

B and T cell values in peripheral blood in Polish mixed breed rabbits

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Abstract

Due to the lack of reference values for immunological parameters in Polish mixed breed rabbits, the study was aimed at developing standards for B-cells with CD19+ receptor, and T-cells with CD5+ receptor, and their subpopulations, namely T-cells with receptors CD4+, CD8+ and CD25+ in peripheral blood of Polish mixed breed rabbits, as well as at assessing the impact of four seasons and sex of the animals on such values. The results of the study not only are the source of reference values, but also revealed that the season of the year and sex of the rabbits affect the percentage of B- and T-cells and their subpopulations in peripheral blood.

Key words: rabbits, lymphocytes, reference values.

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Introduction

Rabbits are animals of a high economic and laboratory value, whereas in Poland, principally in scientific and diagnostic research, mixed breed rabbits are generally used [1]. It must also be added that although researches involving experiments on laboratory animals are conducted on mice, rats and Guinea pigs, still rabbits are more “convenient” as laboratory animals, for example as more blood can be drawn. Nevertheless, when performing observations on animals, including rabbits, including mixed breed rabbits, it is necessary to know the reference values for immunological factors due to an increased possibility of changes in the elements of the immune system, while in the case of rabbits, including Polish mixed breed rabbits, there are no standards for B- and T-cells. Previous studies in the volume of B- and T-cells in peripheral blood were carried out abroad on rabbits of New Zealand, Fauve de Bourgogne races, as well as LP and V genetic lines (Table 1), while in Poland, exclusively on mixed breed rabbits (Table 2). Such observations were not oriented at determining reference values and no impact of physiological properties of rabbits was analysed, such as sex, age and race, as well as environmental conditions (seasons), even though they were considered, and despite the fact that their impact was documented when analysing haematological factors [2-19]. The studies carried out in Poland on Polish mixed breed rabbits evidenced the impact of age [3, 8] and sex [3] of

the animals, as well as season of the year [10], while foreign studies on New Zealand and Angora rabbits, as well as Czech races and mixed breed animals also pointed out to the impact of the age [5, 6, 11-13, 15, 19], sex [4, 11, 14, 16, 17, 19], and race [7, 9, 11, 18, 19], as well as season [2, 16, 19].

The study was aimed at developing standards for B-cells with CD19+ receptor, and T-cells with CD5+ receptor, and their subpopulations, namely T-cells with receptors CD4+, CD8+ and CD25+ in peripheral blood of Polish mixed breed rabbits, as well as at assessing the impact of four seasons and sex of the animals on such values.

Material and methods

The study involved 200 Polish mixed breed rabbits, labelled as conventional animals, originating from a licensed farm, remaining under continuous veterinary and zootechnical supervision [20], weighing 3.2-4.2 kg, aged 6-8 months, females and males, in four seasons of the year: spring, summer, autumn, and winter. During the experiment, the animals remained at the vivarium of the Department of Microbiology and Department of Immunology of the Biology Faculty at the University of Szczecin, where zootechnical parameters were in line with the recommended Polish standards developed in line with the European Union Directive as regards temperature and humidity, as well as lighting and size of cages for animals [21]. After transportation to

Table 1. Percentage of B and T cells and subpopulations in peripheral blood of rabbits – foreign data

Reference	Age	Sex	Number of animals	Season	Breed	Percentage of B and T cells and subpopulations				
						B (receptor CD19+)	T (receptor CD5+)	T (receptor CD4+)	T (receptor CD8+)	T (receptor CD25+)
28	no data	female	65	no data	genetic lines LP and V	151.7 × 10 ⁶ /l	1306 × 10 ⁶ /l	734.2 × 10 ⁶ /l	328.6 × 10 ⁶ /l	38.77 × 10 ⁶ /l
29	11-15 mth	female	22	no data	Spanish mixed breed	96 × 10 ⁶ /l	1191 × 10 ⁶ /l	543 × 10 ⁶ /l	216 × 10 ⁶ /l	14 × 10 ⁶ /l
30	1 day and 2, 4, 6, 8 weeks	no data	28	no data	New Zealand	no data	NS	29.1%	10.9%	NS
31	no data	male	no data	no data	Fauve de Bourgogne	NS	6314/mm ³	NS	NS	NS

NS – not studied

Table 2. Percentage of B and T cells and subpopulations in peripheral blood of rabbits – Polish data

Reference	Age	Sex	Number of animals	Season	Breed	Percentage of B and T cells and subpopulations					
						B (receptor CD19+)	T (receptor CD5+)	T (receptor CD4+)	T (receptor CD8+)	T (receptor CD25+)	
22	no data	male	20	no data	Polish mixed breed	\bar{x}	1.6	40.3	32.9	NS	0.75
						$\pm SD$	NS	NS	NS	NS	NS
23	3-5 mth	male and female	20	spring (March-June)	Polish mixed breed	\bar{x}	19.23	55.61	39.35	18.47	20.58
						$\pm SD$	1.75	1.42	2.54	1.52	1.54
24	3-4.5 mth	male and female	60	spring (March-June)	Polish mixed breed	\bar{x}	11.4	55.7	39.6	14.5	11.2
						$\pm SD$	4.51	15.09	5.75	2.53	3.84
25	3-4.5 mth	male and female	60	spring (March-June), autumn (September-October)	Polish mixed breed	\bar{x}	11.40	55.70	39.60	14.50	11.20
						$\pm SD$	4.51	15.09	5.75	2.53	3.84
26	no data	no data	20	no data	Polish mixed breed	\bar{x}	1.6	40.3	32.9	NS	0.75
						$\pm SD$	NS	NS	NS	NS	NS
27	3-4.5 mth	male and female	60	spring (March-June), autumn (September-October)	Polish mixed breed	\bar{x}	11.4	55.7	39.6	14.5	11.2
						$\pm SD$	4.51	15.09	5.75	2.53	3.84

NS – not studied; \bar{x} – mean value, $\pm SD$ – standard deviation

the Department vivarium, the animals were provided with a two-week adaptation period. The animals were fed with all-mash rabbit feed (16% Królik z Motycza), at a volume of 0.15-0.20 kg/day, and had unlimited access to water.

The study was carried out twice (seven days each) in four seasons. Blood for tests was drawn by establishing a port from the marginal vein of the ear, in 24-hour intervals,

for three consecutive days, at 8:00 AM, namely at hours 0, 24 and 48 h from commencement of the study. In blood of rabbits, the percentage of CD19+ B-cells, and CD5+ T-cells, as well as their subpopulations – T-cells with receptors CD4+, CD8+ and CD25+ was determined, according to the method described by Deptuła *et al.* [22] using monoclonal antibodies (mouse anti-rabbit) (Serotec, USA).

Table 3. Percentage of B and T cells and subpopulations in peripheral blood of rabbits taking into consideration season and sex

B and T cells and subpopulations		Spring			Summer			Autumn			Winter		
		female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)	female (25)	male (25)	together (50)
B receptor CD19+	\bar{x}	13.01	11.44	12.23 ^{b1}	11.12	10.39	10.76	12.28	12.02	12.15 ^{b4b6}	10.85	11.03	10.94
	\pm SD	1.26	1.15	1.11	0.94	0.93	0.52	0.76	2.28	0.18	1.34	1.23	0.013
T receptor CD5+	\bar{x}	48.99	48.99	48.99 ^{b2}	52.68	50.24	51.46 ^{b4}	41.48	44.64	43.06	43.57	53.28	48.43 ^{b5b6}
	\pm SD	4.43	5.43	4.56	4.50	4.35	6.23	1.83	3.85	4.18	5.08	3.17	6.78
T receptor CD4+	\bar{x}	39.13 ^a	21.53	30.33	40.16	39.93	40.01 ^{b1}	35.78	40.02 ^a	37.90 ^{b2}	34.15	44.74 ^a	39.45 ^{b3}
	\pm SD	4.14	3.04	6.54	2.57	4.07	4.16	2.39	4.11	3.48	4.03	1.67	2.49
T receptor CD8+	\bar{x}	15.49	14.78	15.14	13.00	14.97	13.99	13.64	13.47	13.56	19.84 ^a	11.69	15.77
	\pm SD	2.14	3.29	2.50	1.16	2.67	1.39	0.70	2.75	1.12	5.25	4.57	3.26
T receptor CD25+	\bar{x}	12.07	12.34	12.20	14.86 ^a	10.83	12.85	12.06	12.24	12.15	11.69	13.23	12.46
	\pm SD	0.62	0.18	2.19	4.13	4.23	2.85	1.05	1.06	2.13	1.86	2.05	1.97

() – number of animals; \bar{x} – mean value; SD – standard deviation, ^a – statistically significant difference between males and females, ^b – statistically significant difference between seasons (together), where ^{b1} – statistically significant difference between spring and summer; ^{b2} – statistically significant difference between spring and autumn; ^{b3} – statistically significant difference between spring and winter; ^{b4} – statistically significant difference between summer and autumn; ^{b5} – statistically significant difference between summer and winter; ^{b6} – statistically significant difference between autumn and winter

The analysed samples were incubated for 45 minutes in ice, rinsed three times with Cell Wash (BD Biosciences, USA) by centrifugation at 200 xg. To such prepared cellular sediment, 10 µl of rabbit antibodies were added marked against mouse IgG with fluorescein isothiocyanate (FITC). After triple repetition of the rinsing procedure in Cell Wash, 2000 µl of lysing solution was added to samples to eliminate erythrocytes (BD FACS Lysing Solution, BD Biosciences, USA). After ten minutes of incubation in the dark, at room temperature, measurement was performed on FACScan flow cytometer by Becton Dickinson (USA) using FACSDiva software.

The results of the study on B- and T-cells and their subpopulations in percentage, as obtained from three blood draws from each rabbit (at 0, 24, 48 h), performed twice at the interval of seven days in each season of the year, are presented as average values and standard deviations in Table 3 and in Figs. 1-3, as previously subjected to statistical analysis using *t*' Student test at *p* = 0.05.

Results and discussion

When analysing the results obtained as regards the percentage of CD19+ B-cells and CD5+ T-cells, as well

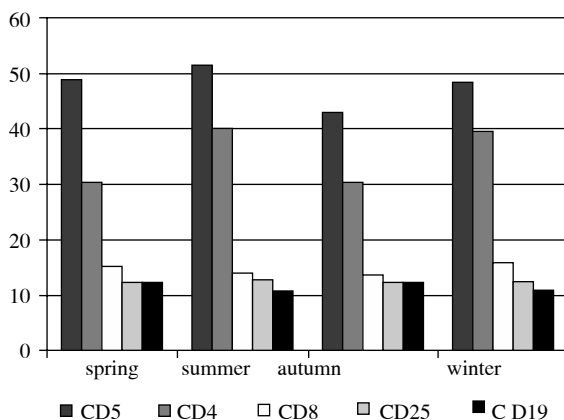


Fig. 1. Values of B and T lymphocytes in Polish mixed breed rabbits in seasons of the year (without considering the sex)

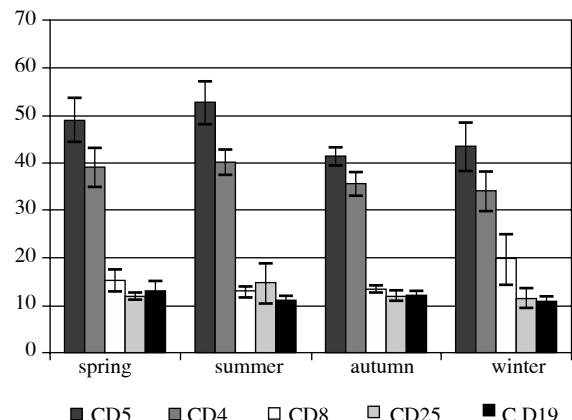


Fig. 2. Values of B and T lymphocytes in females of Polish mixed breed rabbits in seasons of the year

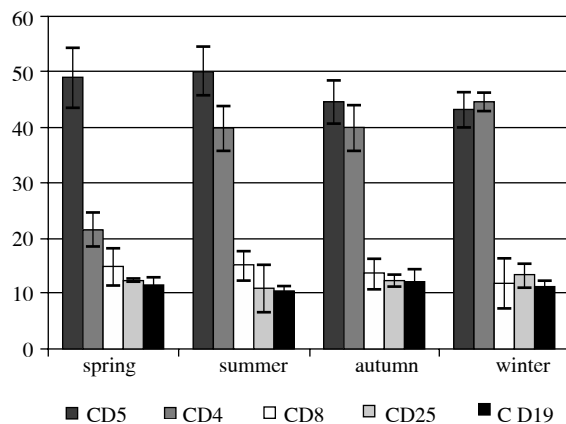


Fig. 3. Values of B and T lymphocytes in males Polish mixed breed rabbits in seasons of the year

as their subpopulations (CD4+ T-cells, CD8+ T-cells, and CD25+ T-cells) (Table 3), it must be stated that the values obtained in Polish mixed breed rabbits can only be compared to the results of studies obtained by Polish authors (Table 2), also carried out on mixed breed rabbits from Poland, which have been presented in the same units as in the present study. In turn, the results of foreign studies (Table 1) regarding such cells, performed on pureblood rabbits (Fauve de Bourgogne and New Zealand) and Spanish mixed breed rabbits, are presented in different units, which renders it impossible to compare them to the present results. When assessing the results in the area of particular blood cells analysed, it must be stated that the values for CD19+ B-cells remain within the range from 10.76 to 12.23% (Table 3), CD25+ T-cells: within the range from 12.15 to 12.85% (Table 3), and are similar to the results previously obtained in Polish mixed breed rabbits (Table 2), although it must be added that both in the case of CD19+ B-cells and CD25+ T-cells, in the latter studies (Table 2), also much lower values and much higher values (Table 2) than the present ones were recorded (Table 3). In turn, as regards CD5+ T-cells, their values in the present study ranged from 43.06 to 51.46%, CD4+ T-cells – from 30.33 to 40.01%, while CD8+ T-cells – from 13.56 to 15.77% (Table 3). The results obtained for CD5+ T-cells, CD4+ T-cells, and CD8+ T-cells are actually also correlated with the results obtained in the previous studies on Polish mixed breed rabbits (Table 2), but no such fluctuations were determined in them, as recorded with reference to CD19+ B-cells and CD25+ T-cells.

When analysing the results obtained for B- and T-cells and their subpopulations in Polish mixed breed rabbits, in the aspect of the impact of the season (Table 3, Figs. 1-3) and sex of the animals (Table 3, Figs. 2, 3), it must be stated that both the season and sex affect values of the parameters analysed.

Detailed analysis of the impact of the seasons on the analysed elements of peripheral blood in rabbits without considering the sex (Table 3) revealed that statistically significant differences between the values obtained in spring and summer refer to CD19+ B-cells and CD4+ T-cells; between spring and autumn – to CD5+ T-cells and CD4+ T-cells, and between spring and winter – to CD4+ T-cells. The assessment also evidenced that the differences between summer and autumn are recorded in the percentage of CD19+ B-cells and CD5+ T-cells, while differences between summer and winter – exclusively in the area of CD5+ T-cells, whereas differences between autumn and winter – as regards CD19+ B-cells and CD5+ T-cells. To recapitulate changes regarding the impact of the seasons on the analysed B- and T-cells and their subpopulations, it can be stated that the season most significantly affects the values of CD5+ T-cells, as for this parameter, four statistically significant changes were recorded; as well as CD19+ B-cells and CD4+ T-cells, where three statistically significant changes were recorded for each of these cells (Table 3). Whereas, when analysing the impact of the seasons on the factors analysed considering sex of the animals (Table 3), it was evidenced that the seasons affect males and females in a different way, as in females, statistically significant values were recorded in spring, summer and winter, while in males exclusively in autumn and winter, and these in females referred to CD4+ T-cells, CD25+ T-cells, and CD8+ T-cells, while in males – to CD4+ T-cells.

The assessment of the impact of sex of the animals on the percentage of B- and T-cells and their subpopulations in peripheral blood of Polish mixed breed rabbits (Table 3) evidenced that more statistically significant values are recorded in the factors analysed in females rather than males, as for females they were recorded in spring for CD4+ T-cells, summer for CD25+ T-cells, and winter for CD8+ T-cells, while in males they were only recorded in autumn and winter and exclusively for CD4+ T-cells. This would confirm observations recorded when assessing the impact of the seasons on the analysed factors in females and males, and points out to the fact that this physiological property (sex) most strongly affects CD4+ T-cells.

To conclude, it must be stated that the values of B- and T-cells and their subpopulations in peripheral blood in Polish mixed breed rabbits remain within the following ranges: for CD19+ B-cells: 10.76-12.23%, for CD5+ T-cells: 43.06-51.46%, CD4+ T-cells: 30.33-40.01%, CD8+ T-cells: 13.56-15.77%, whereas for CD25+ T-cells: 12.15-12.85%. Due to the large material which the study involved, the results should constitute reference values for such animals, the more so that they correlate to the values obtained in the previous studies also on mixed breed rabbits (Table 2). The present study also revealed that both the season of the year and sex of the rabbits affect the percentage of B- and T-cells and their subpopulations in peripheral blood. In the case of season of the year, it was observed that this factor principally affects the values of

CD5+ T-cells and CD19+ B-cells, as well as CD4+ T-cells, differently in males and females. In turn, in the case of sex of the animals, it was recorded that it affects the analysed immunological factors, causing more changes in females, but in both males and females these referred to CD4+ T-cells, whereas in females the changes took place in spring, summer and winter, while in males in autumn and winter.

Authors declare no conflict of interest.

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