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Original article

B-cell and T-cell values in peripheral blood in Polish mixed-breed rabbits with addition of blood of meet breeds

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Abstract

In Poland, rabbit is a highly valued animal, due to dietetic and flavour values of its meat, but above all, rabbits tend to be commonly used laboratory animals. The aim of the study was developing standards for counts of B-cells with CD19+ receptor, T-cells with CD5+ receptor, and their subpopulations, namely T-cells with CD4+, CD8+ and CD25+ receptor in the peripheral blood of mixed-breed Polish rabbits with addition of blood of meet breeds, including the assessment of the impact of four seasons of the year and animal sex on the values of the immunological parameters determined. The results showed that the counts of B- and T-cells and their subpopulations in peripheral blood remain within the following ranges: for CD19+ B-cells: 1.05 – 3.05%, for CD5+ T-cells: 34.00 – 43.07%, CD4+ T-cells: 23.52 – 33.23%, CD8+ T-cells: 12.55 – 17.30%, whereas for CD25+ T-cells: 0.72 – 2.81%. As it comes to the season of the year, it was observed that it principally affects the values of CD25+ T-cells, while in the case of rabbit sex, more changes were found in females.

Key words: rabbits, laboratory animals, standard values, B and T lymphocytes

Introduction

Rabbits are in Poland, apart from mice and rats, widely used for research (Anon 2013). In Poland, besides laboratory use, rabbits, especially mixed breed rabbits with addition of blood of meat breeds – usually Belgian Hare, are also breeding animals. These facts cause a need for developing reference blood values for those rabbits, including immunity factors, which are pivotal in diagnostic tests and scientific research. So far, studies on the number of B- and T-cells in peripheral blood have only been carried out in Poland on typical Polish mixed-breed rabbits (Table 1), while analogical foreign studies were performed on New Zealand and Fauve de Bourgogne rabbits, as well as LP and V genetic lines, and on Spanish mixed-breed rabbits (Table 2). Despite the fact that the studies were performed on a limited material, they are not determining reference values, and they were not assessed in the context of physiological and environmental conditions, which importance in rabbits was evidenced in the previous studies on haematologi-

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Table 1. The percentage of B and T cells and subpopulations in peripheral blood of rabbits – Polish data.

Reference	Age	Sex	Number of animals	Season	Breed	The percentage of B and T cells and subpopulations					
							B (receptor CD19+)	T (receptor CD5+)	T (receptor CD4+)	T (receptor CD8+)	T (receptor CD25+)
Deptuła and others 1998a	no data	male	20	no data	Polish mixed breed	$\bar{x}$	1.6	40.3	32.9	ns	0.75
						SD±	ns	ns	ns	ns	ns
Deptuła and others 2009	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
						SD±	1.75	1.42	2.54	1.52	1.54
Deptuła and others 2004a	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
						SD±	4.51	15.09	5.75	2.53	3.84
Deptuła and others 2004b	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
						SD±	4.51	15.09	5.75	2.53	3.84
Deptuła and others 1998b	no data	no data	60	no data	Polish mixed breed	$\bar{x}$	1.6	40.3	32.9	ns	0.75
						SD±	ns	ns	ns	ns	ns
Tokarz-Deptuła and Deptuła 2005	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
						SD±	4.51	15.09	5.75	2.53	3.84

Legend: ns – not studied; $\bar{x}$ – mean value, SD ± – standard deviation

Table 2. The percentage of B and T cells and subpopulations in peripheral blood of rabbits – abroad data.

Reference	Age	Sex	Number of animals	Season	Breed	The percentage of B and T cells and subpopulations				
						B (receptor CD19+)	T (receptor CD5+)	T (receptor CD4+)	T (receptor CD8+)	T (receptor CD25+)
Ferrian and others 2012	no data	female	65	no data	genetic lines LP and V	151,7 x 10^6/L	1306 x 10^6/L	734,2 x 10^6/L	328,6 x 10^6/L	38,77 x 10^6/L
Guerrero and others 2011	11-15 mth	female	22	no data	Spanish mixed breed	96 x 10^6/L	1191 x 10^6/L	543 x 10^6/L	216 x 10^6/L	14 x 10^6/L
Jeklova and others 2007	1-day and 2,4,6, 8-weeks	no data	28	no data	New Zealand	no data	ns	29.1%	10.9%	ns
Sabolovic and others 1977	no data	male	no data	no data	Fauve de Bourgogne	ns	6314/mm^3	ns	ns	ns

Legend: ns – not studied

Table 3. The 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		Values of the parameters											
		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+	$\bar{x}$	1.52	1.43	1.48	2.62	3.05	2.85^{b1b4b5}	1.06	1.38	1.25	1.05	1.77^{a}	1.30
	SD±	0.31	0.39	0.38	0.75	0.93	0.82	0.51	0.46	0.47	0.31	0.35	0.32
T receptor CD5+	$\bar{x}$	42.71	42.18	42.50^{b2}	40.60	37.36	38.89	35.58	34.00	34.70	40.15	43.07	41.67^{b6}
	SD±	7.38	5.23	6.46	7.72	6.39	6.77	7.58	7.73	7.65	7.60	5.42	6.65
T receptor CD4+	$\bar{x}$	33.23	27.36	30.89^{b2}	29.44	28.00	28.68	23.52	26.05	24.88	28.37	31.67	29.96
	SD±	6.13	6.97	6.39	6.83	5.30	6.09	4.18	4.93	4.68	5.59	4.23	5.21
T receptor CD8+	$\bar{x}$	12.91	12.55	12.77	12.62	13.88	13.28	15.63	12.57	13.25	16.34	17.30	16.82^{b3}
	SD±	2.88	2.96	2.93	2.53	2.70	2.64	2.48	2.65	2.47	2.76	2.41	2.56
T receptor CD25+	$\bar{x}$	2.55	2.70	2.8^{b1b1b2b3}	2.19^{a}	1.08	1.61^{b5}	1.72	1.67	1.69^{b6}	1.35^{a}	0.72	1.06
	SD±	0.45	0.41	0.44	0.33	0.22	0.29	0.15	0.32	0.26	0.20	0.13	0.17

Legend: ( ) – number of animals; $\bar{x}$ – mean value; SD – standard deviation, ^{a} – statistically significant difference between male and female, ^{b} – statistically significant difference between seasons (together), where ^{b1} – statistically significant difference betweenspring and summer; ^{b2} - statistically significant difference between spring and autumn; ^{b3} – statistically significant difference betweenspring and winter; ^{b4} – statistically significant difference between summer and autumn; ^{b5} – statistically significant difference summer and winter; ^{b6} – statistically significant difference between autumn and winter.

cal factors (Pinna Pintor and Grassini 1957, Fox and Baird 1970, Laird et al. 1970, Bortolotti et al. 1989, Jain 1994, Rohilla et al. 2000, Nowaczyk et al. 2004, Nowaczyk et al. 2005, Burnet et al. 2006, Chineke et al. 2006, Olayemi et al. 2007, Archetti et al. 2008, Black et al. 2009, Çetin et al. 2009, Jeklova et al. 2009, Poljičak-Milas et al. 2009, Abdel-Azeem et al. 2010, Martinec et al. 2012, Özkan et al. 2012, Yaqub et al. 2013). The observations carried out in Poland on typical Polish mixed-breed rabbits showed the impact of animal age (Deptuła et al. 1995) and season of the year (Nowaczyk et al. 2004, 2005), while foreign studies performed on New Zealand, Angora, Czech rabbits and Sylvilagus Bachmani Riparius breeds, as well as mixed-breed rabbits, not only evidenced the impact of the age (Laird et al. 1970, Bortolotti et al. 1989, Chineke et al. 2006, Olayemi and Nottidge 2007, Archetti et al. 2008, Jeklova et al. 2009, Yaqub et al. 2013), but also of the season of the year (Pinna Pintor and Grassini 1957, Black et al. 2009, Çetin et al. 2009, Abdel-Azeem et al. 2010, Yaqub et al. 2013), sex (Fox and Baird 1970, Burnett et al. 2006, Chineke et al. 2006, Black et al. 2009, Çetin et al. 2009, Poljičak-Milas et al. 2009, Abdel-Azeem et al. 2010, Özkan et al. 2012, Yaqub et al. 2013), and race (Jain 1994, Rohilla et al. 2000, Burnett et al. 2006, Chineke et al. 2006, Martinec et al. 2012, Yaqub et al. 2013).

The fact that in Poland mixed-breed rabbits with an addition of blood of meat breeds are commonly used as breeding or laboratory animals, has caused undertaking of research regarding developing standards for B-cells with CD19+ receptor, T-cells with CD5+ receptor, and their subpopulations, such as T-cells with CD4+, CD8+ and CD25+ receptor in peripheral blood of such animals, including the impact of four seasons of the year and animal sex.

Materials and Methods

The study involved 200 Polish mixed-breed rabbits with an addition of blood of meat breeds, originating from a licensed farm, remaining under continuous veterinary and zoo-technical supervision (Anon 1987), 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 zoo-technical parameters were in accordance with the recommended Polish standards and the European Union Directive as far as temperature, humidity, lighting and size of cages for animals are concerned (Anon 2006). After transportation to the Department vivarium, the animals underwent a two-week adaptation period. The animals were fed with all-mash rabbit feed (16% Królik z Motycza), at volume of 0.15-0.20 kg/day, and had unlimited access to water.

Blood drawing was carried out twice a season at seven days intervals. Blood for tests was drawn by inserting a port into 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. In the blood of rabbits the percentage of B-cells with CD19+ receptor, and T-cells with CD5+ receptor, as well as their subpopulations – T-cells with receptors CD4+, CD8+ and CD25+ was determined, according to the method described by Deptuła et al. (1998) using monoclonal antibodies (mouse anti-rabbit) (Serotec, USA). The analysed samples were incubated for 45 minutes on ice, rinsed three times with Cell Wash (BD Biosciences, USA) by centrifugation at 200 x g. To such prepared cellular sediment, 10 µl of rabbit antibodies against mouse IgG labelled 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 have been presented as average values and standard deviations in Table 3, as previously subjected to statistical analysis using Student t-test.

Results

The values for CD19+ B-cells were ranging of from 1.05 to 3.05%, while CD25+ T-cells from 0.72 to 2.81% (Table 3). Values for CD5+ T-cells were ranging from 34.00 to 43.07% (Table 3), CD4+ T-cells from 23.52 to 33.23%, while CD8+ T-cells from 12.55 to 17.30% (Table 3).

Detailed analysis of the impact of the seasons, without considering the sex of the animals (total for females and males) (Table 3), revealed that statistically significant differences between the values obtained in spring and summer refer to CD19+ B-cells and CD25+ T-cells; between spring and autumn – to CD5+ T-cells, CD4+ T-cells, and CD25+ T-cells, while between spring and winter – to CD8+ and CD25+ T-cells. Also statistically significant differences between summer and autumn were found for the percentage of CD19+ B-cells, while differences between summer and winter – for CD19+ B-cells and CD25+ T-cells, whereas differences between autumn and winter – for CD5+ and CD25+ T-cells. The analysis of the impact of the seasons considering sex of the animals (Table 3), showed that in females, statistically significant values were recorded in summer and winter, while in males exclusively in winter, and these in females referred to CD25+ T-cells, while in males – to CD19+ B-cells.

Discussion

The analysis of the results concerning the percentage of CD19+ B-cells and CD5+ T-cells, as well as their subpopulations (CD4+ T-cells, CD8+ T-cells, and CD25+ T-cells) (Table 3) can only be compared to the results of studies performed by Polish authors (Table 2), carried out on typical mixed-breed rabbits from Poland, as the results of foreign studies (Table 1), performed on pureblood rabbits (Fauve de Bourgogne and New Zealand), genetic lines and Spanish mixed-breed rabbits, are presented in different units.

When assessing the results for CD19+ B-cells, and CD25+ T-cells (Table 3) it may be stated that they are similar to the results previously obtained in typical Polish mixed-breed rabbits (Deptuła et al. 1998ab in Table 1), although much higher values were recorded in the previous studies (Deptuła et al. 2004ab, 2009, Tokarz-Deptuła and Deptuła 2005 in Table 1). The results for CD5+ T-cells, CD4+ T-cells and CD8+ T-cells (Table 3), concord the data obtained in typical Polish mixed-breed rabbits (Deptuła et al. 2004a,b, 2009, Tokarz-Deptuła and Deptuła 2005 in Table 1).

The values of parameters obtained for B- and T-cells and their subpopulations in Polish mixed breed rabbits with an addition of blood of meat breeds (Table 3), are influenced by both, the season and sex.

The season of the year most significantly affects the values for CD25+ T-cells, as for this parameter five statistically significant changes were recorded; as well as CD19+ B-cells, where three statistically significant changes were recorded, and to a lesser extent CD5+ T-cells (two changes), as well as CD4+ and CD8+ T-cells (one change for each) (Table 3).

Moreover, it was shown that the seasons affect males and females in a different way, as in females statistically significant values were recorded in summer and winter, while in males exclusively in winter, and these in females referred to CD25+ T-cells, while in males – to CD19+ B-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 with addition of blood of meat breeds remain within the following ranges: for CD19+ B-cells: 1.05 – 3.05%, for CD5+ T-cells: 34.00 – 43.07%, CD4+ T-cells: 23.52 – 33.23%, CD8+ T-cells: 12.55 – 17.30%, whereas for CD25+ T-cells: 0.72 – 2.81% (Table 3). It must be added that the results obtained on such a large number of animals generally used for laboratory tests and scientific research, should become reference values for such animals as this is the first such an extensive analysis of those parameters. The present study also

revealed that both, the season of the year and sex of the rabbits affect the percentage of analysed lymphocytes in peripheral blood. In the case of season of the year, it was observed that this factor principally affects the values for CD25+ T-cells in summer and winter, while in the case of sex, more changes were found in females.

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