Indices of white and red blood cells in rabbits experimentally infected with chosen antigenic variants of rabbit haemorrhagic disease virus

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Abstract

Among rabbit haemorrhagic disease virus (RHDV) strains, there are some of them, nominated as antigenic variants, which have not been studied to the extent of haematological parameters. Moreover, antigenic variants differ in haemagglutination capacity. The paper aim at examining the dynamic changes in the values of white and red blood cells indexes in four haemagglutinating (Vt97 and Hartmannsdorf) and non-haemagglutinating (Pv97 and 9905) antigenic variants of RHDV. The study indeed showed differences among examined strains that were not depending on haemagglutination property.

Key words: *rabbit haemorrhagic disease, antigenic variant, haemagglutination.*

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Introduction

The haematological picture of the animals infected with different strains of rabbit haemorrhagic disease virus (RHDV) lead to the conclusion, that the strains differ from one another, nevertheless the differences are not sufficient to divide the virus into haemotypes [1, 2]. What is worth mentioning, so far there is also no information about the RHDV antigenic variants in the context of haematological indexes of rabbits infected with them, so as with non-haemagglutinating strains, while it has been previously demonstrated that the mechanism of RHDV pathogenic effect on macroorganism is regulated by the haemagglutinating properties of the virus [1, 2]. Moreover, the RHDV antigenic variants cause not only a higher mortality rate of infected rabbits in comparison to the strains which are not classified as antigenic variants, but also they exhibit a different antibody-binding properties [3], which may be connected with the pathogenicity of the virus.

The purpose of this study was to describe the dynamic changes in the values of white (WBC) and red blood cells (RBC) indexes infected antigenic variants of RHDV – haemagglutinating, e.i. originating form Italy – Vt97 and from Germany – Hartmannsdorf and non-haemagglutinating, e.i. originating from Italy – Pv97 and from France – 9905.

Material and methods

The study was performed on 80 mixed breed rabbits of both sexes, weighing in the range of 3.2-4.2 kg, marked as conventional animals, coming from a licensed breeding farm, under a constant veterinary and zootechnique supervision [4]. During the experiment, the animals were kept at the vivarium with standards recommended in Poland [5]. After transportation to the vivarium, the animals were subject to a two-week adaptation period and were fed with full-portion rabbit feed (brand: 16% *królik z Motycza*) in the quantity of 0.15-0.20 kg/day, and an unlimited access to water.

For the purpose of this study rabbits were divided into 8 groups, each comprising 10 animals. There were 4 experimental groups in which rabbits were infected with one of the investigated RHDV antigenic variants: haemagglutinating (Vt97, or Hartmannsdorf), and non-haemagglutinating (Pv97, or 9905), and 4 control groups, each corresponding to one of the experimental groups. The animals from the experimental groups were infected with a dose of appropriate RHDV strain (Vt97, 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. All RHDV strains originat-

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ed from naturally dead animals from the area of Italy, Germany and France. Livers were collected *post mortem* from the naturally infected animals, and served as a source of virus for the experimental infection of rabbits, in which the collected strains were propagated. After the death of animals the livers were collected again to prepare homogenates. 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 [6]. Centrifugation of the samples in caesium chloride gradient resulted in obtaining comparable concentrations of virus particles, estimated to be 1.34 g/dm^3. The rabbits from the 4 experimental groups were infected with the prepared RHDV samples.

Blood samples were collected from all groups of rabbits (control, and experimental) from the peripheral 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. Additionally mortality rate was registered. Further observations were not conducted due to mortality of the animals, or occurrence of the disease symptoms, which was in accordance with the Local Ethical Committee recommendations.

The blood samples were analysed in respect to haemoglobin concentration, erythrocytes, leucocytes and trombocytes quantity, and WBC picture (lymphocytes, neutrophilic, eosinophilic and basophilic granulocytes, and monocytes). The haemoglobin concentration was determined in EDTA blood with the use of 5 ml Drabkin's reagent (Aqua-Med., Poland). After 20 min incubation with the reagent, at room temperature, the blood samples were thoroughly mixed and the extinction values were measured at 540 nm wavelength in comparison to blank sample (Drabkin's reagent). Additionally an extinction value of reference sample (cyanmethemoglobin) was also measured (Aqua-Med., Poland). The erythrocytes quantity was determined by multiplying the haematocrit value [%] by a 0.105 coefficient. In order to determine the leucocytes quantity 0.02 ml of the EDTA blood was suspended in 0.38 ml of Tűrk solution, constantly aspirating. After 5 min the suspension was administered into Bűrker chamber, and 5 min later the number of leucocytes were counted on the area of 1 mm^2 (a square of 1 mm side). The obtained number of cells was multiplied by 20, and 10^9, and the results were presented as an average number of leucocytes in 10^9/l units. The quantity of thrombocytes was measured after incubation of 0.02 ml blood with 2 ml of lysis buffer in Thrombo Plus tubes (Sarstedt, Germany). After 20 min incubation the suspension was administered into Bűrker chamber, and 10 minutes later the cells were counted in the large square of the net (including 16 small squares), with the length of 1 mm each side. The obtained results were multiplied by 10^9, and values were presented as an average number of thrombocytes in 10^9/l units. Additionally a smear was performed from the EDTA blood samples, which was next stained according to Pappenheim method, with the use of two stains: May-Grűnwald and Giemsa. Microscopic analysis was performed with the use of light microscope, and oil immersion, 100× objective. One hundred cells were counted, with the division on lymphocytes, neutrophilic, eosinophilic and basophilic granulocytes, and monocytes.

The obtained results of haematological analysis were subjected to statistical analyses by *t*-Student test with $p = 0.05$, with the use of Statistica software ver. 6.0, and presented in Tables 1-4. Additionally the mortality rate was presented as a percentage of rabbits' death registered during the time of experiment.

Results

The analysis of the changes observed during the infection with individual antigenic variants showed that the Italian haemagglutinating antigenic variant – Vt97 (Table 1) caused a decrease in the haemoglobin concentration 36 h after infection, while at the same time point the French non-haemagglutinating antigenic variant – 9905 (Table 4) caused an increase in this parameter. The German haemagglutinating antigenic variant – Hartmannsdorf (Table 2) evoked an increase in the number of leucocytes, and a decrease in the lymphocytes number 24 h after the infection. In case of the Italian non-haemagglutinating antigenic variant – Pv97 (Table 3) the decreased number of thrombocytes was observed in every registered time point after infection (4, 8, 12, 24, 36 h), the quantity of lymphocytes was declining in the 12, 24, 36 hours, while the number of neutrophils increased 12, 24, 36 h after the infection.

To conclude on the haematological indexes analysis in rabbits infected with RHDV, one may say that the investigated antigenic variants of the virus – haemagglutinating, or non-haemagglutinating – differ in regard to the analysed WBC and RBC indexes. Three out of four investigated variants: haemagglutinating strains Vt97 and Hartmannsdorf, and non-haemagglutinating strain 9905 caused only slight changes in these parameters, whereas the non-haemagglutinating RDHV variant: Pv97 evoked almost 10-fold changes, mainly in the number of lymphocytes, neutrophils, and thrombocytes. Similar differences among the investigated RDHV strains were observed in respect to the mortality rate, which oscillated between 30-100% depending on the viral strain.

Detailed analysis of the mortality rate caused by rabbits' infection with the 4 investigated RDHV strains (Pv97, 9905, Hartmannsdorf, Vt97) showed that in the 36th h of the infection 90% mortality was registered in animals infected with the non-haemagglutinating variants (Pv97 and 9905), 100% in the case of the haemagglutinating variant Hartmannsdorf, while the infection with haemagglutinating variant Vt97 evoked death only in 30% of the animals.

Table 1. Haematological parameters in rabbits infected with haemagglutinating antigenic variant Vt97 of the RHD virus

Parameters			Values of parameters in hours											
			0		4		8		12		24		36	
			I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (7)	C (10)
haemoglobin concentration [mmol/l]		$\bar{x}$	6.83	7.73	6.98	7.11	6.81	6.80	6.35	6.41	5.29	6.47	5.23	7.73*
		± SD	0.91	0.27	0.86	0.38	0.61	0.28	0.30	0.26	0.66	0.36	0.45	0.07
leucocytes [10^9/l]		$\bar{x}$	5.10	3.84	5.30*	3.90	5.10*	3.54	3.90	3.74	3.50	3.68	4.40	3.30
		± SD	0.49	0.27	0.54	0.21	0.50	0.28	0.54	0.36	0.36	0.33	0.30	0.05
erythrocytes [10^{12}/l]		$\bar{x}$	3.10	3.79	3.40	3.44	3.10	3.51	4.00	3.14	2.40	3.19	2.20	3.83
		± SD	0.40	0.25	0.40	0.18	0.40	0.16	0.40	0.16	0.18	0.02	0.00	0.13
trombocytes [10^9/l]		$\bar{x}$	511.00	493.8	539.00	476.90	572.00	456.00	576.00	456.60	560.00	483.60	600.00	483.00
		± SD	47.00	29.85	52.00	30.61	51.00	35.35	52.00	37.35	34.00	33.06	18.00	31.92
WBC picture	**lymphocytes**	$\bar{x}$	0.55	0.64	0.51	0.57	0.57	0.59	0.50	0.61	0.54	0.61	0.48	0.66
		± SD	0.050	0.022	0.050	0.018	0.040	0.025	0.050	0.013	0.050	0.016	0.090	0.011
	granulocytes – **neutrophilic**	$\bar{x}$	0.46	0.34	0.42	0.38	0.36	0.38	0.39	0.36	0.39	0.35	0.44	0.34
		± SD	0.050	0.032	0.050	0.013	0.040	0.022	0.060	0.010	0.093	0.016	0.011	0.011
	granulocytes – **eosinophilic**	$\bar{x}$	0.04	0.01	0.04	0.02	0.04	0.02	0.04	0.02	0.04	0.01	0.04	0.02
		± SD	0.012	0.005	0.023	0.006	0.025	0.004	0.022	0.006	0.01	0.006	0.002	0.006
	granulocytes – **basophilic**	$\bar{x}$	0.02	0.01	0.01	0.01	0.04	0.01	0.02	0.01	0.06	0.02	0.00	0.04
		± SD	0.011	0.005	0.002	0.003	0.002	0.002	0.001	0.002	0.003	0.003	0.000	0.002
	monocytes	$\bar{x}$	0.04	0.02	0.02	0.02	0.03	0.01	0.03	0.01	0.02	0.01	0.04	0.01
		± SD	0.021	0.005	0.001	0.003	0.010	0.003	0.001	0.002	0.002	0.003	0.001	0.005

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

Table 2. Haematological parameters in rabbits infected with haemagglutinating antigenic variant Hartmannsdorf of the RHD virus

Parameters			Values of parameters in hours									
			0		4		8		12		24	
			I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (9)	C (10)
haemoglobin concentration [mmol/l]		$\bar{x}$	7.88	7.73	7.45	7.11	7.24	6.80	6.81	6.41	5.15	6.47
		± SD	0.89	0.27	1.44	0.38	1.19	0.28	1.79	0.26	1.36	0.36
leucocytes [10^9/l]		$\bar{x}$	3.40	3.84	3.40	3.90	4.60	3.54	3.40	3.74	2.00*	3.68
		± SD	0.80	0.27	0.80	0.21	1.60	0.28	1.10	0.36	0.60	0.33
erythrocytes [10^{12}/l]		$\bar{x}$	3.92	3.79	3.82	3.44	3.42	3.51	3.17	3.14	3.36	3.19
		± SD	0.55	0.25	0.65	0.18	0.58	0.16	0.58	0.16	0.55	0.02
trombocytes [10^9/l]		$\bar{x}$	480.00	493.80	482.00	476.90	469.00	456.00	460.00	456.60	396.00	483.60
		± SD	8.50	29.85	11.30	30.61	14.10	35.35	18.90	37.35	34.30	33.06
WBC picture	**lymphocytes**	$\bar{x}$	0.65	0.64	0.59	0.57	0.50	0.59	0.50	0.61	0.40	0.61
		± SD	0.010	0.022	0.040	0.018	0.050	0.025	0.120	0.013	0.080	0.016
	granulocytes **neutrophilic**	$\bar{x}$	0.35	0.34	0.41	0.38	0.50	0.38	0.40	0.36	0.50	0.34
		± SD	0.010	0.032	0.040	0.013	0.050	0.022	0.120	0.010	0.080	0.011
	eosinophilic	$\bar{x}$	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.01
		v	0.000	0.005	0.000	0.006	0.000	0.004	0.000	0.006	0.000	0.006
	basophilic	$\bar{x}$	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.02
		± SD	0.00	0.005	0.000	0.003	0000	0.002	0.000	0.002	0.000	0.003
	monocytes	$\bar{x}$	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.01
		± SD	0.000	0.005	0.000	0.003	0.000	0.003	0.000	0.002	0.000	0.003

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

Table 3. Haematological parameters in rabbits infected with non-haemagglutinating antigenic variant Pv97 of the RHD virus

Parameters			Values of parameters in hours											
			0		4		8		12		24		36	
			I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (1)	C (10)
haemoglobin concentration [mmol/l]		$\bar{x}$	8.20	7.73	8.00	7.11	7.30	6.80	6.80	6.41	5.80	6.47	6.50	7.73
		± SD	0.40	0.27	0.60	0.38	0.30	0.28	0.60	0.26	0.70	0.36	0.23	0.07
leucocytes [10^9/l]		$\bar{x}$	4.00	3.84	3.90	3.90	3.40	3.54	3.10	3.74	2.70	3.68	2.80	3.30
		± SD	0.20	0.27	0.10	0.21	0.20	0.28	0.30	0.36	0.20	0.33	0.80	0.05
erythrocytes [10^{12}/l]		$\bar{x}$	3.30	3.79	2.90	3.44	3.00	3.51	2.50	3.14	2.90	3.19	2.60	3.83
		± SD	0.90	0.25	0.70	0.18	0.70	0.16	0.60	0.16	0.30	0.02	0.20	0.13
trombocytes [10^9/l]		$\bar{x}$	341.0	493.80	291.00	476.90*	265.00	456.00*	317.00	456.60*	260.00	483.60*	193.00	483.00*
		± SD	22.00	29.85	41.00	30.61	44.00	35.35	24.00	37.35	49.00	33.06	38.00	31.92
WBC picture	**lymphocytes**	$\bar{x}$	0.52	0.64	0.53	0.57	0.46	0.59	0.37	0.61*	0.32	0.61*	0.24	0.66*
		± SD	0.070	0.022	0.040	0.018	0.090	0.025	0.050	0.013	0.110	0.016	0.080	0.011
	granulocytes – **neutrophilic**	$\bar{x}$	0.44	0.34	0.44	0.38	0.50	0.38	0.55*	0.36	0.58*	0.35	0.70*	0.34
		± SD	0.070	0.032	0.060	0.013	0.070	0.022	0.170	0.010	0.090	0.016	0.040	0.011
	granulocytes – **eosinophilic**	$\bar{x}$	0.01	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.02
		± SD	0.020	0.005	0.030	0.006	0.030	0.004	0.010	0.006	0.010	0.006	0.030	0.006
	granulocytes – **basophilic**	$\bar{x}$	0.01	0.01	0.02	0.01	0.02	0.01	0.04	0.01	0.01	0.02	0.04	0.04
		± SD	0.010	0.005	0.010	0.003	0.000	0.002	0.010	0.002	0.010	0.003	0.080	0.002
	monocytes	$\bar{x}$	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.01	0.02	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: $\bar{x}$ – mean value, ± SD – standard deviation, I – infected animals, C – control animals, () – number of animals, * – statistically significant change*

Table 4. Haematological parameters in rabbits infected with non-haemagglutinating antigenic variant 9905 of the RHD virus

Parameters			Values of parameters in hours											
			0		4		8		12		24		36	
			I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (10)	C (10)	I (7)	C (10)	I (1)	C (10)
haemoglobin concentration [mmol/l]		$\bar{x}$	8.35	7.73	7.50	7.11	8.06	6.80	7.92	6.41	7.81	6.47	9.00*	7.73
		± SD	0.56	0.27	0.49	0.38	0.64	0.28	0.93	0.26	1.43	0.36	0.00	0.07
leucocytes [10^9/l]		$\bar{x}$	5.40	3.84	4.50	3.90	3.70	3.54	3.50	3.74	2.30	3.68*	2.20	3.30*
		± SD	0.90	0.27	0.96	0.21	0.30	0.28	0.20	0.36	0.70	0.33	0.00	0.05
erythrocytes [10^{12}/l]		$\bar{x}$	4.11	3.79	3.93	3.44	4.03	3.51	3.82	3.14	3.72	3.19	2.94	3.83
		± SD	0.32	0.25	0.58	0.18	0.14	0.16	0.20	0.16	0.67	0.02	0.00	0.13
trombocytes [10^9/l]		$\bar{x}$	480.00	493.80	474.00	476.90	460.00	456.00	457.00	456.60	392.00	483.60	398.00	483.00*
		± SD	8.50	29.85	9.96	30.61	17.35	35.35	16.40	37.35	8.48	33.06	0.00	31.92
WBC picture	**lymphocytes**	$\bar{x}$	0.68	0.64	0.58	0.57	0.49	0.59	0.30	0.61	0.25	0.61	0.21	0.66*
		± SD	0.050	0.022	0.180	0.018	0.180	0.025	0.120	0.013	0.030	0.016	0.000	0.011
	granulocytes **neutrophilic**	$\bar{x}$	0.32	0.34	0.42	0.38	0.51	0.38	0.70	0.36	0.74	0.35	0.79	0.34
		± SD	0.050	0.032	0.180	0.013	0.180	0.022	0.120	0.010	0.030	0.016	0.000	0.011
	eosinophilic	$\bar{x}$	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.02
		± SD	0.00	0.005	0.000	0.006	0.000	0.004	0.000	0.006	0.000	0.006	0.000	0.006
	basophilic	$\bar{x}$	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.02	0.00	0.04
		S± SD	0.000	0.005	0.000	0.003	0.00	0.002	0.000	0.002	0.000	0.003	0.000	0.002
	monocytes	$\bar{x}$	0.00	0.02	0.00	0.02	0.00	0.04	0.00,	0.01	0.00	0.01	0.00	0.01
		± SD	0.000	0.005	0.000	0.003	0.000	0.003	0.000	0.002	0.000	0.0003	0.000	0.005

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

Discussion

The results of the up-to-date studies on the RHD virus infection are lacking information about the changes in haematological indexes caused by the different antigen variants of RDHV. Some data can be found regarding 14 haemagglutinating strains originating from France (Fr-1, Fr-2) [2], Czech Republic (CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558) [7] and Poland (K-1, SGM, MAŁ, KGM, ŻD, PD, GSK, Kr-1) [2, 8], and one Polish non-haemagglutinating strain BLA [2], however, the described strains are not classified as antigenic variants. These studies describe mostly a decline of investigated WBC parameters, especially in respect to the number of neutrophils, leucocytes, and lymphocytes, observed within 30-60 h after infection. The greatest changes in the haematological indexes were observed in the case of infection with CAMP V-351 strain, and the less pronounced effect was seen in animals infected with French Fr-1 strain [2, 7].

It should be mentioned that the studies on the picture of the haematological indexes during rabbits' infection with RDHV haemagglutinating strains, conducted worldwide, and concerning mainly the unclassified strains of this virus from the regions of China [9-13], Austria [14, 15], Israel [16], Italy [17], Germany [18], Korea [19], Spain [20] and France [21], showed that these strains cause, in most cases, a decrease in the WBC parameters, observed mainly 35-56-60 h after the infection, and sporadically within 4-8 h after administration of the virus. The changes concerned especially the number of neutrophils, lymphocytes, and the general number of leucocytes, which confirms the previous observations conducted in Poland [2, 7, 8].

It should be noted that only a small amount of changes in haematological indexes, registered in the present study, in animals infected with the haemagglutinating variants: Vt97, and Hartmannsdorf, and the non-haemagglutinating variant 9905, closely resemble the haematological picture caused by the RHDV haemagglutinating strain Fr-2 [2], while almost 10-fold higher number of changes evoked by infection with non-haemagglutinating variant Pv97 of the virus is in accordance with the picture described in the studies on haemagglutinating RHDV strains: KGM, PD [2], K-1 [8], CAMP V-562, and CAMP V-558 [7].

The results presented in our study, concerning the mortality rate in rabbits infected with the analysed RHDV strains, are also difficult to compare with similar researches, as in the present experiment the mortality was registered only until 36th hour of infection, whereas in case of the 13 previously described RHDV strains (Fr-1, Fr-2, CAMP V-351, CAMP V-561, CAMP V-562, CAMP V-558, K-1, SGM, MAŁ, KGM, ŻD, GSK, Kr-1) the observations were conducted until 60-72 h after infection, in which case the mortality amounted to 25-100%. The high mortality rate caused by haemagglutinating Hartmannsdorf antigenic variant (100%), and non-haemagglutinating variants: Pv97 (90%) and 9905 (90%) is consistent with the percentage of deaths quite commonly registered during infections with French, Czech, and some Polish strains of the RHD virus [2, 7, 8]. On the other hand the low percentage of death (30%) caused by haemagglutinating Vt97 antigenic variant is similar to the result obtained during the study on the Polish RHDV haemagglutinating strain: PD, where the mortality rate of rabbits amounted only to 25% [2].

Conclusions

In the summary of the present results it should be concluded that the investigated variants of RHD virus, which show different haemagglutinating properties, also differ in regard to the haematological changes they cause. Three (Vt97, 9905, Hartmannsdorf) out of four analysed strains, defined as antigenic variants, evoked only small changes in haematological parameters, while the non-haemagglutinating strain Pv97 caused 10 times more changes in the investigated parameters. Also the high mortality rate was observed during the infection with three out of four investigated variants (Pv97, 9905, Hartmannsdorf) in the 36th hour of the experiment, while the Vt97 strain caused relatively low mortality in rabbits. Therefore it can be concluded that the investigated haemagglutinating (Vt97 and Hartmannsdorf), and non-haemagglutinating (Pv97 and 9905) RHDV antigenic variants have different effect on the haematological parameters, and cause a different death rate of animals. Moreover the analysed RHDV variants cause a different haematological picture from the previously described 8 haemagglutinating strains: Fr-1, CAMP V-351, CAMPV-561, K-1, SGM, MAŁ, ŻD, GSK [2, 7, 8], and one non-haemagglutinating strain BLA [2].

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