UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF INTERNAL PARASITES IN BORNEAN SUN BEARS (*HELARCTOS MALAYANUS*) AT BORNEAN SUN BEAR CONSERVATION CENTRE, SABAH.

ONG YU QI

PREVALENCE OF INTERNAL PARASITES IN BORNEAN SUN BEARS (*HELARCTOS MALAYANUS*) AT BORNEAN SUN BEAR CONSERVATION CENTRE, SABAH.

ONG YU QI

A project paper submitted to the

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Prevalence of Internal Parasites in Bornean Sun Bears (*Helarctos Malayanus*) at Bornean Sun Bear Conservation Centre, Sabah.", by Ong Yu Qi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project

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I could not possibly thank everyone enough. Lastly, I hope this final product could be of help to the field of wildlife medicine and conservation as well as to motivate further related research to be conducted.

Sincerely,

Ong Yu Qi

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
BSBCC	Bornean Sun Bear Conservation Centre
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic Acid
DPX	Dibutylphthalate Polystyrene Xylene
IACUC	Institutional Animal Care and Use Committee
IUCN	International Union for Conservation of Nature
NGOs	Non-Governmental Organizations
PCR	Polymerase Chain Reaction
QAC	Quaternary Ammonium Compounds
SaBC	Sabah Biodiversity Centre
SSC	Species Survival Commission

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek Ilmiah Tahun Akhir.

PREVALENS PARASIT DALAMAN TERHADAP BERUANG MATAHARI BORNEO (*HELARCTOS MALAYANUS*) DI PUSAT PEMULIHARAAN BERUANG MATAHARI BORNEO, SABAH.

Oleh

Ong Yu Qi

2022

Penyelia: Dr. Azlan Che' Amat

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Sebagai spesies beruang terkecil dan satu-satunya beruang yang berasal dari Malaysia, beruang matahari juga disenaraikan sebagai *vulnerable* dalam *International Union for Conservation of Nature* (IUCN) *Red List*. Oleh itu, penilaian kesihatan rutin dalam kurungan sangat penting dalam memulihara spesies ini. Walaupun parasit diketahui berbahaya bagi haiwan, terdapat penyelidikan yang sangat terhad mengenai beruang matahari. Tujuan kajian ini adalah untuk menentukan prevalen parasit dalaman dan menilai perkaitan faktor risiko dengan serangan parasit yang mungkin mempengaruhi status kesihatan beruang matahari Borneo yang terlindung di Pusat Pemuliharaan Beruang Matahari Borneo (BSBCC), Sabah. Sampel najis dari semua 45 beruang matahari Borneo dikumpulkan dua kali dan dianalisis dengan pemeriksaan koproskopi menggunakan teknik rutin iaitu pengapungan dan pemendapan. Slaid calitan darah dan pewarnaan menggunakan *Diff-Quik* dilakukan ke atas 21 ekor beruang. Pensampelan mudah dilakukan dari beruang matahari dan jantina, pelbagai keadaan badan, usia dan amalan pengurusan direkodkan. Analisis deskriptif prevalens dan ujian *Fisher's exact* dijalankan untuk perkaitan pemboleh ubah risiko dianalisis dalam perisian GraphPad. Keseluruhan prevalens parasit dalaman adalah 68.89% (95% CI: 53.35-81.83). Dari 45 sampel, 31 (68.89%) beruang dikesan mempunyai parasit gastrointestinal, 28 (90.3%) daripadanya mempunyai beban parasit ringan. *Trichuris* spp. adalah parasit yang paling lazim (42.22%), diikuti oleh *Ancylostoma* sp. (31.11%), protozoa (8.89%), *Strongyloides* sp. (6.67%), cestod (4.44%) dan *Babesia* sp. yang hanya dijumpai dalam 3 beruang (14.28%). Kehadiran parasit gastrousus terbukti berkaitan secara signifikan dengan jenis perumahan secara kumpulan. Kesimpulannya, walaupun memiliki prevalen parasit dalaman yang tinggi, tetapi beban parasit adalah rendah, oleh itu tidak ada penyakit klinikal yang direkodkan. Oleh itu, amalan pengurusan yang baik dan rawatan pencegahan yang betul harus disarankan untuk mengawal perkembangan penyakit klinikal dan parasitisme.

Kata Kunci: *Beruang matahari Bornean, parasit gastrousus, parasit darah, captive, pemuliharaan*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

PREVALENCE OF INTERNAL PARASITES IN BORNEAN SUN BEARS (*HELARCTOS MALAYANUS*) AT BORNEAN SUN BEAR CONSERVATION CENTRE, SABAH.

By

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2022

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Being the smallest bear species and the only bear native to Malaysia, sun bears are also listed as vulnerable in the International Union for Conservation of Nature (IUCN) Red List. Hence, routine health evaluation in the captive setting is imperative in conserving this species. Despite the fact that parasites are known to be harmful to animals, there has very limited research conducted on sun bears. The aim of this study is to determine the prevalence of internal parasites and evaluate the association of risk factors with parasitic infestations that might impact the health status of Bornean sun bears sheltered at Bornean Sun Bear Conservation Centre (BSBCC), Sabah. Faecal samples from all 45 captive Bornean sun bears were collected twice and analysed by coproscopic ova examination utilising standard simple floatation and sedimentation techniques. Blood smears and Diff-Quik staining were performed in 21 bears. Convenience sampling was conducted from sun bears and genders, various body conditions, ages and management practices were recorded. Descriptive analysis of prevalence and Fisher's exact test for the association of risk variables were analysed in GraphPad Software. The overall prevalence of internal parasites was 68.89% (95% CI: 53.35-81.83). Out of 45 samples, 31 (68.89%) bears were detected for gastrointestinal parasites with 28 (90.3%) of them having a mild parasitic load. *Trichuris* sp. was the most prevalent parasite (42.22%), followed by *Ancylostoma* sp. (31.11%), protozoa (8.89%), *Strongyloides* sp. (6.67%), cestode (4.44%) and the only haemoparasite, *Babesia* spp. was found in 3 bears (14.28%). Furthermore, the presence of gastrointestinal parasites was shown to be significantly associated with group-living housing. In conclusion, despite having a high prevalence of internal parasites, the parasite burden was low, hence no clinical diseases were observed. Thus, good management practice and proper preventive medicine should be advised to control the progression of clinical disease and parasitism.

Keywords: *Bornean sun bear, gastrointestinal parasites, haemoparasites, captive, conservation.*

1.0 INTRODUCTION

Internal parasites, also known as endoparasites are defined as organisms that inhabit a significant portion of their lives inside another organism, known as a host (Taylor *et al*., 2015). Internal parasites are further divided into intercellular and intracellular parasites (Britannica, 2022). Intercellular parasites populate in the cavities of a host, for instance, helminths that live in the intestines whereas intracellular parasites populate in the cells of a host such as the intraerythrocytic protozoa *Babesia* spp. Wild animals can contract parasitic infections just like other domestic animals. It is well known that parasitic infections are harmful to the health of captive wildlife oftentimes resulting in fatalities when a hazardous parasite species is present in large numbers, or predisposing the animal to secondary deficiencies and infectious diseases (Panayotova-Pencheva, 2013).

Animal health status including parasitic infestations is frequently taken into consideration when dealing with wildlife conservation to ensure the animals' maximum well-being. In Southeast Asia, sun bears have been reported to have five parasites, including a cestode (*Pentorchis arkteios*), a hookworm (*Ancylostoma malayanum*), and three ticks (*Haemaphysalis semermis*, *H. leachi*, and *H. hystricis*) (Rogers and Rogers, 1976). Additionally, faecal examination reports of *Trichuris* spp. and *Ancylostoma* spp. in sun bears exist (Jenantika *et al*. 2019).

The present study is conducted due to the lack of empirical data from research and reports pertaining to internal parasites of Bornean sun bears. As sun bears are listed as vulnerable in the International Union for Conservation of Nature (IUCN) Red List, this makes any possible pathogens like parasitic infestations a significant threat to the conservation of sun bears. At the moment, there is only one published report on internal parasites in Bornean Sun Bear Conservation Centre (BSBCC). There is still more to learn about internal parasites in sun bears as well as the risk factors correlated with their parasite burden.

The main objective of this study was to determine the prevalence of internal parasites in captive Bornean sun bears at BSBCC, Sandakan. The specific objectives include, (i) identification and quantification of faecal parasite ova and blood protozoa in Bornean sun bears, and (ii) determination of the correlation of risk factors such as sex, age and management practices that could predispose Bornean sun bears to internal parasite infestation and correlation of parasite burden with clinical manifestation.

The null hypotheses in this study were (i) internal parasites in captive Bornean sun bears at BSBCC are absent, (ii) age, sex and management practices do not influence parasite burden, and (iii) body condition and faecal score are not correlated with parasite burden. Inversely, the alternative hypotheses were, (i) internal parasites in captive Bornean sun bears at BSBCC are present, (ii) age, sex and management practices do influence parasite burden, and (iii) body condition and faecal score are correlated with parasite burden.

2.0 LITERATURE REVIEW

2.1 Basic information on Bornean sun bears

Sun bears (*Helarctos malayanus*) are believed to be named so due to their individually distinct orange-yellow crescent-shaped chest mark that stands out among their black fur. The Malayan sun bear (*Helarctos malayanus malayanus*), which is found in Mainland Asia and Sumatra, and the Bornean sun bear (*Helarctos malayanus euryspilus*), which is only found in Borneo, are the two sun bear subspecies currently recognized. The Bornean sun bear is reported to have a smaller skull, a lower maximum body length and weight. (Scotson *et al*., 2020; Meijaard, 2004). Sun bears are the smallest species out of the eight living bear species and they have the longest tongue of all bear species which is useful for acquiring invertebrate prey (AZA Bear Taxon Advisory Group, 2019; Stirling and Derocher, 1990).

Sun bears live in tropical evergreen rainforests with uniformly distributed heavy precipitation throughout the year in Borneo, Sumatra, and Peninsular (West) Malaysia. There are various forest types in tropical evergreen rainforests where sun bears could be found but they prefer lowland and hill forests owing to the abundance of fruiting trees (Scotson *et al*., 2020; Steinmetz *et al*., 2011).

Sun bears are thought to be omnivores who eat a variety of things, including honey from bee hives and fruits like figs and invertebrates like termites and beetle larvae

(Augeri, 2005; Wong, 2002). Sun bears forage on land, but they have evolved to have skilful climbing abilities which allow them to nest and reach fruits and insects on trees (Fredriksson *et al*., 2006; Wong *et al*., 2004).

2.2 Sun Bear Status and Conservation Actions

There is a lack of direct empirical estimates of population trends but subjective estimates of rates of population loss over three generations have been made by country experts from the IUCN SSC (Species Survival Commission) Bear Specialist Group. The assessment has suggested that the numbers of sun bears are decreasing throughout their range which has resulted in a decline of about 35% over the past 30 years, and 40% or more for future time periods. Hence, sun bears are listed as vulnerable in the IUCN Red List (Scotson *et al*., 2017). Threats to sun bears include hunting, trade in bear and bear parts, conflict with humans, habitat destruction and establishment of plantations and these all contribute to the decline of sun bear numbers in the wild (Gomez *et al*., 2020; Meijaard, 2007). Primary forest protection, stricter law enforcement, non-lethal mitigation to reduce human-bear conflict, expanding scientific knowledge of Sun Bear ecology, population distribution, status and effects of threats are some of the other conservation actions conducted in order to put a stop to the decline of this species (Scotson *et al*., 2017).

In Cambodia, Vietnam, Laos, Thailand, Malaysia, and Indonesia, non-governmental organizations (NGOs) have established bear-specific rescue facilities with the main objective of providing a haven for bears seized from the illegal wildlife trade. The importance of bear rescue centres includes increasing local awareness regarding threats of sun bears as well as the conservation value of ecological services provided by the bear habitat. Structured education programs are also offered by certain rescue centres' dedicated outreach teams which can reach tens of thousands of people per year (Scotson *et al*., 2017). Bornean Sun Bear Conservation Centre (BSBCC), the only sun bear-specific conservation centre in the world, is the main player in sun bear conservation in Malaysia. It was established in Sabah, Malaysia, in 2008 with two goals in mind: to raise awareness of sun bears worldwide and to provide care and rehabilitation for rescued bears. To date, 11 sun bears have now been released into the wild (Tan, 2020).

2.3 Parasitic Infestations

Parasites can be categorised into internal (endoparasites) and external parasites (ectoparasites), both have a dependency on the host for nutrition and other benefits for instance a safe environment to grow and reproduce (Taylor *et al*., 2015). Internal parasites can be further grouped into intercellular parasites and intracellular parasites, the former resides in the cavities of a host, for instance, helminths that inhabit the intestines while intracellular parasites populate in host cells such as the intracellular parasite infecting mononuclear cells, *Ehrlichia* spp.

Helminth is one of the most abundant parasites which is part of intercellular internal parasites. It has two major phyla, Nematoda and Platyhelminthes (Wakelin, 1996). Some common types of helminths include hookworms, roundworms, whipworms, tapeworms, and flukes and they can cause various clinical signs and lesions. For example, hookworms can cause anaemia, diarrhoea, weakness and emaciation while roundworms may cause pot-belly, mucoid diarrhoea, coughing and so on (Peregrine, 2022).

For intracellular parasites, blood parasites are common which include single-celled protozoa to more complex bacteria and rickettsiae with the common transmission method through bites of ticks or flies (Tabor *et al*., 2017). Examples of clinical signs caused by blood parasite infestations are anaemia and thrombocytopenia in cases of babesiosis (Vishwakarma and Nandini, 2019).

There are various factors that affect parasitic infestation, most of which can be explained using the epidemiologic triad. The triad comprises an external agent, a host, and an environment which aids in the contact of the host and agent causing the manifestation of disease. An organism that spreads infection by transporting the pathogen from host to host is called a vector and can be involved in the infectious process (Penn State Eberly College of Science, n.d.). The host's immunity level, the humidity and temperature of the environment, the tenacity of the parasitic agent and so forth also contribute to the occurrence of parasitic diseases. For instance, the hatching and development of nematode eggs occur slower at lower temperatures while the development rate rises to a maximum at higher temperatures, after which development will be hampered leading to the death of larvae (Stromberg, 1997). In the wild, animals have a certain inherent resistance to parasitic infections, and there is a state of equilibrium between the parasite and the host, which rarely leads to severe infection unless stressed (Gaur *et al*., 1979). In captive animals, they are frequently subjected to considerable stress such as limitations in space and changes in living conditions, which weakens their resistance to parasitic infestations (Manjunatha *et al*., 2019; Atanaskova *et al*., 2011).

Currently, there are only eleven studies that had found internal parasites in sun bears from 1916 to 2022, six in eleven of them are from more than thirty years ago. Among those eleven studies, only two studies were conducted in Malaysia. In six studies, sun bears were reported as being in captivity, while the others did not provide such details. There were no findings of clinical signs in sun bears associated with the detection of parasitic infestation except for a case study done by Zubaidah *et al*. (2020) which found pale mucous membrane due to babesiosis. Risk factors of parasitic infestations in sun bears have not been evaluated but Jenantika *et al.* (2019) proposed some possible causes of parasitic infestations which include inconsistency of anthelmintic administration and the lack of a quarantine system. Description of parasitic infestation treatment has only been described in the case study by Zubaidah *et al.* (2020) showing the usage of diminazene aceturate in successfully treating babesiosis in a

Malayan sun bear. Therefore, more research on sun bear parasitic infestations is needed to gain a better understanding of sun bear physiology and to help strategize parasitic preventive medicine.

Table 1: Internal parasites that were reported in sun bears

Nematode	*Ancylostoma malayanum* (Baylis and Daubney, 1922; Lane, 1916)
	Ancylostoma sp. (Jenantika *et al*., 2019; Mahannop *et al*., 1984)
	Baylisascaris transfuga (Canavan, 1929)
	Trichuris sp. (Jenantika *et al*., 2019)
	Toxocara transfuga (Panayotova-Pencheva, 2013)
Cestode	*Pentorchis arkteios* (Meggitt, 1927)
Haemoparasite	*Babesia* sp. (Chua *et al*., 2022; Zubaidah *et al*., 2020)

Others	*Enterocytozoon bieneusi* (Li *et al*., 2016)
	Cryptosporidium oocysts (Wang and Liew, 1990)

2.4 Zoonotic Potential

Zoonosis is defined as any disease or infection that is naturally transmissible from vertebrate animals to humans (World Health Organization, 2020). Parasitic zoonotic diseases are common all over the world and there are currently over 15 protozoa and 50 other parasitic diseases that are zoonotic in nature (Dhaliwal and Juyal, 2013). For instance, *Ancylostoma* sp. has been found in sun bear faeces and might cause transmission to humans as cases of Ancylostomatidae infection are well-documented in humans (Di Salvo and Chomel, 2019). Another example of a parasitic agent that is transmissible to humans is *Babesia* spp. Thus, the prevention of an outbreak of babesiosis in bears residing in proximity to humans can diminish the chances of zoonotic transmissions, such as in a conservation centre (Chua *et al*., 2022).

2.5 Diagnostic Methods

The faecal floatation method is conducted using faecal samples of a studied animal mixed with a liquid with a specified specific gravity which can cause parasite ova to float on the surface of the liquid. Different solutions can be used for the liquid such as saturated sodium chloride (NaCl), sodium nitrate ($NaNO_3$), sugar (e.g. Sheather's solution), magnesium sulfate ($MgSO_4$), and zinc sulfate ($ZnSO_4$) (Burton, n.d.). Passive floatation and centrifugation are the two most common methods of faecal examination with the primary distinction being the method of separating faecal debris from parasite ova. Centrifugal floatation reduces the time required for parasite eggs to rise to the surface, increases the yield of parasite ova in the sample and is ultimately considered a more sensitive procedure when compared to the passive floatation method (Haller, 2021; Blagburn and Butler, 2006).

Faecal sedimentation is another method to find ova in faecal samples, it is utilized to detect ova that do not float in usual floatation solutions due to their higher specific gravity. This technique is most useful to identify fluke ova which are heavier and denser than other ova (Samples, 2013). One alternative method other than simple sedimentation is formalin-ether sedimentation technique which has the advantage of lesser distortion of organisms and enhanced recovery of *Schistosoma* eggs and operculated eggs (Truant *et al*., 1981).

Although microscopic examination is still regarded as the “gold standard” for faecal parasitic disease diagnosis, the faecal sample still can be analyzed using molecular techniques such as polymerase chain reaction (PCR) (Centers for Disease Control and Prevention, 2020). PCR is an efficient way of rapidly amplifying deoxyribonucleic acid (DNA) which uses the enzyme DNA-polymerase to multiply a single molecule of DNA several million times in a matter of hours. It is useful not solely in fundamental molecular biological research but advantageous in clinical diagnostics (Giasuddin, 1995). For detecting haemoparasites, PCR is also an effective method for getting a definitive diagnosis of blood parasite infection due to its high sensitivity and specificity (Rucksaken *et al*., 2019).

3.0 MATERIAL AND METHODS

3.1 Site of Study

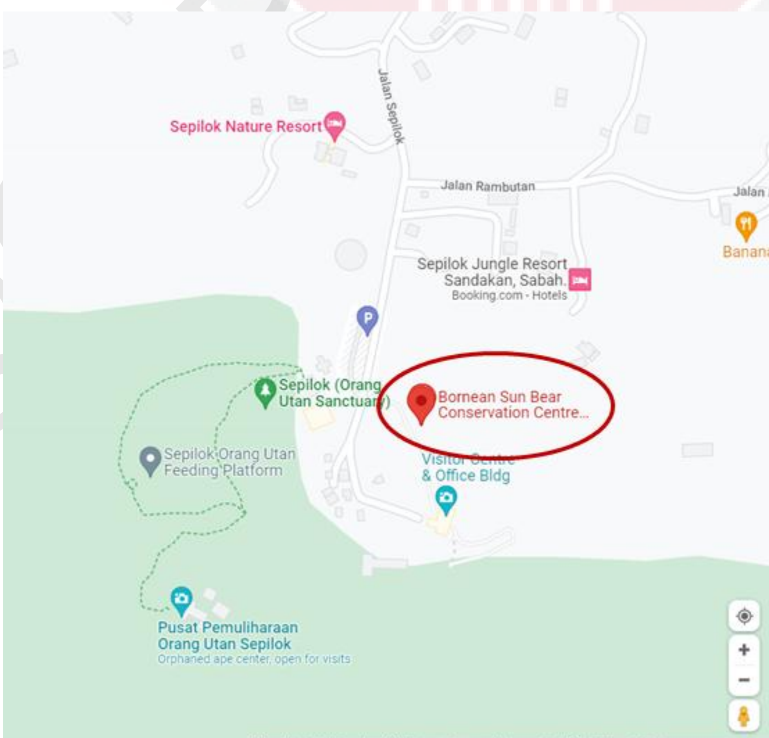


Figure 1: Map of Sepilok. The red circle indicates the place of interest in this study, which is Bornean Sun Bear Conservation Centre, Sabah.

Bornean Sun Bear Conservation Centre (BSBCC) is situated in a forest area named Sepilok, approximately 26 km away from Sandakan city in the state of Sandakan. The coordinates for BSBCC in decimal degrees are 5.864887018204693, 117.94984431411996 and it is located just opposite Sepilok Orangutan Rehabilitation Centre. BSBCC was founded in 2008 in Sabah, Malaysia, with purposes including increasing global awareness of sun bears and offering care and rehabilitation for rescued bears. BSBCC is housing 45 sun bears as of September 2022.

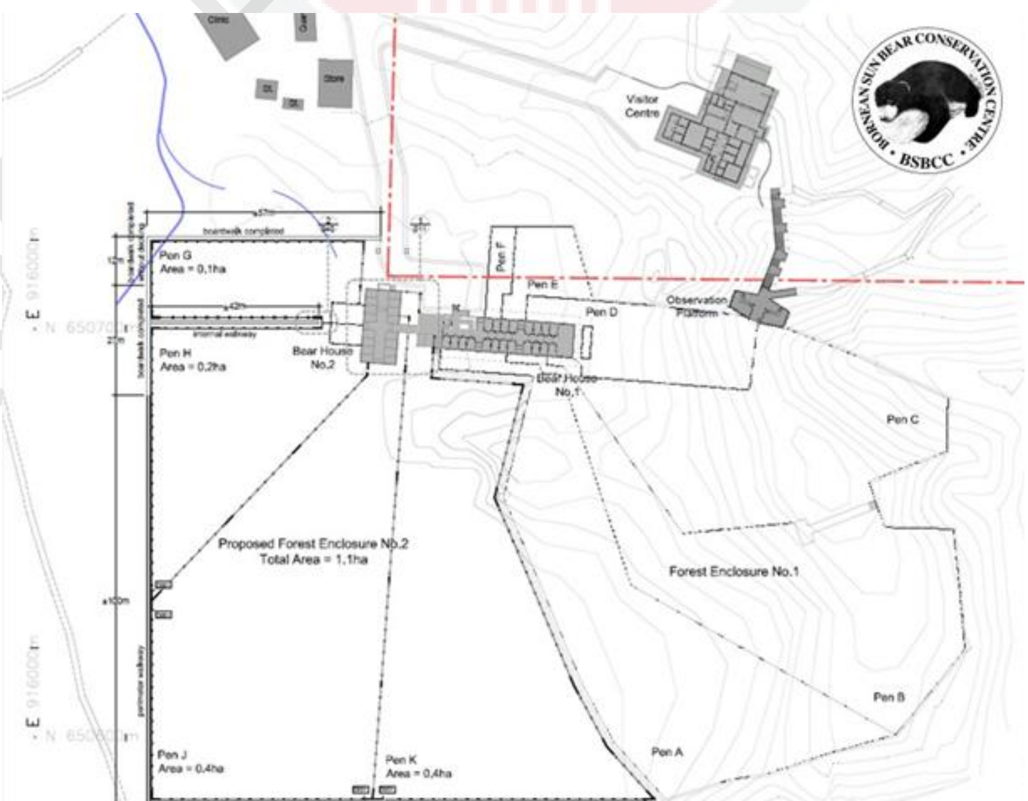


Figure 2: Floor plan of BSBCC. The red circle indicates the indoor housing is surrounded by a fenced outdoor forest enclosure.

3.2 Animal and Management

This study involves all 45 bears in BSBCC, which is considered as convenience sampling that has been given access licenses from Sabah Biodiversity Centre (SaBC) and Institutional Animal Care and Use Committee (IACUC). All the bears are clinically healthy except for one bear that has signs of cerebellar disease and is immunocompromised.

Table 2: Information on the sun bears in BSBCC used in this study.

	Category	Number
Sex	Males	18
	Females	27
Age	Juvenile: ≤5	9
	Subadult: 6 to 10	9
	Adult: >10	27

BCS	<3	32
	≥3	13
Management	Indoor	13
	Outdoor	32
Housing	Individual	17
	Group	28

Cleaning and brushing of the bear cages were done once every day in the morning using water. Moreover, quaternary ammonium compounds (QAC) were used once every week to disinfect the cages. Deworming was administered every six months using Praziplus® (active ingredients: praziquantel and albendazole) and Drontal® (active ingredients: febantel, pyrantel and praziquantel).

3.3 Sample Collection

All the procedures have been approved by Sabah Biodiversity Centre (SaBC) and Institutional Animal Care and Use Committee (IACUC) prior to the period of study. Fresh faecal samples were collected either in the morning or in the afternoon from the cages of the bears using latex gloves labelled with bear names. The gloves containing faeces were then stored in the fridge at 4°C. Sample collection was done twice for each bear with intervals of 1 to 3 days.

For blood samples, immobilization and sedation were conducted by the resident veterinarian using Tiletamine-Zolazepam HCl, (3 mg/kg, Zoletil® 100, Virbac, Carros, France) and Xylazine HCl (1 mg/kg, Xylazil-100, NSW, Australia) intramuscularly. Ketamine HCl (2 mg/kg, Narketan® 10, Buckingham, UK) was used for induction top-up if necessary and the bear was then maintained with isoflurane. Six mL of blood samples were collected using venipuncture of the cephalic vein into blood tubes. It should be noted that the blood sampling is done during their routine health check-up under general anaesthesia with the frequency of collection being once per bear for this study. Recovery of the animal from anaesthesia was monitored using an anaesthesia record form (Figure 13 and 14) until fully recovered.

3.4 Sample Processing and Examination

3.4.1 Gastrointestinal parasites

Faecal floatation and sedimentation were carried out using the same procedures for diagnosing gastrointestinal parasites in Parasitology Lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia. For faecal floatation, 1 g of faeces was put into a beaker and 1 mL of tap water was added. The faeces was emulsified with a spatula followed by adding 40 mL of sodium nitrate. The mixture was then strained through into another beaker using a tea sieve. Subsequently, the filtered mixture was poured into a glass vial until a meniscus appeared at the mouth of the vial. A coverslip was placed on the meniscus and the mixture was left to stand for 30 minutes. When the time was up, the coverslip was lifted and placed on a clean glass slide followed by an examination of the whole coverslip under a light microscope (10x and 40x objective lens).

For simple sedimentation, 4 g of faeces was placed into a beaker and emulsified using a spatula mixed with 100 mL of tap water. The suspension was poured through a tea sieve into a conical jar and allowed to sediment for 15 minutes. Consecutively, the supernatant was decanted to about 10 mL of sediment. The jar was then refilled with tap water and allowed to sediment again for 15 minutes. The suspension was repeatedly allowed to sediment, decant and refilled until the supernatant turns clear. The clear supernatant was then removed to the 10 mL mark and the sediment was emulsified. A few drops of sediment were diluted into a glass slide and covered with a cover slip. The glass slide was then observed under a light microscope (10x and 40x objective lens).

The parasite burden grading used was the same for diagnosing gastrointestinal parasites in Parasitology Lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Table 3: Parasite Burden Grading

Grade	Characterization
+	1 ovum present in some fields
+ +	1 ovum present in almost all fields
+ + +	>1 ova present in almost all fields
+ + + +	Too many to count

3.4.2 Haemoparasites

The blood samples collected were processed into thin blood smears and stained with Diff-Quik stain (Rapidiff™, Kuala Lumpur, Malaysia). The procedures of Diff-Quik staining were done following procedures by the University of Bristol. The first step was to dip the glass slide into the first pot containing fixative solution for one second for five times then the excess fluid was drained onto a paper towel. Next, the slide was dipped into the second pot containing the eosinophilic stain for five times, each time lasting one second. Excess fluid was also drained onto a paper towel followed by dipping into the third pot containing basophilic stain for one second for five times. Excess fluid was then drained onto a paper towel again, the slide was subsequently rinsed under gently running tap water. The slide was allowed to air-dry and then mounted with dibutylphthalate polystyrene xylene (DPX) solution to prevent fading of the stain.

The glass slides were brought back to Parasitology Lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia to be examined under a light microscope (100x objective lens). Parasite burden was counted using a formula by Centers for Disease Control and Prevention (CDC): % parasitemia = (parasitized RBCs/total RBCs) × 100.

3.5 Data Analysis

The first part of data analysis includes descriptive statistics on prevalence using the formula: Prevalence = (Number of positive cases/Number of sample population) × 100. The second part of the data analysis was using Fisher's Exact Test on GraphPad Software to determine if there are non-random associations between two categorical variables. The variables of interest were age, sex, body condition score, faecal score, management and housing.

4.0 RESULTS

4.1 Types of internal parasites

This study found 5 different types of gastrointestinal parasite eggs and 1 type of haemoparasite namely *Trichuris* sp. (**Figure 3**), *Ancylostoma* sp. (**Figure 4**), protozoa oocyst (**Figure 5**), *Strongyloides* sp. (**Figure 6**), a cestode (**Figure 7**) and *Babesia* sp. (**Figure 8 and 9**).

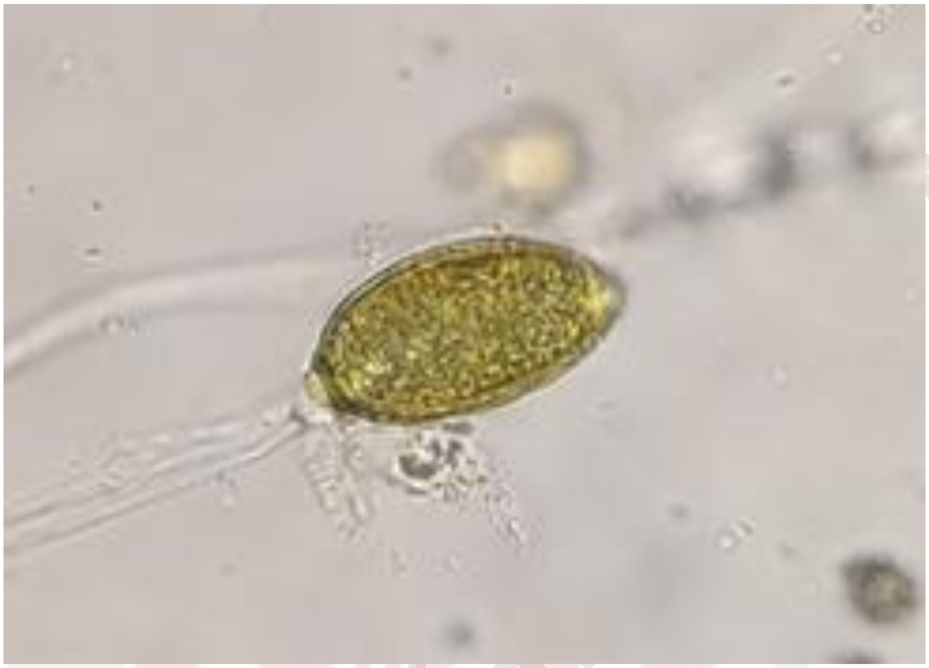


Figure 3: *Trichuris* sp. (400x total magnification)

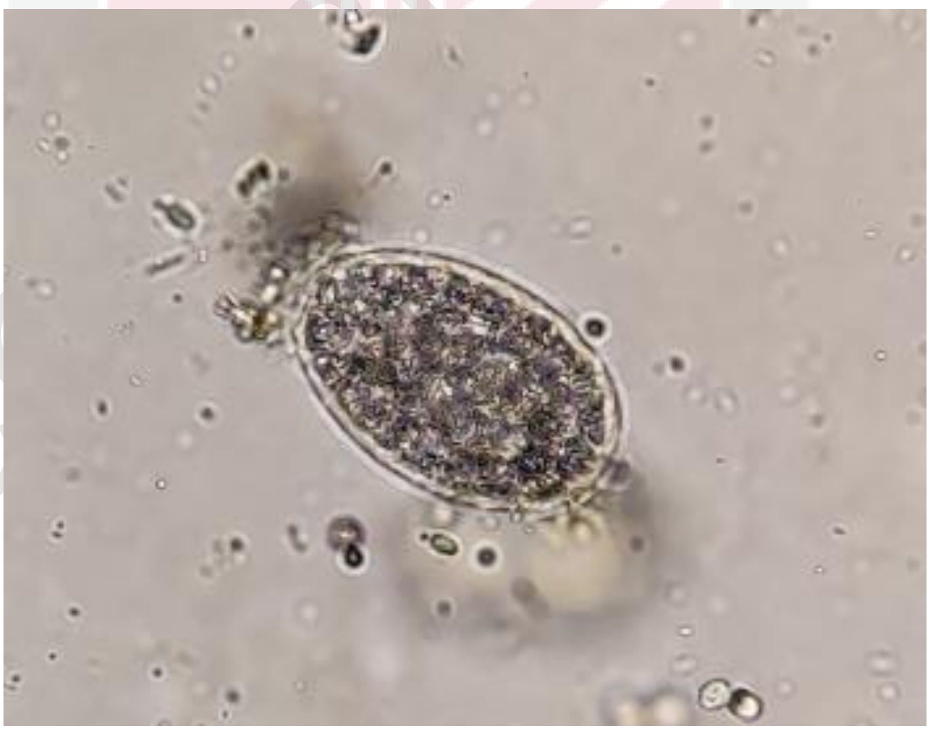
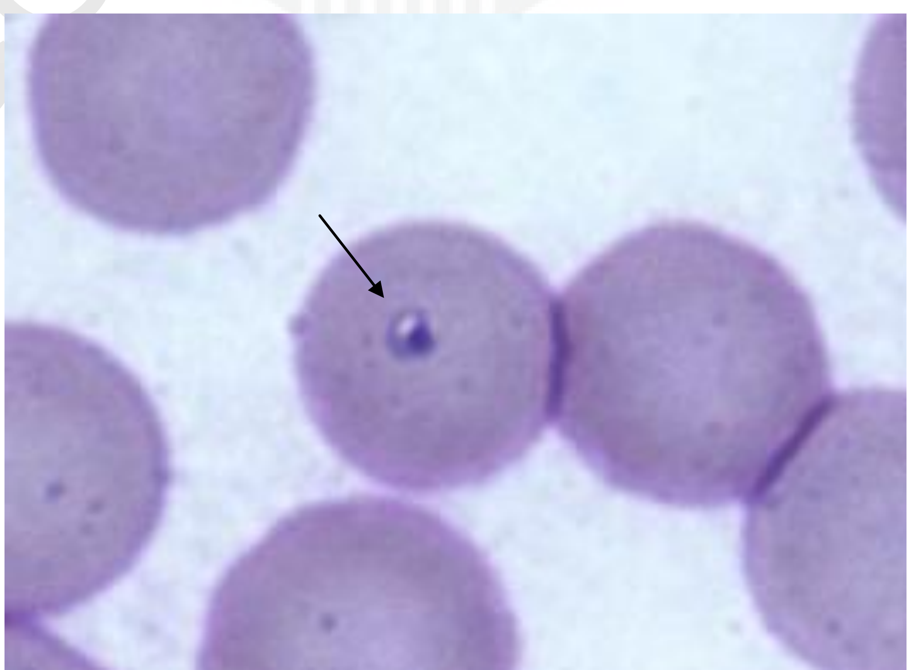
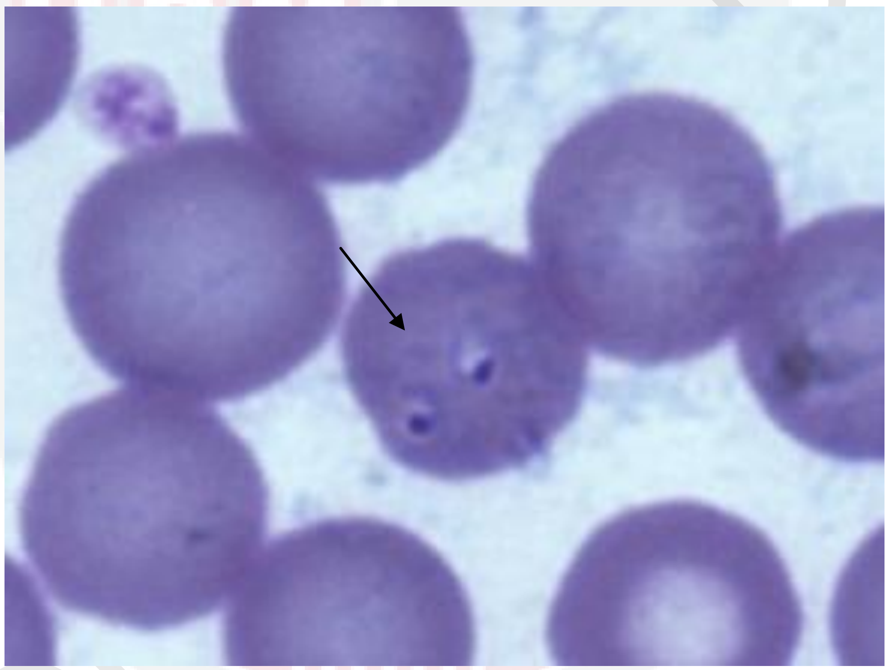


Figure 5: Protozoa oocyst (400x total magnification)

Figure 4: *Ancylostoma* sp. (400x total magnification)

Figure 7: Cestode (400x total magnification)





4.2 Prevalence of internal parasitic infestations

The overall prevalence of internal parasites in Bornean sun bears at BSBCC was 68.89% (95% CI: 53.35-81.83). Out of 45 samples, 31 (68.89%) bears were detected for

Figure 8: *Babesia* sp. (1000x total magnification)

gastrointestinal parasites with 28 (90.3%) of them having a mild parasitic load (1+). *Trichuris* sp. was the most prevalent parasite with 19 cases (42.22%), followed by *Ancylostoma* sp. with 14 cases (31.11%), protozoa account for 4 cases (8.89%), *Strongyloides* sp. with 3 cases (6.67%) and cestode with 2 cases (4.44%). For haemoparasites, *Babesia* sp. was found in 3 out of 21 Bornean sun bears (14.28%). 2 out of 3 cases have 0.5% parasitism while the other has 0.4% parasitism.

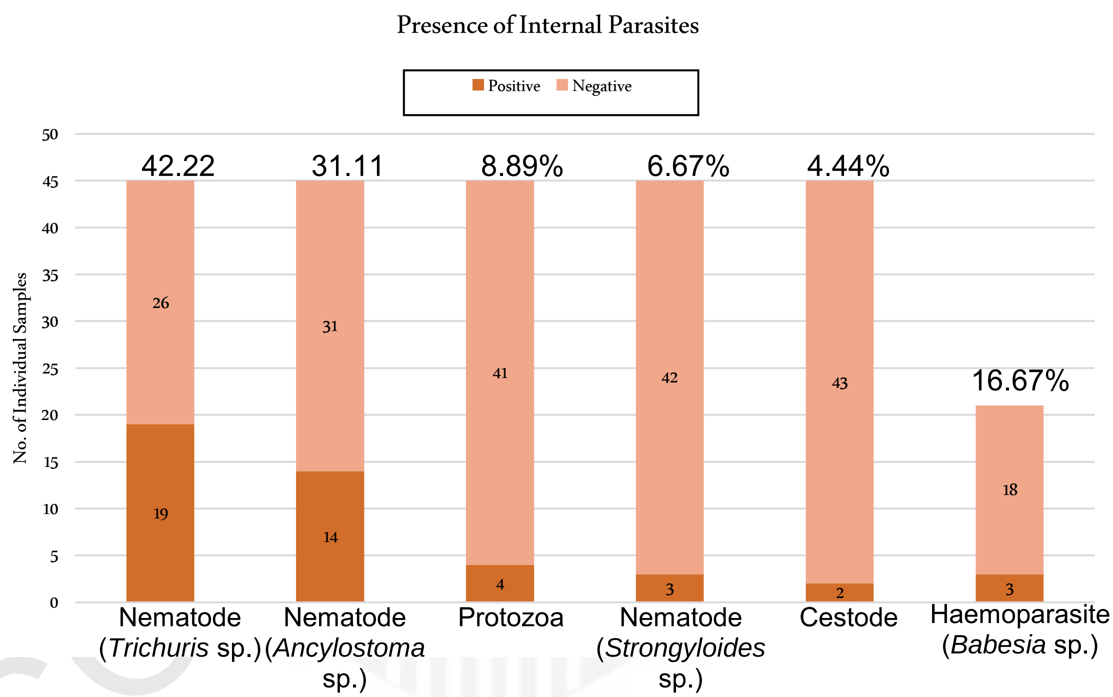


Figure 10: Presence of internal parasites in Bornean sun bears in BSBCC

Out of 31 sun bears that were found to be positive for gastrointestinal parasites, 28 (90.3%) were graded as 1+ while 3 (9.7%) were graded as 2+. Additionally, 22 cases (71%) were single infestations whereas 9 cases (29%) were mixed infestations.

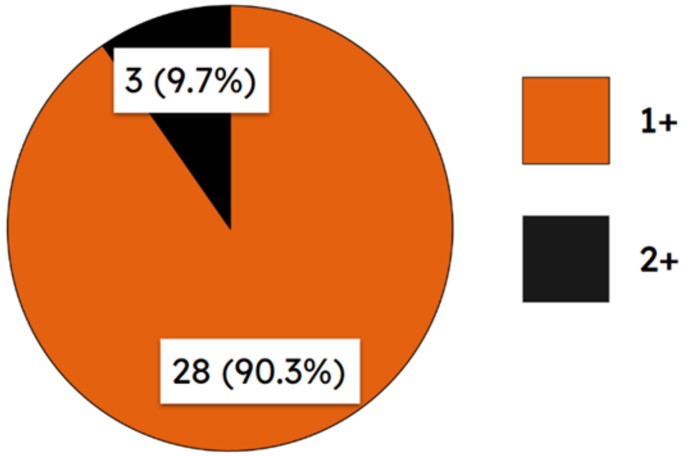


Figure 11: Gastrointestinal parasite burden in Bornean sun bears in BSBCC

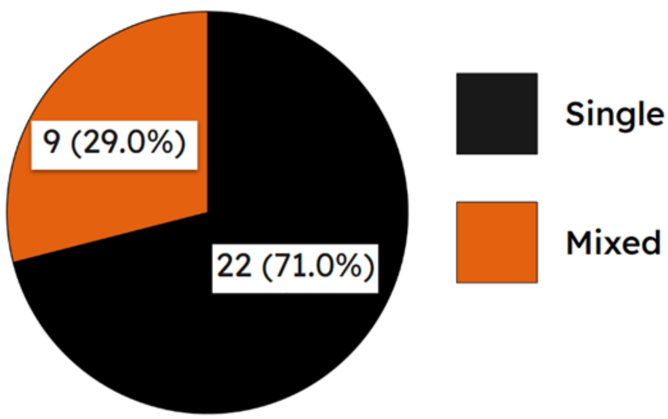


Figure 12: Types of gastrointestinal parasite infestation in Bornean sun bears in BSBCC

4.3 Association of different variables with internal parasitic infestations

Based on the Fisher's Exact Test, statistically significant associations were shown between the prevalence of gastrointestinal parasitic infestations and the variable of housing (p=0.0210). However, the prevalence of gastrointestinal parasitic infestations was not associated with sex, age, body condition score and management (**Table 4**). Furthermore, the prevalence of *Trichuris* sp. parasitic infestations was shown to be significantly associated with group-living housing (p=0.0001) (**Table 5**). On the other hand, there were no variables that were found to be associated with the prevalence of haemoparasitic infestations (**Table 6**). Faecal score was excluded from Fisher's Exact Test due to the lack of distinct differences between the samples.

Table 4: Factors associated with the prevalence of gastrointestinal parasitic infestations in Bornean sun bears in BSBCC

Variables	Categories	Parasitic Infestations		p-value
		Positive N (%)	Negative N (%)	
Sex	Male	12 (66.6)	6 (33.3)	1.0000
	Female	19 (70.4)	8 (29.6)	
Age	Juvenile: ≤5	7 (77.8)	2 (22.2)	0.7363
	Subadult: 6 to 10	7 (77.8)	2 (22.2)	
	Adult: >10	17 (63.0)	10 (37.0)	
BCS	<3	24 (75)	8 (25)	0.2861
	≥3	7 (53.8)	6 (46.2)	
Management	Indoor	7 (53.8)	6 (46.2)	0.2861
	Outdoor	24 (75)	8 (25)	
Housing	Individual	8 (47.1)	9 (52.9)	**0.0210**
	Group	23 (82.1)	5 (17.9)	

Table 5: Association between housing and the prevalence of gastrointestinal parasitic infestations in Bornean sun bears in BSBCC

	Individual		Group		
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	p-value
Ancylostoma sp.	5 (29.4)	12 (70.6)	9 (32.1)	19 (67.9)	1.0000
Trichuris sp.	1 (5.9)	16 (94.1)	18 (64.3)	10 (35.7)	**0.0001**
Strongyloides sp.	0	17 (100)	3 (10.7)	25 (89.3)	0.2788
Cestode	1 (5.9)	16 (94.1)	1 (3.6)	27 (96.4)	1.0000
Protozoa	2 (11.8)	15 (88.2)	2 (7.1)	26 (92.9)	0.6262

Table 6: Factors associated with the prevalence of haemoparasitic infestations in Bornean sun bears in BSBCC

Variables	Categories	Parasitic Infection		p-value
		Positive N (%)	Negative N (%)	
Sex	Male	1 (14.3)	6 (85.7)	1.0000
	Female	2 (14.3)	12 (85.7)	
Age	Juvenile: ≤5	0	7 (100)	0.2594
	Subadult: 6 to 10	0	4 (100)	
	Adult: >10	3 (0.3)	7 (0.7)	
BCS	<3	2 (13.3)	13 (86.7)	1.0000
	≥3	1 (16.7)	5 (83.3)	
Management	Indoor	2 (25)	6 (75)	0.5308
	Outdoor	1 (7.7)	12 (92.3)	
Housing	Individual	2 (0.4)	3 (0.6)	0.1278
	Group	1 (6.3)	15 (93.8)	

5.0 DISCUSSION

The two most abundant parasites in my study were *Trichuris* sp. and *Ancylostoma* sp., these two parasites were analogous to the findings of Jenantika *et al.* (2019). Despite having a high prevalence of internal parasites, the parasite burden is low. This could be one of the reasons to explain the absence of clinical signs related to parasitic infestations. Another contributing factor to the lack of clinical manifestations could be the regular deworming practices in BSBCC, which is approximately once every 6 months. Based on a previous study by Parsani *et al*. (2001), captive animals typically do not display alarming signs of parasitism because of the deployment of routine deworming.

The presence of 29% of mixed infestations can be attributed to the environment that the bears share which promotes the transmission of different parasites between the bears. In 5 out of 9 cases of mixed parasitic infestations, *Ancylostoma* sp. and *Trichuris* sp. are present. This is aligned with my findings which found these two genera being the most abundant.

The consequences of *Trichuris* spp. and *Ancylostoma* spp. infestations in sun bears have not been documented. In the faeces or gastrointestinal tracts of other bears such as Asiatic black bears and brown bears, *Trichuris* spp. was previously reported (Zoumin et al., 2022). The center for Food Security and Public Health states that many infected animals show no clinical symptoms but heavy burdens can lead to diarrhoea, weight loss, unthriftiness and/or anaemia. For *Ancylostoma* sp. infestations, it was reported in all bear species except Andean bears according to Borka-Vitális *et al.* (2017) and can give rise to iron-deficiency anaemia and protein malnutrition in humans and animals (Xie et al., 2017).

The type of housing was the only variable that was found to be associated with the prevalence of gastrointestinal parasitic infestations with group housing yielding more positive cases. A previous study by Moudgil *et al*. (2020) observed similar results, animal kept in herds showed greater prevalence compared to individually housed animals. This can be connected to the fact that overcrowding and competition for feed and water typically cause stress and decreased immunity, making animals more susceptible to parasitic infestations (Singh et al., 2009; Dhoot et al., 2002).

Pertaining to *Trichuris* sp. infestation was detected to be associated with the type of housing, there can be several reasons for it but the eggs of *Trichuris* spp. could be the one to blame. They are hardy, have the ability to persist for years and are resistant to disinfection. The only entirely efficacious way to exterminate whipworm eggs from the environment is desiccation or incineration (Dysko et al., 2022).

For haemoparasites, the presence of *Babesia* sp. was analogous to a previous study by Chua *et al.* (2022) which also found *Babesia* sp. in a Bornean sun bear at BSBCC. The predisposing factor could be the existence of Ixodes ticks found on the sun bears in the same study. In addition, Chua *et al.* (2022) discovered that the *Babesia* isolates found are sharing a common node with *Babesia gibsoni* but with a low bootstrap value (<70%) after conducting DNA analysis. It is most likely that the *Babesia* sp.

found in this current study are of the same isolate as results by Chua *et al.* (2022). Furthermore, a previous study by Mohammed *et al*. (2017) found *Babesia gibsoni* and *Babesia vogeli* in stray dogs in Sabah but there are currently no reports on babesiosis in Sabah wild animals. One of the treatment options that can be given is diminazene aceturate (2mg/kg) which was used in a case study by Zubaidah *et al.* (2020). It was found to be successful in resolving infestation in the case of babesiosis in a Malayan sun bear after three times of intramuscular injections at one-week intervals. Some of the ways to prevent and control babesiosis include prophylactic therapy and vector controls like acaricides (Pfeffer et al., 2018; Khan et al., 2015).

6.0 CONCLUSION

This study revealed the presence of internal parasites in Bornean sun bears at BSBCC (68.89%) with nematodes being the predominant parasite found. Despite having a high prevalence of internal parasites, the parasite burden was low hence they did not cause any clinical signs related to the infestation. Group-living housing was the only risk factor found to be associated with the presence of gastrointestinal parasitic infestations. Thus, good management practice and preventive medicine should be advised to control the progression of clinical disease.

7.0 RECOMMENDATIONS

A molecular approach such as PCR could be used to identify the internal parasites up to the species level which would greatly help in the documentation of internal parasites in sun bears. In addition, other more robust faecal techniques can be used, for instance, formalin-ether sedimentation. Giemsa staining instead of Diff-Quik could also be utilized as it is preferable for the detection of haemoparasites. Studies on sun bear parasites could be conducted at other locations in Malaysia which could subsequently be used for results comparison. Research could also be done on the resistance of gastrointestinal parasites of sun bears against different anthelmintics used.

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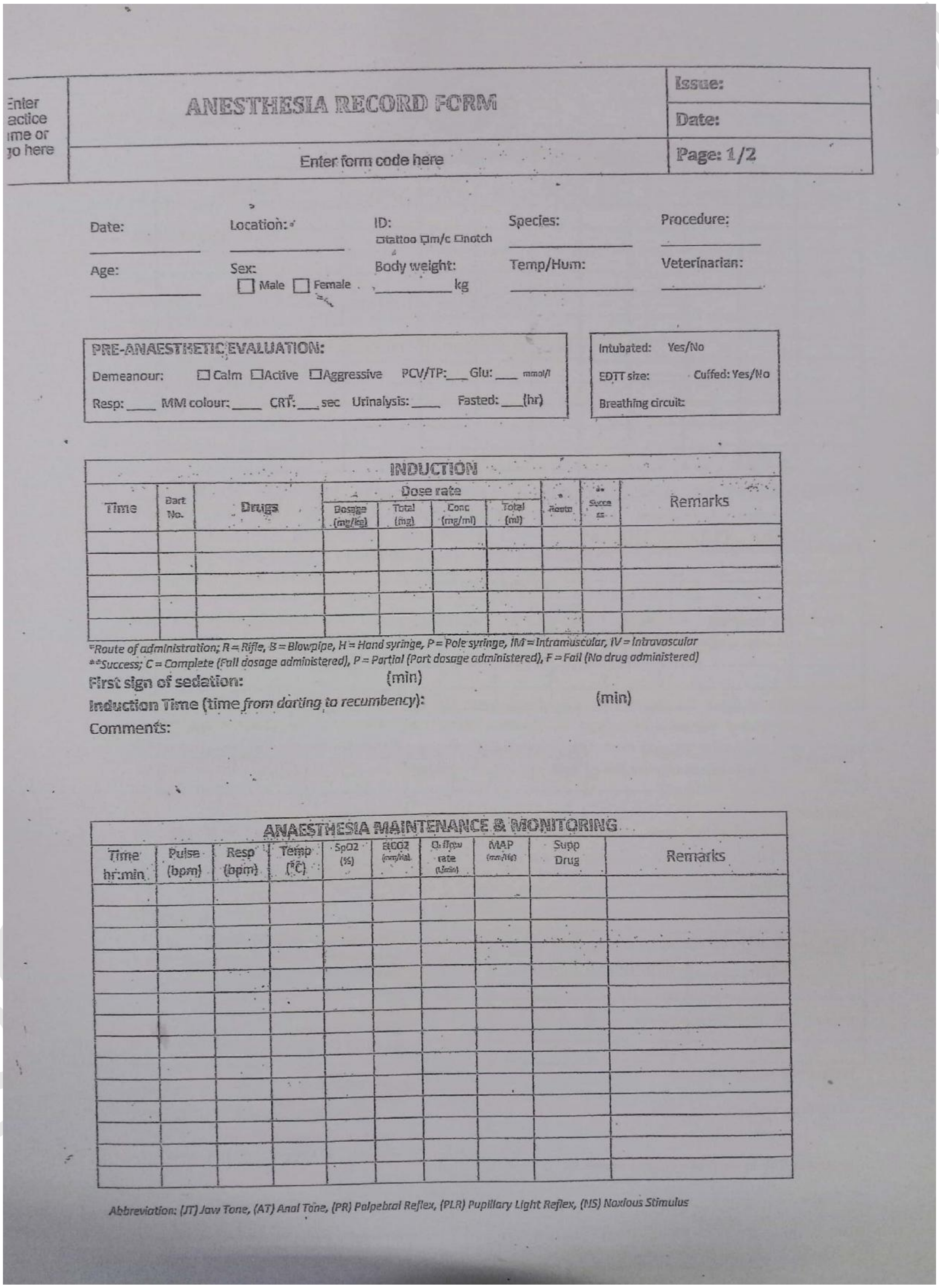
9.0 APPENDICES



Enter practice name or logo here

ANESTHESIA RECORD FORM

Issue:
Date:
Page: 1/2

Enter form code here

Date: ____ Location: ____ ID: ☐tattoo ☐m/c ☐notch Species: ____ Procedure: ____

Age: ____ Sex: ☐ Male ☐ Female Body weight: ____ kg Temp/Hum: ____ Veterinarian: ____

PRE-ANAESTHETIC EVALUATION:

Demeanour: ☐ Calm ☐Active ☐Aggressive PCV/TP:___ Glu: ___ mmol/l

Resp: ____ MM colour: ____ CRT: ___ sec Urinalysis: ____ Fasted: ___(hr)

Intubated: Yes/No

EDTT size: Cuffed: Yes/No

Breathing circuit:

INDUCTION

Time	Dart No.	Drugs	Dose rate				Route*	Success**	Remarks
			Dosage (mg/kg)	Total (mg)	Conc (mg/ml)	Total (ml)			

Route of administration; R = Rifle, B = Blowpipe, H = Hand syringe, P = Pole syringe, IM = Intramuscular, IV = Intravascular

***Success; C = Complete (Full dosage administered), P = Partial (Part dosage administered), F = Fail (No drug administered)*

First sign of sedation: (min)

Induction Time (time *from darting to recumbency*): (min)

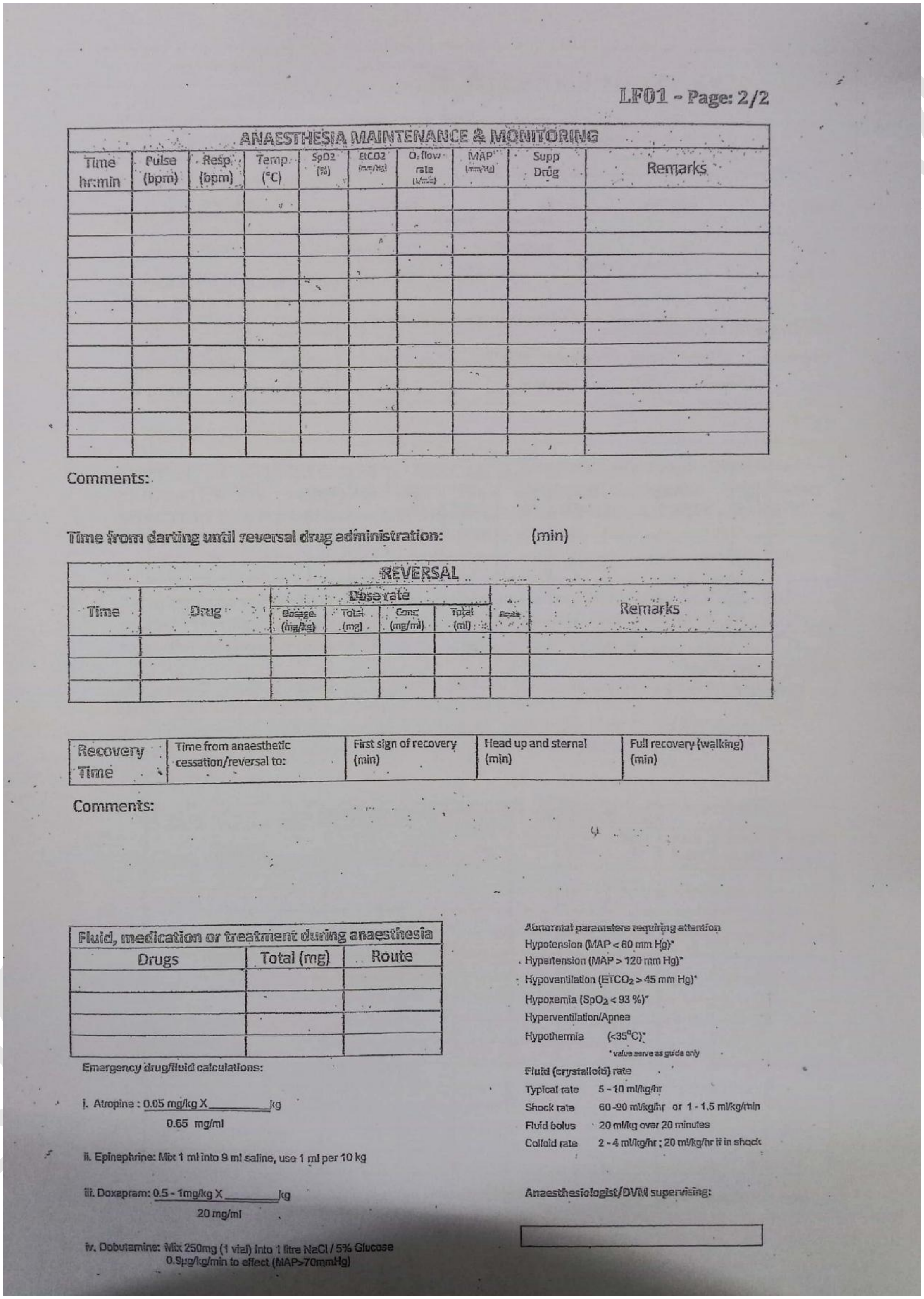
Comments:

ANAESTHESIA MAINTENANCE & MONITORING

Time hr:min	Pulse (bpm)	Resp (bpm)	Temp (°C)	SpO2 (%)	EtCO2 (mmHg)	O2 flow rate (L/min)	MAP (mmHg)	Supp Drug	Remarks

Abbreviation: (JT) Jaw Tone, (AT) Anal Tone, (PR) Palpebral Reflex, (PLR) Pupillary Light Reflex, (NS) Noxious Stimulus

Figure 13: Anaesthesia Record Form page 1



LF01 - Page: 2/2

ANAESTHESIA MAINTENANCE & MONITORING									
Time hr:min	Pulse (bpm)	Resp (bpm)	Temp (°C)	SpO2 (%)	EtCO2 (mmHg)	O_2 flow rate (L/min)	MAP (mmHg)	Supp Drug	Remarks

Comments:

Time from darting until reversal drug administration: (min)

REVERSAL							
Time	Drug	Dose rate				Route	Remarks
		Dosage (mg/kg)	Total (mg)	Conc (mg/ml)	Total (ml)		

Recovery Time	Time from anaesthetic cessation/reversal to:	First sign of recovery (min)	Head up and sternal (min)	Full recovery (walking) (min)

Comments:

Fluid, medication or treatment during anaesthesia		
Drugs	Total (mg)	Route

Emergency drug/fluid calculations:

i. Atropine : 0.05 mg/kg X ________ kg / 0.65 mg/ml

ii. Epinephrine: Mix 1 ml into 9 ml saline, use 1 ml per 10 kg

iii. Doxapram: 0.5 - 1mg/kg X ________ kg / 20 mg/ml

iv. Dobutamine: Mix 250mg (1 vial) into 1 litre NaCl / 5% Glucose 0.5μg/kg/min to effect (MAP>70mmHg)

Abnormal parameters requiring attention

Hypotension (MAP < 60 mm Hg)*

Hypertension (MAP > 120 mm Hg)*

Hypoventilation ($ETCO_2$ > 45 mm Hg)*

Hypoxemia (SpO_2 < 93 %)*

Hyperventilation/Apnea

Hypothermia (<35°C)*

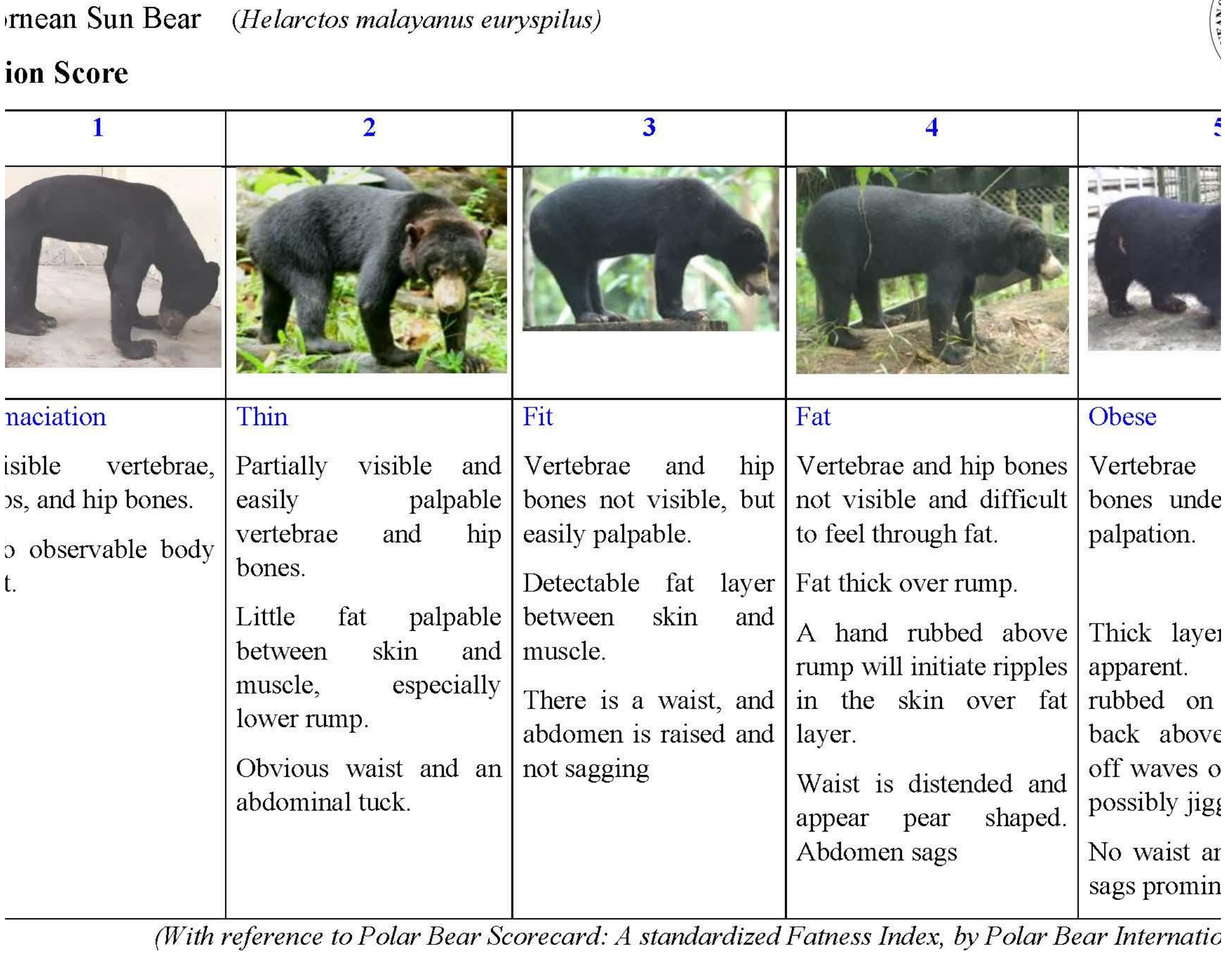
* value serve as guide only

Fluid (crystalloid) rate

Typical rate	5 - 10 ml/kg/hr
Shock rate	60 - 90 ml/kg/hr or 1 - 1.5 ml/kg/min
Fluid bolus	20 ml/kg over 20 minutes
Colloid rate	2 - 4 ml/kg/hr ; 20 ml/kg/hr if in shock

Anaesthesiologist/DVM supervising:

Figure 14: Anaesthesia Record Form page 2



ɪrnean Sun Bear (*Helarctos malayanus euryspilus*)

ion Score

1	2	3	4	5
naciation	Thin	Fit	Fat	Obese
isible vertebrae, ɔs, and hip bones. ɔ observable body t.	Partially visible and easily palpable vertebrae and hip bones. Little fat palpable between skin and muscle, especially lower rump. Obvious waist and an abdominal tuck.	Vertebrae and hip bones not visible, but easily palpable. Detectable fat layer between skin and muscle. There is a waist, and abdomen is raised and not sagging	Vertebrae and hip bones not visible and difficult to feel through fat. Fat thick over rump. A hand rubbed above rump will initiate ripples in the skin over fat layer. Waist is distended and appear pear shaped. Abdomen sags	Vertebrae bones unde palpation. Thick layeɪ apparent. rubbed on back above off waves o possibly jigg No waist aɪ sags promin

(With reference to Polar Bear Scorecard: A standardized Fatness Index, by Polar Bear Internatic

Figure 15: Body Condition Score Guidelines

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	30th August 2022
AUP No.:	UPM/IACUC/AUP-U042/2022
Project Title:	Prevalence of Internal Parasites in Bornean Sun Bears (*Helarctos malayanus*) at Bornean Sun Bear Conservation Centre, Sabah.
Principal Investigator:	Dr. Azlan Che' Amat
Members:	Dr. Nor Azlina Abdul Aziz, Ong Yu Qi
Attending Veterinarian:	Dr. Yeoh Boon Nie
Committee Decision:	The committee has reviewed and approved the proposed animal utilisation protocol, subject to relevant permit and/ or owner's consent.
Project Classification:	Acute
Category of Invasiveness:	B
Source of Animals:	Bornean Sun Bear Conservation Centre (BSBCC), 90000 Sandakan, Sabah.
Number of Animals Approved:	44 Bears
Housing:	Bornean Sun Bear Conservation Centre (BSBCC).
Duration:	30th August 2022 – 30th August 2023

Ethical approval is required in the case of amendments to the approved animal utilisation protocol (AUP). Please apply using Form 105. Kindly submit a final/annual report (Form 106) upon study completion, or before expiry of approval.

PROF. DR. ABDUL RAHMAN OMAR
Chairman
Institutional Animal Care and Use Committee
Universiti Putra Malaysia.

Telephone: +6088 369 000/ +6088 369 312
Facsimile: +6088 250 753
Email address: sabc@sabah.gov.my
Website URL: www.sabc.sabah.gov.my

(Please quote your licence reference number and date if you have any queries)

SABAH BIODIVERSITY COUNCIL
(Majlis Biodiversiti Sabah)
c/o Sabah Biodiversity Centre (SaBC)
Chief Minister's Department,
19th Floor, Block A,
Sabah State Administrative Centre,
88400 Kota Kinabalu
SABAH, MALAYSIA

LICENCE REF.NO. : JKM/MBS.1000-2/13 JLD. 2 (2)

DATE OF APPROVAL : 30TH AUGUST 2022

ACCESS LICENCE

(Section 8(1) & 15 of Sabah Biodiversity Enactment 2000)

This is to certify that:

Ong Yu Qi

Passport No./ MyKad No.:

991012-07-6468

of

Universiti Putra Malaysia

is authorized to access the following biological resources from the place(s) specified below, for **ACADEMIC PURPOSES**, upon terms and conditions hereinafter stipulated:

Research Title:
Final Year Project - Prevalence Of Internal Parasites In Bornean Sun Bears (*Helarctos malayanus*) at Bornean Sun Bear Conservation Centre, Sabah.

No.	Common name	Scientific name	Descriptions		Place(s) where access is permitted
1.	Bornean Sun Bear	*Helarctos malayanus*	Minimum 10, maximum 44 bears (6mL of blood and 10g of feces per bear)	Fecal collection after natural defecating and blood sampling during routine health checks.	Bornean Sun Bear Conservation Centre **(BBSBCC)**

Terms and conditions
(Section 24 of Sabah Biodiversity Enactment 2000)

1. This licence is valid from ..30 AUG 2022......for a period of 12 months.
2. The holder must apply relevant pass from the State Immigration of Sabah.
3. This licence is not transferable. The holder must produce this licence for inspection by the Secretary or any person authorized by him.
4. Transfer of biological resources is not allowed without the written consent of the Sabah Biodiversity Council.
5. All biological resources collected shall be taken to, stored and preserved at:
   Bornean Sun ear Conservation Centre.
6. The holder must submit progress report, dissertation or publications to Sabah Biodiversity Council.
7. Application for patent or other intellectual property rights to the relevant authority in or outside Malaysia subject to first obtaining the prior written consent of the Sabah Biodiversity Council.
8. Principle Investigator and Local collaborator is fully responsible for the conduct of their research associate/assistant under supervision; to ensure they are only allowed within the permitted areas and

LICENCE REF.NO. : JKM/MBS.1000-2/13 JLD. 2 (2)

DATE OF APPROVAL : 30TH AUGUST 2022

amount samples of biological resources taken are as approved by The Sabah Biodiversity Council.

9. Any offence committed under this Enactment may be compound under Section 36(1) of The Sabah Biodiversity Enactment (Amendment) 2017.

New: *X* Renewal:

(GERALD JETONY, PhD)
Secretary
of Sabah Biodiversity Centre