HAEMATOLOGY AND SERUM BIOCHEMISTRY PROFILE OF PHYSICALLY RESTRAINED *Cervus timorensis*

ZAWIDA Z.*, ADNAN M., PREMAALATHA B., ZAINI C M., JAMNAH O., CHANDRAWATHANI P., NURULAINI R., NORLINDAWATI A.P., NORAZURA A.H. AND MOHD BOHARI J.

Veterinary Research Institute, Department of Veterinary Services,
Jalan Sultan Azlan Shah, 31400 Ipoh, Malaysia
* Corresponding author: zawida82@hotmail.com

ABSTRACT. Normal haematology and biochemistry values are of clinical significance for the diagnosis of diseases and abnormal conditions. As such it can be used as reference data, especially for Timor deer (*Cervus timorensis*) where these data is limited. Routine sampling was conducted on healthy, adult deer in captivity. Blood samples from 45 animals and serum samples from 29 animals were collected monthly over a period of six months by physical restraint and was followed up with laboratory analysis using conventional methods and commercial kits to obtain the values of important haematology and serum biochemistry parameters. The lower and upper bound values are suggested to be the reference values of *Cervus timorensis* considering the samples were taken from animals with no clinical signs of disease. This data will be useful for monitoring the health of Timor deer towards achieving an efficient and profitable deer farming enterprise.

Keywords: Deer, *Cervus timorensis*, haematology, biochemistry, reference values

INTRODUCTION

Timor deer or Java deer (*Cervus timorensis*) originates from the island of Java and is now well distributed throughout the Southeast Asian countries including Malaysia (Hedges *et al.,* 2010). It can be recognised by its medium size body, covered with coarse, reddish brown coat with a paler colour at the ventral part of the body. Males have three-tined antlers and will shed and re-grow once a year (www.ozanimals.com, 2011). The main purpose of breeding in captivity is for their exotic meat which has high economic value as it is a delicacy among local folks. Although deer in captivity is still considered a semi wild animal, the health status should not be taken for granted. This breed is tolerant to local weather and local pathogens, however, they may succumb to disease under stressful conditions like poor nutrition or stressful management. The deer are susceptible to various kinds of diseases that may cause economic losses such as trypanosomiasis and malignant cattarhal fever (Reid *et al.,* 1999, Nurulaini *et al.,* 2007, Denholm *et*

al., 1982). Therefore, to maintain the health status of the animals, it is important to be knowledgeable about the normal range of haematology and blood biochemistry values (Ramchurn, 2001) as this will allow for immediate detection of any abnormality in the animals. The results of this study will make available relevant information that will empower veterinarians and animal health care workers to assess the health of animals in the farm and to carry out the necessary treatment to prevent morbidity and mortality. Knowledge of normal blood parameters will aid in better management and preventive measures for animal health. These results can be used for other large scale deer farms such as this. (Gupta *et al*., 2007).

There are several methods that can be practised for the capture and restraint of deer to enable blood and serum sampling, such as physical restraint, tranquilization and shooting, depending on management of the farm. The different practices may cause differences in the biochemical and haematological parameter values due to different levels of stress that may affect the blood circulation and enzyme secretions (Gorazd, 2006). Although the closest to normal haematology and serum biochemistry profile readings are when the body is at rest, the most common method practiced in the field for blood and serum sampling from any living animal is by physical restraint using an animal crush, which may stress the animal. This method takes less time and cost and will be the most appropriate method for sample collection in this study.

Thus, the objective of this study is to develop the haematology and serum biochemistry reference values for *Cervus timorensis* bred in captivity and under physical restraint so that it can be used to evaluate the health status of deer in Malaysia.

MATERIALS AND METHOD

A total of 45 adult Timor deer *(Cervus timorensis)* were individually identified and followed through monthly for a period of 6 months in this study. The animals were managed in a semi-captive farm in Pusat Ternakan Haiwan, Lenggong Perak, one of the government farms under the perview of the Department of Veterinary Services, Malaysia. A total of 45 blood samples and 29 serum samples from Timor deer were individually collected from the jugular vein monthly. The deer were physically restrained to facilitate collection of blood from the jugular vein and for general assessment. The deer were first gathered in a dark house situated in the vicinity of the improved grass paddocks where they graze. They were ushered in a single file to fit into the long trevis. A body crusher attached to the trevis was used to immobilise the deer physically. Extra caution was practised for stags with large antlers. Rope was used to tie antlers to the iron wall to provide extra safety to the handlers. As the deer were easily alarmed with human handling, the handlers covered the deer's eyes with

a piece of cloth to calm them and then exposed the neck area for blood collection from the jugular.

Blood was collected from the jugular vein using 1.5 inch, 18 gauge vacujet needle, with a needle holder and vacum blood tube. In this study, plain tubes and EDTA tubes were used to collect 5–10 ml of blood from each deer. The blood samples were chilled and transported to the haematology and biochemistry laboratory in VRI, Ipoh for further laboratory analysis. Parameters evaluated for haematology were red blood cell count (RBC), packed cell volume (PCV), Haemoglobin (Hb), White blood cell count (WBC) and Neutrophil, Lymphocyte, Monocyte, and Eosinophil percentage. Parameters evaluated for kidney functions were; Calcium, Creatinine, Inorganic phosphorus and Urea-Nitrogen. Parameters evaluated for liver functions were; Alkaline phosphatase (ALKP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Total bilirubin, Ammonia, Total protein, Albumin (A), Globulin(G), and A/G ratio.

Conventional methods and commercial kits were used to conduct the tests. The percentage of packed cell volume (PCV) was determined by haematocrit centrifugation method (Christopher *et al.,* 1992). Whole blood from the EDTA tube were withdrawn into capillary tube and then sealed at one end, the tube was then centrifuged at 1200 rpm for 5 minutes. The PCV in the centrifuged capillary tube were measured using haematocrit reader to determine the percentage of blood cells. RBC and WBC counts were done by using haemacytometer with blood dilution, 1:20 with Acetic acid and gentian violet mix for WBC and 1:200 with Dacies fluid for RBC (O.W. Schalm *et al.,* 1975). Haemogolobin evaluation was done using cyanmethaemoglobin method (Christopher *et al.*, 1992) with spectrophotometer. Differential white blood cell was assessed in percentage from thin blood smears (Schalm *et al.*, 1975).

All kidney and liver function tests for this study was conducted using a commercial kit consisting of an automated machine; that is the VETTEST 8008, Blood Chemistry Analyser with the help of VETTEST slides designed for each specific value (VETTEST 8008 Laboratory Manual). All data analysis was performed using SPSS version 16.0.

RESULTS

Results from 45 blood samples and 29 serum samples were obtained after haematology and serum biochemistry evaluation. The lower and upper bound values for each parameter will indicate the range of their normal values. Table 1 shows the results for haematology parameters with standard deviations. Table 2 shows the results for kidney function parameters. Table 3 shows the results for the liver function parameters.

DISCUSSION

In order to obtain haematology as well as kidney and liver function parameter

Table 1: Haematological values in Timor deer (*Cervus timorensis*) that are restrained and bred in captivity.

Parameters	Unit	n	Range	Mean ± sd
RBC	x 10^6/µl	45	11.84 – 13.35	12.59 ± 2.45
PCV	%	45	39.10 – 43.16	41.13 ± 6.17
Hb	g/dL	45	12 – 13.11	12.56 ± 1.84
WBC	x 10^3/µl	45	9.65 – 13.16	11.35 ± 5.69
Neutrophil	%	45	41.54 – 48.02	44.78 ± 10.79
Lymphocyte	%	45	45.75 – 54.07	49.91 ± 13.84
Monocyte	%	45	2.13 – 3.12	2.63 ± 1.60
Eosinophil	%	45	4.34 – 5.98	5.17 ± 2.62

Table 2: Serum kidney function values in Timor deer (*Cervus timorensis*) that are restrained and bred in captivity.

Parameters	Unit	n	Range	Mean ± sd
Calcium	mmol/L	29	2.4765 – 2.5490	2.5128 ± 0.0953
Creatinine	µmol/L	29	155.30 – 167.87	161.59 ± 16.526
Inorganic phosphorus	mmol/L	29	2.2593 – 2.5121	2.3857 ± 0.326
Urea nitrogen	mmol/L	29	7.391 – 7.934	7.662 ± 0.7138

Table 3: Serum liver function values in Timor deer (*Cervus timorensis*) that are restrained and bred in captivity.

Parameters	Unit	n	Range	Mean ± sd
ALKP	U/L	29	91.6742 – 129.52	110.60 ± 48.799
AST	U/L	29	82.8054 – 92.3715	87.5885 ± 11.842
ALT	U/L	29	82.0799 – 90.6354	86.3577 ± 10.590
Total Bilirubin	µmol/L	29	7.0313 – 10.8918	8.9615 ± 4.7790
Ammonia	µmol/L	29	241.4 – 263.34	252.37 ± 27.724
Total protein	g/L	29	72.14 – 77.79	74.97 ± 7.428
Albumin	g/L	29	32.25 – 36.3	34.28 ± 5.324
Globulin	g/dL	29	38.144 – 42.863	40.503 ± 6.2031
A/G ratio	-	29	0.7854 – 0.9046	0.8450 ± 0.1477

values, the animal must be restrained to allow for blood collection. In this study, physical restraint was done to immobilize the animals for safe blood collection at the same time creating a calm environment for the animal, as stress can alter the normal values. Semi captive deer are still considered wild animals compared to other domesticated livestock such as cattle and goats, therefore, physical restraint will elevate their stress level and may alter the haematology and biochemistry values when compared at rest. Care must be practiced during animal handling to minimize the stress for the animals. The suitable time for sample collection using physical restraint is in the morning when the environmental temperature is lower and it is comfortable for the animal. The animal should be humanely restrained with care taken not to allow too much struggling. Handlers must be accurate and fast during sample collection. A body crusher was used in this study to aid and safeguard the handlers during animal restraint. Chemical immobilisation is also one of the methods to restrain animals (Gorazd *et al.,* 2006) however, this method is costly and not widely practiced in local deer farms. This study will be useful to evaluate blood and serum parameters in deer using physical restraint as is usually done in many deer farms.

Similar previous studies show that there are slight differences in blood parameters in deer. The differences may be due to many factors such as farm conditions, management, environment and stress factors. Other factors that may contribute to differences in these values are animal handling, methods of sampling and laboratory test methods. Comparison of results with physically restrained grey-brocket deer originating from Brazil, (*Mazama gouazoubira)* shows similar results in haematology values (Estevam *et al.,* 2010). Studies using chemical restraint show differences in haematology and serum biochemistry parameters where physical restraint results have higher values. Stressors such as physical handling might cause spleen contraction leads to elevation of blood cells in the circulation (Goradz *et al.,* 2006). In comparison with red deer (*Cervus elaphus atlanticus*) the results show similarity in serum biochemistry values, however a slight difference in haematology values was also noted. The red deer seems to have lower haematology values possibly due to tranquilisation used for restraining. In the study by Olaf *et al.,* 2004, chemical restraint was used and the results for serum biochemistry shows lower readings compare to this study. Goradz (2006) explained that for physically active animals, the enzyme secretion is more than rested animals. In a previous study by Estevam (2010), no statistical differences were found between male and female in haematological values, although from the study, WBC value was higher in female than in male.

Each parameter has its own function and any elevation and fluctuation may indicate abnormalities in the circulatory system (Estevam *et al.,* 2010, Cross *et*

al., 1994). Animals infected by pathogens may have elevated amount of WBC that acts as a defense mechanism to fight the invasion. Poor body condition in an animal may show low packed cell volume, glucose and protein level. Haematology and biochemistry values from animals with no clinical signs, may then be used to evaluate health status of a Timor deer. Monitoring and maintaining the health of deer will ensure healthy animals which give economical benefits to the industry.

To determine the most suitable range for normal value of haematology and clinical chemistry, stem and leaf plot with 95% confidence interval was chosen. From the plotting of data of each parameter, only the values within 95% confidence interval should be considered for the range of reference values. Rejection of too extreme values was done so as to ensure that they are not within the normal range. As this was the first study to evaluate haematology as well as kidney and liver function parameters in Timor deer locally, more work could be carried out on other aspects of clinical biochemistry to help in the diagnosis of diseases for this species of animals.

CONCLUSION

Haematology and biochemistry values from blood and serum were determined from deer with no clinical signs. These results are the reference values for future evaluation of the health status of *Cervus timorensis.*

REFERENCE

1. Cross J.P., Mackintosh C.G. and Griffin J.F.T. (1994). Haematological reference values for farmed red deer (*Cervus elaphus*) in New Zealand. *Comp Haematol Int*. **4**(2): 76-85.
2. Christopher R., Chandrawathani P. and Cheah T.S. (1992). Manual on Parasitology, Jabatan Perkhidmatan Haiwan Malaysia. pp 23–28.
3. Denholm L.J. and Westbury H.A. (1982). Malignant catarrhal fever in farmed Rusa deer (*Cervus timorensis*). Clinico-pathological observations. *Aust Vet J*. **58**(3): 81–87.
4. Estevam H., Elizabeth S., Felipe Z., José D. and Adjair N. (2010). Haematology of captive grey-brocket deer *Mazama gouazoubira* (Fischer, 1814) (Cervidae: Odocoileinae). *Comp Clin Path*. **19**(1): 29–32.
5. Gorazd V., Diana Z., Silvestra K. and Andrej B. (2006). Haematological and biochemical values of farmed fallow deer (*Dama dama*) after using different methods of capture. *Vet Archive* **76** (Suppl.):189–S197.
6. Gupta A. R., Patra R. C., Saini M. and Swarup D. (2007). Haematology and serum biochemistry of Chital (*Axis axis*) and Barking Deer (*Muntiacus muntjak*) reared in semi-captivity. *Vet Res Commun*. **31**(7): 801–808.
7. Hedges S., Duckworth J.W., Timmins R.J., Semiadi G. and Priyono A. (2008). *Rusa timorensis*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 03 January 2011.
8. Nurulaini R., Jamnah O., Adnan M., Zaini C.M., Khadijah S., Rafiah A.I. and Chandrawathani P. (2007). Mortality of domesticated java deer attributed to Surra. *Trop Biomed*. **24**(2): 67–70.
9. Olav R., Håvard L.N., Tore S. and Jon M.A. (2004). Haematological and serum biochemical reference values in free-ranging red deer (*Cervus elaphus atlanticus*). *Rangifer* **24**(2): 79–85
10. Ramchurn R. (2001). Haematological studies on *Cervus timorensis russa* (Deer introduced in Mauritius in 1639). *International Bulletin*. **18**(2).
11. Reid S.A., Husein A., Hutchinson G.W. and Copeman D.B. (1999). A possible role for Rusa Deer (*Cervus timorensis* russa) and Wild Pigs in Spread of *Trypanosoma evansi* from Indonesia to Papua New Guinea. *Mem. Inst. Oswaldo Cruz*. **94**(2): 195–197.
12. Schalm O.W., Jain N.C. and Carroll E.J. (1975). Veterinary Haematology (3rd Edition). pp 15-81.
13. VETTEST 8008 Laboratory Manual. (1992) IDEXX Laboratories, Inc. VETTEST is the registered trademark of IDEXX Lab inc. One IDEXX Drive. Westbrook Maine 04092 USA
14. www.ozanimals.com/Mammal/Rusa/*Cervus/timorensis*. html. Downloaded January 2011.