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Hematological values of captive *Indotestudo travancorica* (Boulenger, 1907) and *Batagur kachuga* (Gray, 1931): a short communication

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ABSTRACT

The Indian subcontinent is home to many species of herpetological and conservation interest. The aim of this study was to provide a first assessment of hematological values in Travancore tortoises (*Indotestudo travancorica*) and red-crowned roofed turtles (*Batagur kachuga*), two endangered species on which medical literature is still lacking. In late spring, 19 blood specimens of healthy *I. travancorica* and 17 of *B. kachuga* were sampled. Both populations were housed at the Madras Crocodile Bank Trust – Centre for Herpetology (Tamil Nadu, India). From each animal, a blood sample was obtained from the dorsal coccygeal vein and stored in a lithium-heparin test tube. For both species, manual red and white blood cell counts (RBC and WBC) were performed using a Natt & Herrick stain in a Neubauer chamber. Only for *I. travancorica,* hematocrit values were assessed by centrifugation of capillary tubes, and used to calculate mean corpuscular volume; this investigation was not performed on *B. kachuga* due to equipment malfunction. Due to the small sample size, only descriptive statistics were applied, and the obtained values were compared with the known references of other chelonians. Only for *B. kachuga*, the composition of the sampled population made it possible to run cross sectional comparisons between adult and juvenile individuals, as well as between males and females. No difference was found between males and females, but a significant difference ($P<0,05$) was found for WBC counts between juvenile and adult animals.

Key words: *Batagur kachuga;* hematology; *Indotestudo travancorica;* Red-Crowned Roofed Turtle; Travancore tortoise

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Introduction

Hematological techniques are commonly used in veterinary medicine to assess health status, to monitor the progression of a disease, and to give a prognosis for wild and captive animals, and chelonians are no exception (NARDINI et al., 2013; PENDL, 2015; PERPINAN, 2015). However, hematology in turtles and tortoises is still not as developed compared to domestic mammals or birds. Great variability and subjectivity are expected, due to the different physiological and environmental conditions, and individual adaptations to them. For these reasons, determining reference intervals in these species poses serious challenges (NARDINI et al., 2013; PENDL, 2015), also considering the relatively low number of animals available to be sampled. Moreover, due to the particular features of the erythrocytes in reptiles, and despite technological progress (some impedance counters have been tested to determine red blood cell count, packed cell volume and hemoglobin, but these methods still need validation) (BIELLI et al., 2015; BIELLI, 2017), the evaluation of cell number and morphology still cannot be reliably realized by automated procedures, so that they have to be performed manually by practitioners using specialized tools (TAVARES-DIAS et al., 2008; PERPINAN, 2015). In the present study two rare and endangered turtle species, which both lack medical and physiological studies or reports except for taxonomic end genetic reviews, (FRITZ and HAVAS, 2007; ROHILLA and TIWARI, 2008) are investigated.

The Travancore tortoise (*Indotestudo travancorica*, Family: Testudinidae - Boulenger, 1907) is a medium sized tortoise (body weight up to 4 kg) endemic in southern India, in the States of Karnataka, Kerala and Tamil Nadu, where it inhabits deciduous and evergreen forests up to 850 m elevation, and it is mainly active in late afternoon/evening hours (DEEPAK et al., 2011; DEEPAK and VASUDEVAN, 2014). It is mainly herbivorous and feeds on grasses, herbs, and bamboo blades, but it occasionally integrates mollusks, insects, carcasses, fruit and fungi into its diet. Since these tortoises inhabit tropical monsoon climate zone (PEEL et al., 2007), they do not hibernate, but they can become inactive during the dry season if temperatures spike above 40 °C (DEEPAK et al., 2011). Their mating season is usually from November to March, and females produce 1-5 eggs per clutch. The International Union for Conservation of Nature (IUCN) classifies the Travancore tortoise as "Vulnerable", but no data regarding its population status are available. Major threats for this species are hunting for pets, meat and the traditional medicine market, along with habitat loss and fragmentation (DEEPAK et al., 2011).

The Red-Crowned Roofed Turtle (*Batagur kachuga,* Family: Geoemydidae – Gray, 1831) is a herbivorous, non-hibernating freshwater turtle that was historically widespread in Central Nepal, Northeast India, Bangladesh and North-West Burma, which are classified as monsoon-influenced humid subtropical climate zones (PEEL et al., 2007; PRASCHAG et al., 2019). It dwells primarily in deep flowing rivers with terrestrial nest sites. Like other *Batagur* species, it shows strong sexual dimorphism (females can grow up to 25 kg, while males are considerably smaller), especially during breeding season (January to April), when the head of the male becomes blue and red. With a population concentrated in the watershed of the Ganges River, and an estimated decrease of 80% in the last 50 years, this species is considered the most threatened freshwater turtle in India and is classified by the IUCN as "Critically Endangered" (PRASCHAG et al., 2019). Major threats are poaching for its meat and shells, accidental drowning in fishing gear, water pollution and habitat loss (WHITAKER, 2009).

So far, neither of these species has been included in any international breeding and conservation program, but local projects are being carried on by the Madras Crocodile Bank Trust – Centre for Herpetology (Tamil Nadu, India), which hosts several breeding groups of *B. kachuga* and the only captive breeding group of *I. travancorica* (WHITAKER, 2009; DEEPAK et al., 2011).

In order to contribute to the medical literature and to provide useful data for endangered species conservation, this study aims to provide a first assessment of the hematological values of *I. travancorica* and *B. kachuga*.

Materials and methods

Nineteen adult (>15-year-old, 17 females and 2 males) *I. travancorica* were sampled, with a mean body weight (BW) of 2.63±0.83 kg standard deviation (SD), and mean plastron length (PL) of 19.24±1.8 cm SD. Their health status was assessed by clinical and coprological examination (direct smear, floatation and sedimentation). The animals were housed together outdoors in a 124 m^2 enclosure, with natural substrate, plants and free access to water; their diet included hay, fresh vegetables depending on the season (they were fed early in the morning every day) and spontaneous vegetation. During the sampling season (May-June 2017), day temperatures ranged between 32 and 42°C, (28-32°C by night) with little to absent precipitation. For each animal, 0.5 mL of blood was collected via venipuncture of the dorsal coccygeal vein using a 1 mL syringe with a 26 G needle. Sampling was performed 2-3 hours after feeding. After collection, the blood was transferred to a lithium-heparin test tube and stored at 10°C; total blood cell count was performed using the Natt & Herrick method (CAMPBELL, 2015a; BIELLI, 2017) a few hours after sampling. Packed Cell Volume (PCV) was evaluated by centrifugation of a microhematocrit tube at 15000 RCF for 5 minutes (NARDINI et al., 2013; BIELLI et al., 2015). Mean Corpuscular Volume (MCV) was calculated by applying the standard formulae (MCV = PCV x 10/ RBC).

Regarding *B. kachuga,* two small populations were sampled. The first consisted of 8 juvenile specimens (10-year-old clutch; 5 males and 4 females, mean BW 0.66 ± 0.16 kg, mean PL 14.67 ± 1.07 cm) housed together with 15 young gharials in an 18.1 m^2 pool; their diet included fresh vegetables and thawed freshwater whole fish, that they scavenged from the gharials' leftovers. The second population consisted of 9 adult animals (>20-year-old; males with mean BW 2.4±0.34 kg, mean PL 24.3±1.13 cm, and 5 females with mean BW 18.5±2.68 kg, mean PL 44.52±3.06 cm), housed in a 398.5 m^2 pool with 3 adult gharials; their diet was the same as the first population. Both populations were fed early in the morning every day. Sampling, processing, and performance of blood cell count were the same as recorded for the Travancore tortoises, but PCV and MCV could not be calculated due to equipment malfunction.

According to the American Society for Veterinary Clinical Pathology guidelines, the minimum sample size to determinate *de novo* reference intervals in veterinary species is 20 animals (FRIEDRICHS et al., 2012); for this reason, only descriptive statistics were applied. Statistical analysis was performed using common commercial software (IBM SPSS statistics for Windows, Ver. 26.0). The Tukey method for outlier identification was employed. A Student t test was applied to identify statistically significant (P>0.05) differences between the genders and the ages of *B. kachuga*. This investigation was not performed on *I. travancorica* since the population consisted of 17 females and 2 males, all adults.

Results

All animals were found to be healthy at clinical and parasitological examination. According to the Shapiro-Wilk test, RBC, WBC and PCV from *I. travancorica* were non-normally distributed, while normal distribution was observed for the *I. travancorica* MCV (Fig. 1 and 2). Concerning *B. kachuga,* RBC and WBC were normally distributed (Fig. 3 and 4). Results regarding *I. travancorica* RBC, WBC, PCV and MCV, and *B. kachuga* RBC and WBC are available in Tables 1 and 2. In *B. kachuga*, no gender-associated difference was evident for total RBC (mean values ± standard deviation: 0.78 X 10^6/µL±0.34 in males; 0.66 X 10^6/µL±0.36 in females; P=0.5) and WBC counts (11.6 X 10^3/µL±5.2 in males; 6.87 X 10^3/µL±3.94 in females; P=0.07) (Fig. 5). Regarding age, no difference was found for RBC count between juveniles and adults (0.7 X 10^6/µL±0.43 in juveniles; 0.72 X 10^6/µL±0.25 in adults; P=0.93), while WBC count in adults was significantly higher than in juveniles (6.3 X 10^3/µL±2.63 in juveniles; 12.25 X 10^3/µL±5.36 in adults; P=0.02) (Fig.6).

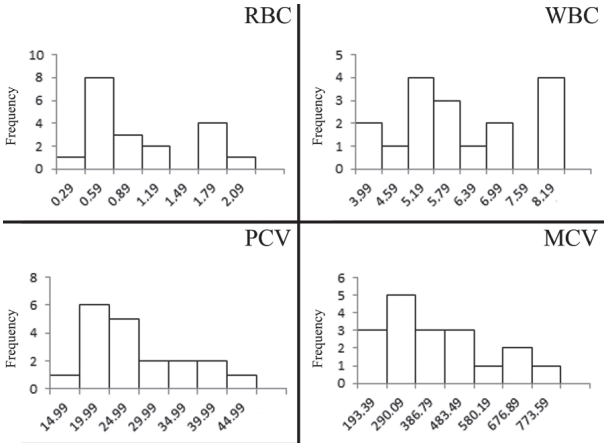

Fig. 1. Histograms depicting the distribution of hematological values measured from a sample of 19 clinically healthy Travancore tortoises (Indotestudo travancorica)

RBC = red blood cell count (106/μL); WBC = white blood cell count (103/μL); PCV = packed cell volume (%); MCV = mean corpuscular volume (fL)

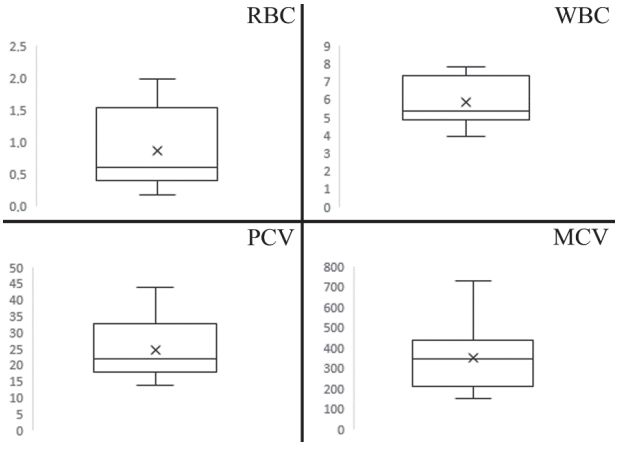

Fig. 2. Box-and-whisker showing red blood cell (RBC) count (106/μL), white blood cell (WBC) count (103/μL) and packed cell volume (PCV, %), and mean corpuscular volume (MCV, fL) from a sample of 19 clinically healthy Travancore tortoises (Indotestudo travancorica)

The boxes represent the values from the first to the third quartile (25–75th percentile). The horizontal line in each box represents the median. The whiskers include values 1.5 times the interquartile range.

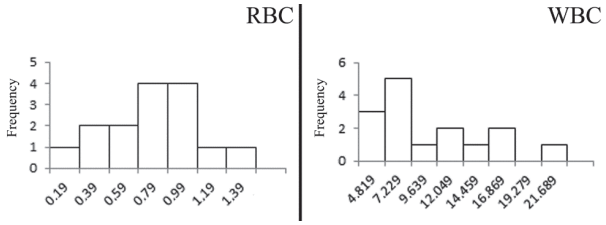

Fig. 3. Histograms depicting the distribution of hematological values measured in a sample of 17 clinically healthy Red-crowned roof turtles (Batagur kachuga)

RBC = red blood cell count (106/μL); WBC = white blood cell count (103/μL)

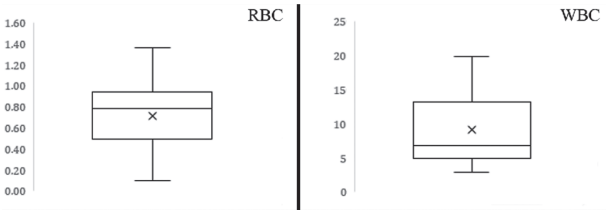

Fig. 4. Box-and-whisker showing red blood cell (RBC) count (106/μL) and white blood cell (WBC) count (103/μL) from a sample of 17 clinically healthy Red-crowned roofed turtles (Batagur kachuga)

The boxes represent the values from the first to the third quartile (25–75th percentile). The horizontal line in each box represents the median. The whiskers include values 1.5 times the interquartile range

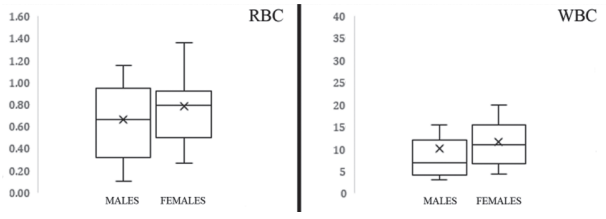

Fig. 5. Box-and-whisker showing the differences in red blood cell (RBC) count (106/μL) and white blood cell (WBC) count (103/μL) between female and male Red-crowned roofed turtles (Batagur kachuga)

The boxes represent the values from the first to the third quartile (25–75th percentile). The horizontal line in each box represents the median. The whiskers include values 1.5 times the interquartile range.

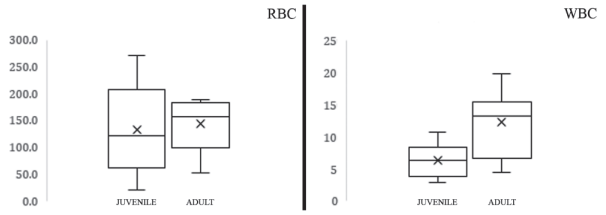

Fig. 6. Box-and-whisker showing the differences in red blood cell (RBC) count (106/µL) and white blood cell (WBC) count (103/µL) between juvenile and adult Red-crowned roofed turtles (Batagur kachuga)

The boxes represent the values from the first to the third quartile (25–75th percentile). The horizontal line in each box represents the median. The whiskers include values 1.5 times the interquartile range.

Discussion

Values of RBC for both *I. travancorica* and *B. kachuga* displayed high variability, possibly related to the low number of animals sampled and to the inherent variability of manual methods (WINTER et al., 2019). Since no clinical alterations were recorded, the presence of anemic conditions associated with the lowest values was unlikely In *I. travancorica*, a relative degree of dehydration due to high seasonal temperatures may be associated with higher values, although no associated clinical signs (e.g. enophtalmos, mucosal dryness, stypsis) were observed, and similarly high values were reported for other reptile and chelonian species (MARKS and CITINO, 1990; CAMPBELL, 2015b). The same trend was also observed for all the other erythroid variables, for the same reason. Determination of plasma protein concentration would help to confirm a different hydration status in animals with extreme RBC values; unfortunately, this was not possible because of the lack of dedicated equipment. Concerning WBC, *I. travancorica* showed a narrower range compared to *B. kachuga* in accordance with what was observed in other mammals, birds or reptiles. The wider WBC count range observed in *B.kachuga,* without any evidence of disease, may reflect a stressful condition (e.g. the sampling procedure), which is known to cause neutrophilia (heterophilia in reptiles and birds) in many animal species (SMITH, 2000). Similar results were previously recorded in similar turtles such as *Mauremys sinensis*, suggesting that the variability of the leukocyte count may be a hematological hallmark for these animals.

Regarding *I. travancorica*, the obtained values were compared with those of the Indian star tortoise (*Geochelone elegans*) (KLAPHLAKE et al., 2018), a species with similar biology that shares the same habitat, and with those of the Hermann's tortoise (*Testudo hermanni*) (BIELLI et al., 2015), which is taxonomically closer (PARHAM et al., 2006) (Table 1). Since no raw data regarding the other species were available, no statistical test was performed to establish significant differences. Compared with *G. elegans*, *I. travancorica* showed a wider RBC range, a narrower WBC range, a lower MCV median value and a wider MCV range. Compared with *T. hermanni boettgeri*, *I. travancorica* showed wider RBC, PCV and MCV ranges and a similar WBC range, lower RBC and WBC median values and similar PCV and MCV median values. This may suggest that specific ranges for *I. travancorica* are needed in order to categorize an animal better as healthy or diseased on the basis of hematological results.

Table 1. Descriptive statistics for hematological parameters in $n = 19$ healthy specimens of Travancore tortoise (*Indotestudo travancorica*) obtained by a manual method, and comparison with reference values for the Indian star tortoise (*Geochelone elegans*) and Hermann's tortoise (*Testudo hermanni boettgeri*) reported in the literature

Analyte	*I. travancorica*	*G. elegans* (Klaphlake et al, 2018)	*T.hermanni* (Bielli et al, 2015)
RBC Median (SD) - 10^6/µL	0.59 (±0.56)	0.37	0.8 - 0.15)
RBC Range - 10^6/µL	0.16 - 1.97	0.240-0.55	0.42 – 1.01
WBC Median (SD) -10^3/µL	5.38 (±1.39)	6.71	9.4 (±2.9)

Table 1. Descriptive statistics for hematological parameters in *n* = 19 healthy specimens of Travancore tortoise (*Indotestudo travancorica*) obtained by a manual method, and comparison with reference values for the Indian star tortoise (*Geochelone elegans*) and Hermann's tortoise (*Testudo hermanni boettgeri*) reported in the literature (continued)

Analyte	*I. travancorica*	*G. elegans* (Klaphlake et al, 2018)	*T.hermanni* (Bielli et al, 2015)
WBC Range - 10^3/μL	3.91 - 7.82	1.35 – 27.9	4.1 – 14.0
PCV Median (SD) - %	22 (±8.73)	23	24 (±5.9)
PCV Range - %	13.7 - 43.8	14-38	11 - 32
MCV Median (SD) - fL	345.18 (±156.79)	621.62	310 (±34.80)
MCV Range - fL	148.27 - 728.72	583.30 – 690.90	193 – 350

RBC = red blood cells; WBC = white blood cells; PVC = packed cell volume; MCV = mean corpuscular volume; SD = standard deviation

The values from *B. kachuga* were compared with those from the Chinese stripe-necked turtle (*Mauremys sinensis*) (CHUNG et al., 2009) (Table 2), a species of the same family (Geoemydidae) with similar habitat and some biological features in common (diet, aquatic behavior, lack of hibernation). This time, the median RBC and WBC ranges were similar, while *B. kachuga* showed a wider RBC range.

Table 2. Descriptive statistics for hematological parameters in *n* = 17 healthy specimens of Red-crowned roofed turtle (*Batagur kachuga*) obtained by a manual method, and comparison with reference values for the Chinese stripe-necked turtle (*Mauremys sinensis*) reported in the literature

Analyte	B. kachuga	*M. sinensis* (Chung et al., 2009)
RBC Median (SD) - 10^6/μL	0.78 (±0.34)	0.48
RBC Range - 10^6/μL	0.10 - 1.36	0.31 – 0.67
WBC Median (SD) - 10^3/μL	6.84 (±5.03)	11
WBC Range - 10^3/μL	2.93 - 19.80	4.21 – 19.63

RBC = red blood cells; WBC = white blood cells; SD= Standard Deviation

As previously stated, chelonians display a great variability in hematological parameters. Both RBC and WBC (and the correlated parameters) are influenced by sex, season, environmental conditions, alimentation, and physiological conditions (DOGUY, 1970; NARDINI et al., 2013; CAMPBELL, 2015b). In this study, in *B. kachuga* no statistical sex-related difference was noted in RBC, nor in WCB counts. Differences in RBC counts between males and females are commonly reported in reptiles, as well as in other species (PENDL, 2015). Among chelonians, sex-related differences in RBC, PCV and hemoglobin have been previously reported for *Astrochelys radiata, Elseya novaeguineae, Aldabrachelys gigantea*, *Gopherus agassizii*, *M. sinensis*, *Terrapene carolina* and *T. hermanni boettgeri* (HART et al., 1991; ANDERSON et al., 1997; DICKINSON et al., 2002; ZAIAS et al., 2008; CHUNG et al., 2009; BIELLI et al., 2015). Sex-based differences have not been found in *Chelonia mydas* (CAMPBELL, 2015b). Regarding WBC, gender-related differences have been reported in crocodiles (STACEY and WHITAKER, 2000). The finding of a sex-based difference in selected parameters may have clinical relevance, and suggests the need for sex-specific reference intervals. Unfortunately, our results are based on a small sized population, thus the possibility of further partitioning of data was not recommended.

Unlike for sex-based difference, an age-related difference was found in *B. katchuga* in our study, where the adult animals had a significantly higher WBC count than the juveniles (Fig. 6). Age-related differences in white and red blood cell parameters are not reported in chelonians, but RBC and lymphocytes were seen to be higher in adult and young marsh crocodiles, respectively (STACEY and WHITAKER, 2000). However, without further data, the difference found in our study cannot be attributed to age.

Conclusions

The present work provides a preliminary assessment of the hematological values of two species of rare and endangered chelonians. The differences found, when compared to other similar chelonian species, suggest that specific ranges are needed in order to categorize animals better as healthy or diseased on the basis of hematological results. Further studies are necessary for a wider sample size, to determine hypothetical seasonal changes, and to investigate other hematological parameters, such as differential white blood cell count, hemoglobin, erythrocyte indices and plasma biochemistry. Nevertheless, the results reported here may serve as a biological dataset useful to compare further data collected from healthy animals of the same species, but especially to identify diseased animals.

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E. BARDI, E. LUBIAN, S. ROMUSSI, N. WHITAKER, A. GIORDANO: Hematološke vrijednosti kornjače Travancore (lat. *Indotestudo travancorica*, Boulenger, 1907.) i crvenokrunaste krovne kornjače (*Batagur kachuga*, Gray, 1931.) u zatočeništvu: kratko priopćenje. Vet. arhiv 93, 591-600 2023.

SAŽETAK

Na indijskom potkontinentu žive mnoge vrste kornjača koje su u središtu zanimanja herpetoloških istraživanja i nastojanja za njihovim očuvanjem. Cilj je ovog rada bio iznijeti prve podatke o hematološkim vrijednostima u kornjače Travancore (*Indotestudo travancorica*) i crvenokrunaste krovne kornjače (*Batagur kachuga*), dvjema ugroženim vrstama o kojima još uvijek u biomedicinskoj literaturi nema dovoljno podataka. Pretkraj proljetne sezone pretraženo je 19 uzoraka krvi zdravih kornjača Travancore i 17 uzoraka crvenokrunaste krovne kornjače. Kornjače uključene u istraživanje su bile smještene u herpetološkom centru Madras Crocodile Bank Trust (Tamil Nadu, Indija). Od svake je životinje uzet uzorak krvi iz dorzalne kokcigealne vene te je pohranjen u epruvetu s litijevim heparinom. U obje je vrste brojenje eritrocita i leukocita (RBC i WBC) provedeno bojenjem Natt and Herrick u komori Neubauer. Razlika je bila samo u tome što su za kornjaču Travancore vrijednosti hematokrita procijenjene centrifugiranjem u kapilarnim cijevima i upotrijebljene za izračun prosječnog volumena korpuskula, dok u crvenokrunaste krovne kornjače ta metoda nije provedena zbog kvara opreme. S obzirom na mali broj uzorka primijenjena je samo opisna statistika te su dobivene vrijednosti uspoređene s poznatim vrijednostima drugih kornjača. Za crvenokrunastu krovnu kornjaču mogla se provesti transverzalna usporedba odraslih i mladih jedinki te mužjaka i ženki, pri čemu nije pronađena razlika između mužjaka i ženki, ali je pronađena znakovita razlika ($P<0,05$) u broju leukocita između mladih i odraslih jedinki.

Ključne riječi: crvenokrunasta krovna kornjača; hematologija; kornjača Travancore