Grządzielska 1976), while stimulation index was determined according to Lechowski (Lechowski et al. 1991).

The obtained results were subjected to statistical analyses by t-Student test with the level of significance set at p=0.05.

Results

The analysis of changes in the phagocytosis indexes, evoked by three antigen variants (Hartmannsdorf, Pv97 and 9905) of the RHD virus showing differential haemagglutinating properties, revealed that most of the examined indexes exhibited de-

Table 2. Chosen parameters of phagocytosis in rabbits infected with non-haemagglutinating antigenic variant Pv97 of RHD virus.

Parameters			Values of parameters 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 (10)	K (10)	Z (1)	K (10)
Adherence ability (%)		$\bar{x}$	40.20	39.19	31.05	40.26	26.88	46.03*	58.35*	39.01	37.33	43.10	21.53	34.45*
		SD±	4.85	2.55	3.08	4.25	4.90	4.17	7.60	2.74	5.86	2.98	0.00	3.21
Adsorption ability	Absorption index (l.b.)	$\bar{x}$	3.82	4.63	3.16	4.55*	3.83	4.98*	2.30	4.67*	2.19	4.41*	1.90	4.62*
		SD±	0.46	0.45	0.25	0.43	0.06	0.48	0.41	0.43	0.30	0.41	0.00	0.42
	% of absorbing cells (%)	$\bar{x}$	60.75	67.89	55.25	70.20	56.50	66.26	39.50	68.70*	36.50	68.09*	38.00	62.25*
		SD±	3.30	4.86	1.71	3.89	4.65	3.91	3.51	4.00	3.00	3.09	0.00	4.22
NBT reduction test	Spectrophotometric (10^9/l)	$\bar{x}$	5.10	4.13	7.00*	4.42	6.33*	4.99	4.74*	5.60	12.41*	4.95	11.81*	4.91
		SD±	1.11	0.88	3.11	0.77	2.62	1.31	1.66	1.16	3.57	0.75	0.00	0.51
	Spontaneous (l.b.)	$\bar{x}$	10.75	10.11	12.50	10.81	10.00	9.93	9.00	9.81	6.75	8.81	6.00	8.22*
		SD±	0.96	0.96	1.00	1.18	1.63	1.00	0.82	0.89	0.96	0.86	0.00	0.65
	Stimulated (l.b.)	$\bar{x}$	21.75	21.11	23.00	20.20	21.00	19.87	20.25	18.85	18.25	18.09	18.00	16.38
		SD±	0.96	1.78	1.41	1.72	1.41	1.69	0.50	1.97	0.96	2.16	0.00	0.61
	Index of stimulation (l.b.)	$\bar{x}$	2.03	2.07	1.85	2.05	2.13	2.09	2.26	1.88	2.73*	2.02	3.00*	2.11
		SD±	0.14	0.30	0.14	0.47	0.23	0.33	0.21	0.20	0.26	0.25	0.00	0.18
Coefficient of the metabolic activity of PMN	Spontaneous (l.b.)	$\bar{x}$	0.25	0.33	0.29	0.29	0.22	0.27	0.18	0.28	0.12	0.25	0.09	0.34*
		SD±	0.04	0.06	0.06	0.07	0.09	0.06	0.06	0.08	0.01	0.06	0.00	0.05
	Stimulated (l.b.)	$\bar{x}$	0.50	0.63	0.53	0.52	0.46	0.53	0.40	0.53	0.32	0.50	0.26	0.69*
		SD±	0.08	0.06	0.09	0.04	0.15	0.05	0.11	0.05	0.04	0.06	0.00	0.05

Legend: – mean value; SD± – standard deviation; Z – infected animals, K – control animals; () – number of animals, * – statistically significant change

creased values, especially in regard to the adherence and absorption abilities of the PMN cells, while the latter changes were observed in respect to the killing abilities of these cells. The changes in chosen immunological parameters were noted in the first few hours after administration of the RHDV strains (4 and 8h after infection), and shortly before the animals' death (at 24, and 36 h after infection). The results are presented in Tables 1-3.

In regard to the adherence abilities of PMN cells, a decrease was noted at the 8th, and 36th hour after infection with the non-haemagglutinating variants: Pv97 and 9905, and at the 8th, and 24th h in case of the exposure to the haemagglutinating RHDV antigenic variant – Hartmannsdorf. However, infection with the non-haemagglutinating variant: Pv97 caused also an increase in the PMN cells' adherence abilities at the 12th h of the experiment. In case of the absorption index the changes were observed only after infection with the Pv97 antigenic variant, which evoked a decrease in values of the parameter measured at 4, 8, 12, 24, and 36 h of the experiment. Additionally, only this strain (Pv97) caused a decrease in the percentage of absorbing cells at 12, 24, and 36 h after infection. Moreover, only infection with the Pv97 non-haemagglutinating variant of the RHD virus caused changes in the values of the NBT spectrophotometric test, however, these values were increased 4, 8, 24, and 36 h after infection. With regard to the spontaneous NBT test, the values dropped at the 24th h after infection with haemagglutinating variant Hartmannsdorf, and at the 36th h in case of infection with non-haemagglutinating antigenic variant Pv9, while at the 4th h of the experiment, the Hartmannsdorf RHDV strain caused an increase in the NBT test results. The 9905 variant did not cause any changes in this parameter, however, in case of the stimulated NBT test the 9905 antigenic variant was the only strain causing decreased values 36 h after virus administration. An increased index of stimulation was characteristic for the infection with the non-haemagglutinating variant: Pv97 after 24, an 36 h of the experiment, while the remaining two RHDV strains did not show any changes in this indicator. In case of both versions of the neutrophil metabolic activity test; spontaneous and stimulated, the declined values were observed

Table 3. Chosen parameters of phagocytosis in rabbits infected with non-haemagglutinating antigenic variant 9905 RHDVa of RHD virus.

Parameters			Values of parameters 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 (10)	K (10)	Z (6)	K (10)
Adherence ability (%)		$\bar{x}$	33.89	39.19	34.33	40.26	37.68	46.03*	30.52	39.01	38.67	43.10	21.34	34.45*
		SD±	1.77	2.55	3.54	4.25	4.62	4.17	4.51	2.74	4.08	2.98	0.00	3.21
Adsorption ability	Absorption index (l.b.)	$\bar{x}$	3.94	4.63	4.03	4.55	4.49	4.98	4.78	4.67	4.84	4.41	5.20	4.62
		SD±	0.10	0.45	0.10	0.43	0.15	0.48	0.27	0.43	0.34	0.43	0.00	0.42
	% of absorbing cells (%)	$\bar{x}$	66.00	67.89	66.80	70.20	65.60	66.26	68.80	68.70	68.00	68.09	68.00	62.25
		SD±	0.00	4.86	1.09	3.89	0.89	3.91	1.09	4.00	0.00	3.09	0.00	4.22
NBT reduction test	Spectrophotometric (10^9/l)	$\bar{x}$	4.32	4.13	5.44	4.42	5.71	4.99	4.74	5.60	5.63	4.95	7.25	4.91
		SD±	0.87	0.88	0.70	0.77	1.19	1.31	0.79	1.16	0.40	0.75	0.00	0.51
	Spontaneous (l.b.)	$\bar{x}$	11.40	10.11	11.20	10.81	10.00	9.93	8.80	9.81	8.50	8.81	8.00	8.22
		SD±	0.55	0.96	0.84	1.18	0.71	1.00	0.45	0.89	0.71	0.86	0.00	0.65
	Stimulated (l.b.)	$\bar{x}$	22.40	21.11	22.00	20.20	20.80	19.87	19.00	18.85	19.00	18.09	18.00	16.38*
		SD±	0.06	1.78	1.00	1.72	1.09	1.69	0.71	1.97	0.00	2.16	0.00	0.61
	Index of stimulation (l.b.)	$\bar{x}$	1.96	2.07	2.48	2.05	1.09	2.09	2.16	1.88	2.22	2.02	2.30	2.11
		SD±	0.10	0.30	0.32	0.47	0.16	0.33	0.06	0.20	0.20	0.25	0.00	0.18
Coefficient of the metabolic activity of PMN	Spontaneous (l.b.)	$\bar{x}$	0.36	0.33	0.27	0.29	0.23	0.27	0.13	0.28	0.11	0.25	0.10	0.34
		SD±	0.06	0.06	0.04	0.07	0.05	0.06	0.02	0.08	0.01	0.06	0.00	0.05
	Stimulated (l.b.)	$\bar{x}$	0.71	0.63	0.59	0.52	0.47	0.53	0.27	0.53	0.25	0.50	0.23	0.69
		SD±	0.12	0.06	0.07	0.04	0.09	0.05	0.05	0.05	0.01	0.06	0.00	0.05

Legend: $\bar{x}$ – mean value; SD± – standard deviation; Z – infected animals, K – control animals; () – number of animals, * – statistically significant change

only at the 36th hour after infection with the Pv97 non-haemagglutinating variant, while other strains used in this study: 9905, and Hartmannsdorf, did not cause any noticeable changes.

The mortality rate of rabbits, determined during the course of the experiment, reached 100% at the 36th h after infection with the RHDV antigenic variants: Hartmannsdorf (haemagglutinating), and Pv97 (non-haemagglutinating), while in case of infection with the non-haemagglutinating variant: 9905, the mortality amounted to 90% of the population after 36 h.

Discussion

In the present study, results regarding the chosen indicators of phagocytosis in rabbits experimentally infected with three antigen variants of the RHD virus: haemagglutinating Hartmannsdorf, and non-haemagglutinating Pv97 and 9905, can be compared with some of the previously described data, which concerned 14 haemagglutinating strains: French (Fr-1, Fr-2) (Niedźwiedzka et al. 2008, Tokarz-Deptuła 2009), Czech (CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558) (Hukowska-Szematowicz 2006, Tokarz-Deptuła et al. 2007, Niedźwiedzka et al. 2008) and Polish SGM (Tokarz-Deptuła 2009), MAŁ (Tokarz-Deptuła 2009), Kr-1 (Tokarz-Deptuła 2009), K-1 (Piekarski 1994), KGM (Tokarz-Deptuła 2009), PD (Tokarz-Deptuła 2009), ŻD (Tokarz-Deptuła 2009), GSK (Tokarz-Deptuła 2009), as well as one non-haemagglutinating strain BLA (Tokarz-Deptuła et al. 2007, Tokarz-Deptuła 2009); however none of the cited strains belong to the antigenic variants of RHDV.

The observed changes regarding the adherence ability of PMN cells included decreased values of this parameter at the 8th, and 24th h after infection with the haemagglutinating variant: Hartmannsdorf, or at the 8th, and 36th h in case of non-haemagglutinating variants: Pv97 and 9905. An increase in the PMN cells' adherence ability was noted at the 12th h after infection with the Pv97 variant. These data can only be compared with the pattern of changes caused by the PD strain, in which a drop of the PMN cells' adher-

ence ability was registered 8, 12, 36, and 56 h after infection (Tokarz-Deptuła 2009).

Lack of changes in the absorption ability of PMN cells during the infection with haemagglutinating variant: Hartmannsdorf, and non-haemagglutinating variant: 9905 is similar to the results obtained in previous studies on haemagglutinating strains: Fr-1 (Niedźwiedzka et al. 2008, Tokarz-Deptuła 2009), Czech CAMP V-561 (Hukowska-Szematowicz 2006) and Polish: SGM (Tokarz-Deptuła 2009), K-1 (Piekarski 1994), KGM (Tokarz-Deptuła 2009), as well as non-haemagglutinating BLA strain (Tokarz-Deptuła et al. 2007, Tokarz-Deptuła 2009). Surprisingly, in case of infection with the non-haemagglutinating antigenic variant Pv97 decreased values of the PMN cells' absorption ability were noted after 4, 8, 12, 24, and 36 h, which is difficult to interpret, as none of the previous studies regarding RHDV strains: French Fr-1, Fr-2 (Tokarz-Deptuła 2009), Czech: CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558 (Hukowska-Szematowicz 2006) or Polish: SGM, MAŁ, Kr-1, K-1, KGM, BLA, PD (Tokarz-Deptuła 2009) described such changes.

Meanwhile, the lack of changes in the percentage of absorbing cells, which was observed during the course of infection with haemagglutinating antigenic variant Hartmannsdorf and non-haemagglutinating variant 9905 is in agreement with observations concerning other haemagglutinating RHDV strains: French Fr-1 and Fr-2 (Tokarz-Deptuła 2009), Polish Kr-1 and PD (Tokarz-Deptuła 2009), as well as the Polish non-haemagglutinating strain of RHD virus: BLA (Tokarz-Deptuła et al. 2007, Tokarz-Deptuła 2009). However, the decreased values of this parameter found at the 12, 24, an 36 h after infection with the non-haemagglutinating variant Pv97, does not confirm any of the previously described investigations.

In the present study, no changes have been observed in the values of the spectrophotometric NBT test, in rabbits infected with the haemagglutinating variant Hartmannsdorf or non-haemagglutinating variant 9905. This is in agreement with data concerning the Czech haemagglutinating strain CAMP V-558 (Hukowska-Szematowicz 2006). An increased level of this indicator was observed 4, 8, 24, 36 h after infection with the non-haemagglutinating variant Pv97, which partially resembles the results obtained in the study concerning the haemagglutinating French strain Fr-1, where the increase was noted at the 4, 8, 12, 24, 48, 52 h of the experiment (Tokarz-Deptuła 2009). In case of the spontaneous NBT test, the decline in values was observed at the 24th hour after infection with the haemagglutinating variant: Hartmannsdorf, and 36 h after administration of the Pv97 non-haemagglutinating variant of the RHD virus. These results were partially similar to those obtained during the course of infection with Polish haemagglutinating strains: Kr-1 (36 h) and PD (4,24 h) (Tokarz-Deptuła 2009). Moreover, the increase in this parameter measured at the 4th h after infection with the haemagglutinating variant Hartmannsdorf is identical with that concerning another haemagglutinating strain: the Czech CAMP V-351 (Hukowska-Szematowicz 2006). In case of haemagglutinating variant Hartmannsdorf, and non-haemagglutinating variant Pv97 no changes have also been observed in regard to the stimulated NBT test, which confirms the previous studies concerning the haemagglutinating strains: Czech CAMP V-561 (Hukowska-Szematowicz 2006), and Polish SGM, MAŁ, and PD (Tokarz-Deptuła 2009). However, the non-haemagglutinating RHDV antigenic variant 9905 caused an increase in the value of this parameter 36 hour after infection, which has never been reported previously. The analysis of data concerning the stimulation index, showed that the lack of changes observed after infection with the haemagglutinating variant Hartmannsdorf, as well as the non-haemagglutinating variant 9905 is in agreement with the results concerning the haemagglutinating strains: Czech CAMP V-562 (Hukowska-Szematowicz 2006) and Polish PD (Tokarz-Deptuła 2009), while the increased values of this index, obtained in the present experiment at the 24th, and 36th h after infection with the non-haemagglutinating variant Pv97, closely resemble the results obtained after infection with the Czech haemagglutinating strain CAMP V-351 (12,24,36 h) (Hukowska-Szematowicz 2006).

In terms of the spontaneous, or stimulated test for metabolic activity of neutrophils, no changes were observed after infection with the Hartmannsdorf haemagglutinating variant, or 9905 non-haemagglutinating variant of the RHD virus, which confirms the previous observations concerning the infection with the haemagglutinating French strain Fr-1 (Tokarz-Deptuła 2009). The decrease in both parameters was found 36 h after infection with the non-haemagglutinating variant Pv97, which is partially in agreement with the changes observed during the course of infection with the Czech haemagglutinating strain CAMP V-561 of the RHD virus (Hukowska-Szematowicz 2006).

The last parameter, which was analysed during this study was the mortality of the rabbits infected with the examined RHDV antigenic variants (Hartmannsdorf, Pv97, 9905). The mortality rate was between 90-100% 36h after infection, which reflects the previous observations (Capucci et al. 1998, Schirrmeier et al. 1999, Grazioli et al. 2000), showing that the RHDV antigenic variants, originating from Italy and Germany, cause the same mortality rate. It should

be noted, that in the group of previously analysed haemagglutinating strains of the RHD virus the mortality varied between 25 and 100% (Piekarski 1994, Hukowska-Szematowicz 2006, Tokarz-Deptuła et al. 2007, Niedźwiedzka et al. 2008, Tokarz-Deptuła 2009), while in case of the described non-haemagglutinating strain: BLA it reached 60% (Tokarz-Deptuła 2009).

The present study, concerning chosen indicators of the phagocytosis process – element of natural immunity, in rabbits infected with RHDV antigenic variants: haemagglutinating Hartmannsdorf, or non-haemagglutinating variants: Pv97 and 9905, has revealed that the strains examined show differences between each other in respect to the chosen immunological parameters, and also differ from the RHDV haemagglutinating, and non-haemagglutinating strains which are not classified as antigenic variants. The non-haemagglutinating variant: Pv97 caused 5-6 fold more changes in the parameters examined (mostly decreasing the measured indexes) in comparison to the haemagglutinating variant Hartmannsdorf, and non-haemagglutinating variant 9905, proving the immunological dissimilarities among the antigenic variants of the RHD virus. The RHDV antigenic variants examined, caused a decrease in most of the chosen indicators of phagocytosis, while the other strains of the virus, not classified as the antigenic variants, increase these immunological parameters, proving the difference between the existing strains of RHD virus. Moreover, the time in which the changes occurred was shorter for the antigenic variants of RHDV. Additionally, the previous studies on RHDV strains, not considered as antigenic variants, revealed changes mainly in regard to the spectrophotometric NBT test, whereas in case of the antigenic variants, the changes concerned mainly the adherence, and absorption abilities of the PMN cells, that constitute the first stages of phagocytosis. It should also be noted, that the mortality rate after infection with all three RHDV antigenic variants (Pv97, 9905 and Hartmannsdorf) reached 90 – 100%, which confirms the high pathogenicity of these strains, characteristic for the defined antigen variants (Capucci et al. 1998).

Therefore it can be concluded, that the differential immunological picture obtained in the course of the present study on the antigenic variants of RHD virus, proves their immunological diversity, which was not included in the definition of antigenic variants introduced by Capucci and co-workers (Capucci et al. 1998). The authors based their definition of antigenic variants only on one property of the antigens – the antigenicity. In the present study, the high mortality of rabbits infected with the RHDV antigenic variants confirms the fact, that the high pathogenecity belongs to the specific properties of antigenic variants, which is in agreement with the hypotheses stated by other authors (Capucci et al. 1998, Schirrmeier et al. 1999, Grazioli et al. 2000).

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