UNIVERSITI PUTRA MALAYSIA

HEALTH EVALUATION OF CAPTIVE MALAYAN SUN BEAR (Helarctos malayanus Horsfield) IN ZOOS IN PENINSULAR MALAYSIA

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By

AZLAN BIN CHE' AMAT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Veterinary Science

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Adult Malayan sun bear

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Veterinary Science

HEALTH EVALUATION OF CAPTIVE MALAYAN SUN BEAR (*Helarctos malayanus* Horsfield) IN ZOOS IN PENINSULAR MALAYSIA

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AZLAN BIN CHE' AMAT

October 2011

Chairman : Siti Suri Binti Arshad, PhD

Faculty : Veterinary Medicine

The health status of 19 captive Malayan Sun Bears, 4 males and 15 females, weighing between 31 – 87 kilograms were used in this study. The objectives of the study include the analysis of blood to obtain baseline blood parameters, blood analysis of some viral, bacteria and faecal analysis of parasitic organisms, and comparison of 2 anaesthetic drugs. Animals in Zoo Negara were immobilized with Zoletil®-Xylazine (ZX) and Zoletil®-Ketamine-Xylazine (ZKX) combination was used in Taiping Zoo & Night Safari. Blood and fresh faecal samples were used for study analysis.

All Sun Bears were sampled to determine their exposure some important pathogens. There was no evidence of exposure to Canine Adenovirus (CAV), Canine Distemper Virus (CDV) by using Serum Neutralization Test (SNT). Seroprevalence of two *Brucella sp*. and 14 *Leptospira* serovars were also not detected by using Rose Bengal Plate Test (RBPT) and Microscopic Agglutination Test (MAT) respectively. Simple faecal floatation technique detected strongyles (hookworm) ova only in two animals but in very low numbers. No blood parasites were found.

Haematology studies includes PCV, RBC count, differential counts for leucocytes, haemoglobin, plasma protein, PT and APTT. A total of 26 biochemistry parameters were analyzed namely Na, K, Cl^-, inorganic phosphate, BUN, creatinine, glucose, cholesterol, total bilirubin, total protein, ALT, ALP, AST, CK, globulin, albumin, globulin-albumin ratio, amylase, LDH, lipase, lactate, uric acid, triglyceride and GGT. Male and female comparison showed only haemaglobin had significant higher value in males. Leucocytes and segmented neutrophil were significantly high in subadult. Current value showed low haemaglobin and leucocytes compared to previous data. Preliminary data on anticoagulation factor namely PT and APTT were recorded. Biochemistry showed ALT, GGT and CK were significantly higher in males. Subadult group

showed higher value of ALT, GGT, CK, LDH and albumin. Our current data showed lower value of calcium, inorganic phosphate, BUN and AST compared to previous data. Additional preliminary data on GGT, amylase, CK, total triglyceride, lipase and lactate were recorded.

The effects of 2 anaesthetic drugs, ZX and ZKX combinations were documented in this species. Both drugs effectively immobilized the sun bears but induction with ZKX produced more rapid, smooth induction and good analgesia. Physiological parameters such as respiratory rate, pulse rate, SpO_2, MAP and rectal temperature were within a good range for both treatment group. The used of yohimbine to reversed the ZKX give a better recovery two times faster than without using yohimbine in ZX group.

Thus, results obtained in the present study suggested that all captive Malayan Sun Bears in the zoo were healthy as shown by their blood haematology and serum biochemistry, were within the data previously documented in the same species. No seroprevalence were detected for CAV, CDV, *Leptospira sp*. and *Brucella sp*. in all bears and this indicates these animals are not protected against these pathogens. Parasites found by faecal examination was not significant but preventive measures should be periodically done. The ZKX may be a better

choice of immobilizing the sun bear due to rapid, smooth induction, good analgesia and rapid recovery by using yohimbine.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PENILAIAN KESIHATAN TERHADAP BERUANG MATAHARI MALAYA (Helarctos malayanus Horsfield) KURUNGAN DI ZOO-ZOO DI SEMENANJUNG MALAYSIA

Oleh

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Penilaian kesihatan 19 ekor beruang matahari dalam kurungan, 4 jantan dan 15 betina, berat di antara 31 – 87 kilogram digunakan di dalam kajian ini. Objektif kajian ini merangkumi analisa darah untuk mendapatkan parameter asas darah, analisa darah untuk beberapa virus, bacteria, dan analisa najis untuk organism parasit serta perbandingan 2 ubat bius. Haiwan di Zoo Negara diimobilisasi menggunakan kombinasi Zoletil®-Xylazine (ZX) dan Zoletil®-Ketamine-Xylazine (ZKX) digunakan di Zoo Taiping. Sampel darah dan najis telah digunakan untuk analisis kajian ini.

Semua sampel darah beruang matahari diukur tahap pendedahan kepada beberapa pathogen penting. Tiada bukti didapati terhadap pendedahan kepada CAV dan CDV menggunakan ujian neutralisasi serum (SNT). Seroprevalen terhadap dua *Brucella sp*. dan 14 serovar *Leptospira* juga tidak dapat dikesan masing-masing menggunakan ujian plat Rose Bengal (RBPT) dan ujian agglutinasi mikroskopi (MAT). Teknik pengapungan najis ringkas didapati mengesan telur cacing kerawit (strongyle) hanya pada dua haiwan tetapi di dalam bilangan yang terlalu sedikit. Tiada parasit darah ditemui.

Kajian hematologi termasuk PCV, jumlah sel darah merah (RBC), perbezaan bilangan leukosit, haemoglobin, protein plasma, PT dan APTT. Sejumlah 26 parameter biokimia dianalisa iaitu Na, Cl^-, fosfat tak organik, BUN, kreatinin, glukosa, kolesterol, jumlah bilirubin, jumlah protein, ALT, ALP, AST, CK, globulin, albumin, ratio globulin-albumin, amilase, LDH, lipase, laktat, asid urik, trigliserida dan GGT. Perbandingan jantan dan betina menunjukkan hanya ukuran hemoglobin lebih tinggi pada jantan. Jumlah leukosit dan neutrofil bersegmen lebih tinggi pada sub dewasa. Jumlah terkini menunjukkan hemoglobin dan leukosit lebih rendah berbanding data terdahulu. Data awal antikoagulasi iaitu PT dan APTT telah diekodkan. Biokimia menunjukkan ALT, GGT dan CK adalah lebih tinggi catatannya pada jantan. Kumpulan sub dewasa

menunjukkan nilai yang tinggi pada ALT, GGT, CK, LDH dan albumin. Kajian ini menunjukan nilai lebih rendah pada kalsium, fosfat inorganic, BUN dan AST berbanding data terdahulu. Data awal tambahan iaitu GGT, amylase, CK, jumlah trigliserida, lipase dan laktat juga direkodkan.

Kesan-kesan 2 ubat bius, kombinasi ZX dan ZKX didokumentasikan di dalam spesis ini. Kedua-dua ubat telah mengimobilisasikan beruang secara efektif tetapi dengan ZKX menghasilkan induksi yang lebih cepat, lancar dan analgesia yang baik. Parameter fisiologi seperti kadar pernafasan, kadar denyutan nadi, SpO_2, MAP dan suhu rektal berada di antara kadar yang baik untuk kedua-dua kumpulan. Penggunaan yohimbine sebagai agen antagonis kepada ZKX memberi pemulihan dua kali lebih cepat berbanding tidak menggunakan yohimbine pada kumpulan ZX.

Oleh itu, kajian ini menunjukkan beruang matahari Malaya kurungan di zoo adalah sihat seperti yang ditunjukkan oleh hematologi dan biokimia, sama seperti data yang telah direkodkan pada spesis yang sama sebelum ini. Tiada seroprevalen CAV, CDV, *Leptospira sp.* dan *Brucella sp*. dikesan dan ini menunjukkan haiwan ini tidak dilindungi terhadap pathogen-patogen ini. Parasit yang ditemui melalui pemeriksaan najis tidak ketara tetapi langkah

pencegahan perlu dilakukan secara berkala. Penggunaan ZKX boleh jadi pilihan yang terbaik untuk mengimobilisasikan beruang matahari kerana induksi yang cepat, lancar, analgesia yang baik dan pemulihan yang pantas dengan menggunakan yohimbine.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Veterinary Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and is not currently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

AZLAN BIN CHE' AMAT

Date: 11 October 2011

I certify that a Thesis Examination Committee has met on 11/10/2011 to conduct the final examination of Azlan Bin Che' Amat on his Master of Veterinary Science thesis entitled "HealthEvaluation of Captive Malayan Sun Bear (*Helarctosmalayanus*Horsfield) in Zoos in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

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LIST OF ABBREVIATIONS

EDTA	ethylenediaminetetracetic
PCV	Packed cell volume
RBC	Red blood cell
WBC	White blood cell
PT	Prothrombin time
APTT	Activated partial thromboplastin time
MCV	Mean cell volume
MCHC	Mean corpuscle haematocrit cels
Hb	Haemoglobin
rpm	revolutions per minute
xg	times gravity
Na	Sodium
K	Kalium
Cl^-	Chloride
Ca	Calcium
PO_4^{3-}	Phosphate
BUN	Blood urea nitrogen
ALT	Alanine aminotransferase

ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
CK	Creatine kinase
LDH	Lactate dehydrogenase
GGT	Gamma-glutamyl transferase
TP	Total protein
°C	Degree Celcius
P	Significant level
e.g.	*Exemplium gratia* (example)
L/L	litre per litre
U/L	Units per litre
μ/L	Micro (10^{-6}) per litre
mmol/L	millimol (10^{-3}) per litre
μmol/L	micromol (10^{-6}) per litre
g/L	gram per litre
fL	femto (10^{-15})litre
10^{12}/L	Tera per litre
10^{9}/L	Giga per litre
CDV	canine distemper virus
CAV	canine adenovirus

MDCK	Madin-Darby canine kidney
MEM	Minimum essential medium
FBS	Fetal bovine serum
CPE	Cytopathic effect
$TCID_{50}$	Median tissue culture infective dose
CO_2	Carbon dioxide
SNT	Serum neutralization test
MAT	Microscopic agglutination test
RBPT	Rose bengal plate test
PBS	Phosphate buffer saline
ATV	Antibiotic trypsine versene
KCT	Knott's concentration technique
mg/kg	milligram per kilogram
µg/kg	microgram per kilogram
ZX	Zoletil®-Xylazine
ZKX	Zoletil®-Ketamine-Xylazine
HCl	Hydrochloride
SpO_2	haemoglobin oxygen saturation
MAP	Mean arterial pressure
CRT	Capillary refill time

bpm	beat per minute / breath per minute
SPSS	Statistical Package for Social Sciences
TP	Total protein
A/G	Albumin globulin ratio
S.I.	International system of units
mmHg	milimetres of mercury
CV	Coefficient variation

CHAPTER I

GENERAL INTRODUCTION

The Malayan sun bear was the most distinguished from other bears which is smallest, have a long slender tongue, more bowed forelimb, the feet turn inward to a greater extent, short fur and have a horseshoe-shaped on the chest with whitish to pale orange yellow in colour (Fitzgerald and Krausman, 2002; Nowak, 1999). The sun bear considered a rare species which is listed in Appendix I in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2011) that the animals are the most endangered. It also listed in International Union for Conservation Nature (IUCN) Red List 2011 as data deficient which the status was unknown for the most countries including Malaysia (Fredrikson *et al.*, 2008).

In Peninsular Malaysia, capture or trade sun bear were prohibited under the Wildlife Conservation Act (2010). The decline in sun bear populations has been attributed to hunting for food and sale of body parts (Servheen, 1999), hunted for gallbladder and other parts for medicinal purposes, habitat loss due to logging

activities and conversion of forest to agricultural plantation (Fitzgerald and Krausman, 2002; Nowak, 1999; Servheen, 1999).

To understand the epidemiology of wildlife disease, surveillance and related study is needed even though conducting research in wildlife area is very hard and challenging. Those challenges include the practicality to handle some of cases, sampling and field data collection, interpretation and the validation of field data through experimental studies (Stallknecht, 2007). As to study the disease prevalence, population data is needed such as population size, density, age structure, sex ratio, recruitment and attrition, home range, habitat utilization, and species composition, among others. All the information is critical to gather in order to understand pathogen transmission and maintenance within wildlife populations (Stallknecht, 2007).

The wildlife conservation community is increasingly aware of the disease risks that threatens wildlife (Deem *et al*., 2001). Populations of domestic carnivores for instance, acts as ideal disease reservoir. High population densities of feral or unvaccinated animals allow even very virulent pathogens to persist in the broader carnivore populations. In addition, some pathogens are able to remain viable in the environment for an extended period of time, which means that the

environment can be a possible source of disease transmission (Fiorello *et al*., 2004).

Serological assay are widely used for epidemiological studies even though they hardly distinguish between the antibodies of active infection, disease exposure or immunity towards vaccination (Evermann and Eriks, 1999). Limitations of serology test can be a problem in determining the disease status of an individual animal, but it is useful to screen for exposure to a certain pathogens on a population level (Christensen and Gardner, 2000).

Zoo or any captive centers usually house many population of varions species of wild animals. This probably may lead to environmental stress or transmission of direct life-cycle pathogens from sources like carriers, reservoirs or infected animals. Thus it is necessary to investigate the disease prevalence because of the existence of mixed population of captive animals (Ramanathan *et al*., 2007).

There are no reported data available in Malaysia with regard to the disease of sun bear. Therefore it is imperative to do a survey of disease exposure to sun bear especially in those in captivity. By doing so, perhaps a more approach to disease prevention and control will emerge for the benefit of animals and

practitioner. For evaluating health aspects of captive sun bear, the survey may includes blood profiles analysis, measuring antibodies for important pathogens such as canine distemper virus (CDV) and canine adenovirus (CAV). Each of these viruses may be an important cause of canine mortality and can also infect other carnivores. This studies also look into the seroprevelence evidence of leptospira and brucella specific serovar, endoparasites and blood protozoa. Selection of anaesthesia used in this study were recorded and evaluated for the reference for the future usage.

1.1 Problems identification

The establishment of reference physiologic parameters, blood cell counts, serum biochemistry profiles, parasites, surveillance of antibody to certain viruses and bacteria is critical for health evaluation of captive animals. Such data can also contribute to improving the health management of captive wildlife populations. Thus, it is important to collect the information on ex-situ management of this species and this study was conducted with the following objectives :

1.2 Objective of study:

1. Determine seroprevalence to important pathogens.

   (a) To determine the seroprevalence of canine distemper virus (CDV) and canine adenovirus (CAV).

   (b) To determine the seroprevalence of *Leptospira spp, Brucella abortus* and *B. melitensis*.

   (c) To identify fecal endoparasites and blood protozoa.

2. To obtain baseline blood parameters of captive sun bear and compare differences between gender and age.

3. To document the effects of anaesthesia by using combination Zoletil®-Xylazine and Zoletil®- Ketamine-Xylazine.

1.3 Output/benefit of study

Captive wildlife can be an invaluable reservoir for systematic research investigations, including generating baseline or preliminary data for managing free-ranging wildlife. The outputs can be used to improve the health aspect of sun bear population especially in terms of control and prevention of disease. By generating and compiling blood profiles and anaesthetic protocol, it will give a guideline to the zoos and the veterinarians to conduct a pre-screening of health evaluation of their animals. From a basis of screening of seroprevalence of

pathogens and as well as from the fecal screening, it might give a platform to them to formulate or suggest herd health programme. In general, the output of this study perhaps will contribute to future conservation programmes.

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