Reference values of blood parameters in rams of the Bosnian Pramenka breed

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A B S T R A C T

Pramenka is a breed of sheep widely distributed on the Balkan Peninsula (over 30 strains). A number of factors affect hematological and biochemical parameters in sheep blood such as breed, age, sex, region, season and health. The aim of this paper is to determine reference ranges for hematological and selected biochemical parameters in the blood of Bosnian Pramenka rams. The experiment included 362 blood samples. The rams were healthy, and were raised under farm conditions in Banat (Serbia). The blood was collected by puncture of v. jugularis, in vacuum cleaners with a purple stopper (EDTA, for hematological analysis) and with a yellow stopper (for biochemical analysis). The reference intervals of the blood parameters in Bosnian Pramenka rams were: WBC 4.34–17.0×109/mL; RBC 4.34–17.0×1012/mL; HGB 72.73–119.8 g/L; HTC 21.97–36.77 %; MCV 26.55–34.23 fl; MCH 8.57–11.34 pg; MCHC 313.32–345.16 g/L; PLT 118–556.45 ×109/mL; TPROT 56.53–70.51 g/L; ALB 29.81–40.05 g/L; GLOB 22.13–35.05 g/L; UREA 2.25–9.57 mmol/L; TBIL 2.21–11.35 μmol/L; GLU 2.41–6.93 mmol/L; CHOL 0.93–1.91 mmol/L; AST 58–244 IU/L; BHB 0.18–0.54 mmol/L and LDH 172–455 IU/L. Normal frequency distribution was determined for RBC, HGB, HGB, and CHOL, while other parameters did not have a normal frequency distribution. A positive correlation was found between body weight and RBC and HGB values, and a negative correlation between body weight and WBC and BHB values. The obtained reference values will be helpful in interpreting the health and productive status of the Bosnian Pramenka population. Further studies should focus on ewes and lambs, as well as on the deviation of blood parameters in the case of various diseases.

Keywords: rams, hematology, biochemistry, Pramenka, reference value.

И З В О Д

Праменка је раса овaca која је најзаступљенија на Балканском полуострву (преко 30 сојева). Велики број фактора утиче на хематолошке и биохемијске параметре у крви овaca, као што су раса, старост, пол, регион, сезона и здравље. Циљ овог рада је да се утврде референтни опсези за хематолошке и одабране биохемијске параметре у крви овнова босанске праменке. У одг提升е је укључено 362 узора крви овнова босанске праменке. Опсези су били здрави, узгајани у фармским условима на територији Баната (Србија). Крв је узимана пункцијом в. jugularis, у вакууматорима са љубичастим чечем (EDTA, за потребе хематолошких анализа) и са жутим чечем (за биохемијске анализе). Утврђене су следеће референтне вредности параметара: РБК 4.34–17,0×109/мл; РКК 4,34–17,0×1012/мл; хгб 72,73–119,8 г/л; ХТС 21,97–36,77 %; МСВ 26,55–34,23 фл; МЧ 8,57–11,34 пг; МЧС 313,32–345,16 г/л; ПЛТ 118–556,45 ×109/мл; Пут 56,53–70,51 г/л; АЛБ 29,81–40,05 г/л; ГЛО 22,13–35,05 г/л; УРЕА 2,25–9,57 ммоль/л; ТБИЛ 2,21–11,35 μмоль/л; ГЛУ 2,41–6,93 ммоль/л; ЧОЛ 0,93–1,91 ммоль/л; АСТ 58–244 ЈУ/л; ББ 0,18–0,54 ммоль/л и ЛДХ 172–455 ЈУ/л. Нормална дистрибуција фракције утврђена је за РБК, ХГБ, ББ и ЧОЛ, док код осталих параметара није утврђена нормална дистрибуција фракције. Нађена је позитивна корелација између телесне масе и вредности РБК и ХГБ, а негативна корелација између телесне масе и вредности ВБ и ББ. Добијене референтне вредности могу бити од користи код интерпретације здравственог и продуктивног статуса популације овнова босанске праменке. У даљим испитивањима потребно је обрадити ове и јачајд као и одступање вредности крвних параметара код појаве различитих обележа.

Кључне речи: овнови, праменка, хематолошка, биохемија, референтне вредности.

1. Introduction

Pramenka is an indigenous sheep breed, the most prevalent on the Balkan Peninsula. This is a very hardy breed raised under extensive conditions and it has no major requirements in the field of nutrition and care (Savić et al., 2007). The effect of different geographical areas has led to the development of more than 30 strains of sheep, including Sjenička (Sjenica sheep), Svrliška (Svrljig sheep), Sarplaninska (Šar Mountain sheep), Ovčepolska (Makedonska) (Ovče Pole (Macedonian) sheep), Pirotka (Pirot sheep), Karakačanski-crni soj (Karakačan Black strain), Krivovirski soj (Kriv Vir strain), Kosovski soj (Kosovo strain), Bardoka (Bela Metohija sheep), Lipaska (Smederevska) (Lipe (Smederevo) sheep), Jezerska Piskva (Lake Piva sheep), Zetska Žuža (Zeta Yellow sheep), Dubeka (Vlaško-Travniki) (Dub sheep (Vlašić Travnik strain)), Vlaška Vitoroga (Corkscrew-horned Wallachian sheep), Kupreška (Kupres sheep) and Licka Pramenka (Lika Pramenka sheep) (Ivanov et al., 2005; Krajnović, 2006).
The determination of reference values in blood has multiple implications in science and the profession. According to international standards, each laboratory should determine its own reference ranges on a number of healthy, strictly controlled and uniform subjects, in order to compare the results with other laboratories and world literature, thereby also checking the quality of the laboratory (CLSI, 2008; Belić and Cincović, 2015, 2019). The reference values are important for differentiation between healthy and diseased individuals, and their comparisons establish similarities and differences in different species and strains of animals. For all these reasons, the Laboratory of Pathophysiology at the Department of Veterinary Medicine in Novi Sad examined reference ranges in different animal species (Cincović et al., 2011, 2019; Belić et al., 2011, 2019; Cincović et al., 2020; Lakić et al., 2020; Nikolić et al., 2020).

In sheep, breed can have a significant effect on the value of blood parameters. In addition, a significant influence of age or gender on different blood parameters has been found (Oramari et al., 2014). In small ruminants, there is metabolic adaptation to the negative energy balance, and therefore knowledge of these parameters and their reference ranges is of great importance (Mohammadi et al., 2016; Milošević-Stanković et al., 2020). The values of blood parameters in sheep can be affected by different infections (Amarante et al., 2004; Lipecka et al., 2010) as well as by heat stress (Wojtas et al., 2014). It has been shown that there is a difference between manual counting of blood cells and counting with an automatic analyzer (Ibrhim, 2014).

The aim of this study is to determine reference values for hematological and selected biochemical parameters in the blood of rams of the Bosnian Pramenka breed. The reference values of the blood parameters of Bosnian Pramenka rams were: WBC 4.34–17.0×10^9/mL; RBC 4.34–17.0×10^{12}/mL; HGB 72.73–119.8 g/L; HTC 21.97–36.77 %; MCV 26.55–34.23 fL; MCH 8.57–11.34 pg; MCHC 313.32–345.16 g/L; PLT 118–556.45×10^9/mL; TPROT 56.53–70.51 g/L; ALB 29.81–40.05 g/L; GLOB 22.13–35.05 g/L; UREA 2.25–9.57 mmol/L; TBIL 2.21–11.35 μmol/L; GLU 2.41–6.93 mmol/L; CHOL 0.93–1.91 mmol/L; AST 58–244 IU/L; BHB 0.18–0.54 mmol/L and LDH 172–455 IU/L (Table 1). Normal frequency distribution was determined for RBC, HCT, PLT, ALB, GLOB, and CHOL, while other parameters did not have a normal frequency distribution. The form of frequency distribution was determined using skewness and kurtosis parameters, and the normality of frequency distribution was determined using the Kolmogorov-Smirnov test and was presented graphically using Q-Q plots. Frequency distribution is presented in the form of histograms and polygons, and the polygon represents an ideal normal distribution. For the parameters for which a linear trend was determined between body weight (which depends directly on age) and biochemical parameters, a regression analysis was performed and the trend was presented graphically.

3. Results

The reference values of the blood parameters of Bosnian Pramenka rams were: WBC 4.34–17.0×10^9/mL; RBC 4.34–17.0×10^{12}/mL; HGB 72.73–119.8 g/L; HTC 21.97–36.77 %; MCV 26.55–34.23 fL; MCH 8.57–11.34 pg; MCHC 313.32–345.16 g/L; PLT 118–556.45×10^9/mL; TPROT 56.53–70.51 g/L; ALB 29.81–40.05 g/L; GLOB 22.13–35.05 g/L; UREA 2.25–9.57 mmol/L; TBIL 2.21–11.35 μmol/L; GLU 2.41–6.93 mmol/L; CHOL 0.93–1.91 mmol/L; AST 58–244 IU/L; BHB 0.18–0.54 mmol/L and LDH 172–455 IU/L (Table 1). Normal frequency distribution was determined for RBC, HCT, PLT, ALB, GLOB, and CHOL, while other parameters did not have a normal frequency distribution. The form of frequency distribution is shown in Figures 1–18, and the deviation of the observed values from the normal distribution is presented for each parameter in Figures 18–36. The values of individual parameters are linearly related to the body weight (age) of the rams. A positive correlation was found between body weight and RBC and HGB values, and a negative correlation between body weight and WBC and BHB values (Figures 37–40). The 95% CI for the population average has been within the known reference value so far, but because of the range of minimum and maximum population values, there was a need to establish a reference value for this population.
Table 1
Descriptive statistics and 95%CI for lower and upper reference values of blood parameters

| Parameter | Lower ref. value | 95%CI for lower ref. value | Higher ref. value | 95%CI for higher ref. value | Mean | SD | Skewness | Kurtosis | Normality |
|-----------|------------------|-----------------------------|-------------------|----------------------------|------|----|----------|----------|-----------|
| WBC       | 4.34             | 4.01–4.67                   | 17.00             | 16.7–17.3                  | 10.67| 3.17| 1.07     | 2.04     | No        |
| RBC       | 7.03             | 6.89–7.17                   | 12.31             | 12.1–12.4                  | 9.67 | 1.32| 0.07     | 0.03     | Yes       |
| HGB       | 72.73            | 71.4–73.8                   | 119.18            | 119–121                    | 95.95| 11.61| 0.61     | 0.71     | No        |
| HTC       | 21.97            | 21.6–22.4                   | 36.77             | 36.4–37.2                  | 29.37| 3.70| 0.50     | 0.72     | Yes       |
| MCV       | 8.57             | 8.5–8.64                    | 11.34             | 11.3–11.4                  | 9.95 | 0.69| 0.03     | 0.92     | No        |
| MCHC      | 311.32           | 310–312                     | 345.16            | 344–346                    | 328.24| 8.46| -0.54    | 0.35     | Yes       |
| PLT       | 118.00           | 107–129                     | 556.45            | 545–567                    | 337.22| 10.6 | 0.18     | -0.59    | Yes       |
| TPROT     | 56.53            | 56.2–56.9                   | 70.51             | 70.2–70.9                  | 63.52| 3.49| -0.14    | 0.83     | No        |
| ALB       | 29.81            | 29.5–30.1                   | 40.05             | 39.8–40.3                  | 34.93| 2.56| -0.22    | 0.37     | Yes       |
| GLOB      | 22.13            | 21.8–22.5                   | 35.05             | 34.7–35.4                  | 28.59| 3.23| 0.23     | 0.22     | Yes       |
| UREA      | 2.25             | 2.06–2.44                   | 9.57              | 9.38–9.76                  | 5.91 | 1.83| 0.00     | -0.67    | No        |
| TBIL      | 0.18             | 0.17–0.19                   | 0.54              | 0.53–0.55                  | 0.36 | 0.09| 1.21     | 1.47     | No        |
| CHOL      | 172.63           | 165–179                     | 455.49            | 448–462                    | 314.06| 70.72| 0.42     | -0.56    | No        |
Figures 1–18: Frequency distribution of ram blood parameters (the histogram shows the obtained values and the polygon shows normal frequency distribution)
Figures 19–36: Q-Q plot of the deviation of the obtained values of the tested parameters from the normal distribution

Figures 37–40: Relationship between body weight and blood parameters in rams (only significant relations are shown)
4. Discussion

Determining the form of frequency distribution helps in the appropriate statistical interpretation of the data obtained. If the distribution is not normal, logarithmic transformation may be used or non-parametric statistics may be used when using the median as a positional mean in the population estimate. Shek Vugrovečki et al. (2017) in the Lika Pramenka sheep determined normal distributions for RBC, HGB, MCV, MCH, UREA and TPROT and log normal or non-normal distributions for other hematology and biochemistry parameters. Our results show that a large number of parameters in the tested rams do not show a normal distribution. The use of smaller groups of sheep, in an appropriate statistical analysis, allows the determination of the reference interval without the transformation of raw data (Vojta et al., 2011), and the method was applied to the Dalmatian sheep.

Hematological parameters were compared with the results of the authors dealing with Pramenka or other local breeds, as well as with data from the internationally recognized literature. Shek Vugrovečki et al. (2017) found the following reference values for Lika Pramenka sheep: WBC 5.6–17 ×10^9/mL; RBC 6.6–9.9 ×10^12/mL; HGB 74–104 g/L; HTC 22–31%; MCV 28–35.8 fl; MCH 9.9–11.9 pg; MCHC 320–354 g/L; and PLT 72–807 ×10^9/mL. Šimpraga et al. (2013) found the method was applied to the Dalmatian sheep. Determining the form of frequency distribution without the sheep, in an appropriate statistical analysis, allows the number of parameters in the tested rams do not show a normal distribution for other hematology and biochemistry parameters. Our results show that a large number of normal distributions for other hematology and biochemistry parameters. The hematological parameters in the present study are consistent with the results of authors in Serbian region. Antunović et al. (2017) found the following reference values for TPROT: 66.7–81 g/L; ALB 28.5–44.7 g/L; UREA 3.5–7.8 mmol/L; GLU 2.9–4.3 mmol/L; AST 66.2–129.3 IU/L. Šimpraga et al. (2013) found the following reference values for the biochemical parameters: TPROT 66.8–87.4 g/L; ALB 28.5–44.7 g/L; UREA 3.5–7.8 mmol/L; GLU 2.9–4.3 mmol/L; AST 85–138 IU/L. Šimpraga et al. (2013) found the following results used in the world literature, which confirms the importance of determining reference ranges in our geographical area. The resulting reference values may be useful in interpreting the health and productive status of the Bosnian Pramenka population. Further studies should focus on ewes and lambs, as well as on the deviation of blood parameters in the case of various diseases.

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