ACTA VETERINARIA INDONESIANA
P-ISSN 2337-3202, E-ISSN 2337-4373

Special Issues: 127-131, May 2021

Research

Blood Biochemistry Reference Values of Javan Slow Loris (*Nycticebus javanicus*) in Rehabilitation Center

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Submitted April 15, 2021 Accepted May 16, 2021

ABSTRACT

The Javan slow loris (*Nycticebus javanicus*) is an endemic primate species to Java Island, Indonesia. Currently, their conservation status is critically endangered due to habitat loss and the illegal wildlife trade. As a consequence of the pet trade, wild-caught slow lorises are confiscated or handed over to centers like IAR Indonesia Rescue Center. Rescued lorises present multiple health issues following stress, trauma, and miss-treatment after being kept as pets. During the medical evaluation, besides physical examination, blood biochemistry provides valuable diagnostic information. However, data on physiological values are unavailable and therefore interpretation of results is difficult. The objective of this study was to establish blood biochemistry reference values for wild, rehabilitant healthy adult Javan slow lorises in captivity. We anesthetized 20 individuals of Javan slow loris (10 males and 10 females) for pre-release check-up procedures. Blood samples were collected for blood biochemistry analysis on an in-house Vetscan VS2 (Zoetis), after which the results were statistically analyzed for mean and standard deviation. Results showed different values between the male and female group, however, they were not significant (p>0,05). Comparison with available biochemistry data (ZIMS) for other loris species in captivity: *Nycticebus pygmaeus* and *Nycticebus coucang,* did not show significant differences. Although the sample size of this study was limited, this study provides the first preliminary reference ranges for healthy adult wild, rehabilitant Javan slow loris in captivity. Further data collection is necessary for more accurate ranges and will be done during the future pre-release health check.

Keyword: blood, analysis, biochemistry, javan slow loris, rehabilitation center

ABSTRAK

Kukang jawa (*Nycticebus javanicus*) merupakan spesies primata endemik Pulau Jawa, Indonesia. Hilangnya habitat dan perdagangan satwa liar ilegal menyebabkan turunnya populasi kukang jawa hingga mencapai status *critically endangered* (terancam punah). Teknik biokimia darah memberikan informasi diagnostik. Akan tetapi, data tentang nilai fisiologis tidak tersedia, sehingga interpretasi hasil menjadi sulit. Tujuan dari penelitian ini adalah untuk mengetahui nilai referensi biokimia darah kukang jawa dewasa sehat yang berada di dalam penangkaran. Penelitian dilakukan dengan membius 20 ekor kukang jawa (10 jantan dan 10 betina) untuk prosedur pemeriksaan pra-pelepasliaran. Sampel darah dikumpulkan untuk analisis biokimia darah dengan menggunakan mesin Vetscan VS2 (Zoetis), setelah itu hasilnya dianalisis secara statistik deskriptif dan statistik non parametrik untuk memperoleh nilai mean dan deviasi standar. Hasil penelitian menunjukkan nilai yang berbeda antara kelompok jantan dan betina, namun tidak signifikan (p> 0,05). Perbandingan dengan data biokimia yang tersedia (ZIMS) untuk spesies kukang lain di penangkaran, seperti *Nycticebus pygmaeus* dan *Nycticebus coucang* tidak menunjukkan perbedaan yang signifikan. Meskipun jumlah sampel penelitian ini terbatas, penelitian ini memberikan rentang referensi biokimia darah pada kukang jawa liar dewasa yang sehat di penangkaran.

Kata kunci: darah, analisis, biokimia, kukang jawa, pusat rehabilitasi

INTRODUCTION

The Javan slow loris (*Nycticebus javanicus*) is a small nocturnal primate, endemic to the island of Java, Indonesia. Listed as Critically Endangered according to IUCN's Red List of Threatened Species, its main threats are habitat loss and capture for the illegal pet trade (Nekaris *et al*. 2020). While, all Indonesian slow loris species are afforded protection under Indonesian law (KLHK 2018), the cute appearance of the Javan slow loris – with its panda-like face, big eyes and weighing only around one kilogram – makes them very desirable pets and consequently, a prime target for illegal traders (Rode-Margono *et al*. 2014). When law enforcement action is taken to mitigate the illegal trade, wild-caught slow lorises are confiscated or handed over to rescue centers like the one run by Yayasan IAR Indonesia (YIARI). Established in 2008, YIARI has rescued hundreds of slow lorises from trade with the aim of returning them to the wild (Moore *et al*. 2015).

On arrival at the YIARI Rescue Centre, rescued slow lorises often exhibit various health problems owing to the stress, trauma, and/or mistreatment while being traded and kept as pets. In fact, dental infections and metabolic problems, which are a consequence of unsuitable care, are among the main causes of morbidity and mortality in slow lorises at rescue centers (Nekaris *et al*. 2009).

A medical evaluation is an important tool for assessing the condition of rescued animals on arrival at the center, for evaluating and monitoring their health status during the rehabilitation period, and finally, prior to their translocation back to the wild. Accordingly, the IUCN Guidelines for Nonhuman Primate Re-introductions recommend that all primates undergo medical assessments and disease screening before release (IUCN, 2013).

During a medical evaluation, in addition to the standard physical examination, a blood biochemistry analysis can also provide valuable diagnostic information. Blood biochemistry is an important indicator of the physical wellbeing of non-human primates (Wu *et al.* 2014), as it provides information on vital organ systems such as in the liver and the kidneys (Villiers and Ristic 2016). Until now, such physiological values for Javan slow lorises have only been documented once before in a study that featured a single individual (Wirdateti et al. 2018); thus, making the interpretation of any future biochemical test results difficult, as there is no standard range for comparison. Our study was the first attempt to establish a range of blood biochemistry reference values for Javan slow lorises. We also compared these reference values with data available on other slow lorises species.

MATERIAL AND METHODS

Animals and Study Site

The study was conducted at IAR Indonesia Rescue Center in Bogor, West Java, Indonesia in March 2019. Twenty healthy adult wild-caught (10 males and 10 females) *N. javanicus* that were undergoing rehabilitation were chosen. Only individuals that did not present any sign of illness were selected. All individuals were anesthetized with zolazepam-tiletamine (Zoletil® 100, Virbac, France; 5 mg/kg BW) for the routine pre-release medical examination, which included a physical examination, body weight and morphometric measurements, radiographs, and blood sampling.

Blood samples and preparation

Blood samples were collected from the cephalic vein for approximately 0,3 ml and stored in a Lithium-heparin tube.

Blood biochemistry analyses

Blood biochemistry analyses were conducted in an in-house VetScan VS2 Chemistry Analyzer (© 2002, Zoetis, Inc., Union City, CA, United States) using the VetScan Comprehensive Diagnostic Profile rotor. The analysis included Albumin, Alkaline Phosphatase, Alanine Aminotransferase, Amylase, Total Bilirubin, Blood Urea Nitrogen, Calcium, Phosphor, Creatinine, Glucose, Sodium, Potassium, Total Protein, and Globulin.

Interspecies comparison

We collected biochemistry reference values of other available slow lorises species (*N. pygmaeus* and *N. coucang*) from a database called Zoo Information Management System (ZIMS) and compared these values to the reference intervals obtained from Javan slow lorises in this current study. The ZIMS database is an online resource that contains data on husbandry, enrichment, medical care, welfare, reproduction, population management, and biodiversity collected from aquaria, zoos, universities, researchers, or governmental members (Species360 2020).

Statistical analyses

Data obtained for each sex group were analyzed. Mean and standard deviation values were calculated using Microsoft Excel. For comparisons between sex groups, the non-parametric Mann-Whitney U test was used. Reference values of the results were made by calculating reference interval with 95% confidence interval (mean ± 1.96SD)

RESULTS AND DISCUSSION

Results of the blood biochemistry analysis from 20 *Nycticebus javanicus* (10 males and 10 females) can be observed in Table 1. All values for both sex groups were summed up and calculated for reference intervals with a 95% confidence interval. Some biochemistry values obtained from females were observed to be higher than in males (amylase, alanine aminotransferase, blood urea nitrogen, calcium, creatinine, and sodium), while some values in males were higher than in females (alkaline phosphatase, phosphor, total protein, globulin, potassium, and glucose). Nevertheless, none of these differences were statistically significant.

Some studies of non-human primates have revealed significant differences in blood biochemistry values between different sexes and ages; this is the case in long-tailed macaques, Tibetan macaques and grey mouse lemurs (Wu *et al.* 2014, Xie *et al.* 2013, Marchal *et al. 2012*). By contrast, no significant differences in the effects of sex on blood biochemistry values were found during this study, which is more consistent with the findings from study of capuchin monkeys (Monteiro *et al.* 2016). As all individuals selected for this study were adults, no age difference could be assessed.

While reliable blood biochemistry references for other species of slow lorises (such as *N. pygmaeus* and *N. coucang*) were found in ZIMS, only one previous publication on the blood biochemistry values of a single individual of Javan slow loris (Wirdateti *et al.* 2018) was found. To our knowledge, our study was the first comprehensive assessment of blood biochemistry reference values in Javan slow lorises that resulted in the definition of a range.

The results of the interspecies comparison using data collected from ZIMS (Table 2), showed that some values in Javan slow loris were lower and the reference value range was also narrower than others, but none of these differences were statistically significant. The range of reference values obtained in our study remained inside the range of those other two species reference values obtained from those obtained from the ZIMS database. This was an important finding as it shows that existing reference values for other species of this genus are also suitable as range values for the Javan species. However,

Table 1 Results of a blood biochemistry analysis in 20 *Nycticebus javanicus* (10 males and 10 females)

Parameter	*N. javanicus* male	*N. javanicus* female	p-value	*N. javanicus* male and female	*N. javanicus* Reference Interval
Albumine (g/dL)	4.7	4.7	0.597	4.7	3.6 - 5.7
Alkaline Phosphatase (U/L)	65.6	58.2	0.874	61.9	14.5 - 109.3
Alanine Aminotransferase (U/L)	76.0	79.2	0.896	77.6	39.9 - 115.3
Amylase (U/L)	402.1	446.0	0.729	424.1	215 - 633.1
Total Billirubine (mg/dL)	0.3	0.3	0.838	0.3	0.1-0.5
Blood Urea Nitrogen (mg/dL)	9.7	10.4	0.896	10.1	2.6 - 17.5
Calcium (mg/dL)	11.4	11.7	0.785	11.6	10-13.2
Phosphor (mg/dL)	4.2	4.0	0.890	4.1	2.5 - 5.7
Creatinine (mg/dL)	0.3	0.4	0.816	0.4	0.1 - 0.6
Glucose (mg/DL)	137.3	133.3	0.896	135.3	61.0 - 209.6
Sodium (mmol/L)	141.9	142.1	0.988	142.0	136.2 - 147.8
Potassium (mmol/L)	4.5	4.4	0.890	4.5	2.7 - 6.2
Total Protein (g/dL)	7.3	7.2	0.324	7.2	5.9 - 8.6
Globulin (g/dL)	2.6	2.5	0.751	2.5	1.5 - 3.6

the ranges obtained in ZIMS were much wider than the ranges obtained in our study. Therefore, we believe that this could be related to the heterogeneity of the samples used for ZIMS, as the data were collected from individuals originating from a diverse range of institutions with different husbandry management systems, different sampling methods, and varying sample sizes for each different parameter (Species360 2020). In contrast, our sample comprised specific individuals, which may suggest that the reference values we have obtained were more accurate. However, as our results could be limited by the smaller sample size, they may not yet fully represent the reference values for the species as a whole, interpretation should still be taken with caution.

Although the sample size of this study was limited, this study provides the first preliminary blood biochemistry reference ranges for healthy adult wild, rehabilitant Javan slow loris. We did not find any significant differences in the range values of *N. javanicus* between males or females or between the other two *Nycticebus* species obtained from the ZIMS database. This is particularly important since the ZIMS database does not currently contain range values for Javan slow lorises. In the future, further data collection would be necessary to obtain more accurate ranges, including from infant or juvenile individuals.

Figure 1 blood sampling in a javan slow loris under anasthesia

Table 2 Blood biochemistry reference interval comparisons between *Nycticebus javanicus, Nycticebus pygmaeus and Nycticebus coucang*

Parameter	**Mean**			**Reference Interval**		
	N. javanicus	*N. coucang* (ZIMS)	*N. pygmaeus* (ZIMS)	*N. javanicus*	*N. coucang* (ZIMS)	*N. pygmaeus* (ZIMS)
Albumin (g/dL)	4.7.			3.6 - 5.7		
Alkaline Phosphatase (U/L)	61.9	237; n=67	63; n=165	14.5 - 109.3	51 - 627	18 -157
Alanine Aminotransferase (U/L)	77.6	80; n=65	80; n=180	39.9 - 115.3	13 - 167	39 -183
Amylase (U/L)	424.1	1233; n=40	641; n=104	215 - 633.1	491 - 2688	262 -1342
Total Billirubine (mg/dL)	0.3	0.2; n=40	0.2; n=120	0.1-0.5	0 - 0.6	0 - 0.6
Blood Urea Nitrogen (mg/dL)	10.1	21.6; n=54	28.6; n=182	2.6 - 17.5	4.76 - 66.9	9.52 - 54.8
Calcium (mg/dL)	11.6	10.4; n=69	10.4; n=172	10-13.2	7.6 - 13.6	8 -13.2
Phosphor (mg/dL)	4.1	4.6; n=65	4; n=156	2.5 - 5.7	1.9 - 10.5	1.5 - 7.8
Creatinine (mg/dL)	0.4	0.4; n=72	0.4; n=150	0.1 - 0.6	0.1 - 1.0	0 -0.7
Glucose (mg/DL)	135.3	148; n=70	185; n=173	61.0 - 209.6	58.7 - 265	58 -280
Sodium (mmol/L)	142.0	146; n=40	146; n=113	136.2 - 147.8	125 - 160	135 -155
Potassium (mmol/L)	4.5	4.2; n=40	4.4; n=111	2.7 - 6.2	2.5 - 7.4	2.3 - 7.8
Total Protein (g/dL)	7.2	7.5; n=60	7.5; n=170	5.9 - 8.6	4.4 - 10.4	5.1 - 8
Globulin (g/dL)	2.5	4; n=41	3.4; n=133	1.5 - 3.6	1.6 - 7.3	1.7 - 5.8

ACKNOWLEDGEMENTS

We thank the Ministry of Environment and Forestry of Republic Indonesia for their support and trust in us, especially in our slow loris conservation efforts. We are also grateful to Baerbel Koehler due to her support and for the sharing of knowledge. We must also show our gratitude for the help we received from of our colleagues, Dr. Ali Anwar bin Ahmad and drh. Wahyu Hananto in accessing data from ZIMS.

"All authors declare that there are no conflicts of interest".

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