UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF GASTROINTESTINAL PARASITES IN CAPTIVE BOVIDAE AT ZOO NEGARA

KASTURI A/P NADARAJAH

PREVALENCE OF GASTROINTESTINAL PARASITES IN CAPTIVE BOVIDAE AT ZOO NEGARA

KASTURI A/P NADARAJAH

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 Gastrointestinal Parasites In Captive Bovidae At Zoo Negara", by Kasturi A/P Nadarajah 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 – Project

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DEDICATIONS

This project paper is dedicated to my support circle that had constructively made it possible for me to work towards developing my competence to serve the field of veterinary medicine

To my family,
Father Mother
Brother,
Aunties and
my extended
family and
friends

And to all my teachers who have both inspired me and driven me while providing the educational opportunity through careful facilitation and guidance

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Indeed this has been a fulfilling experience in which I had a wonderful educational experience and learn to be competent in terms of researching.

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LIST OF ABBREVIATIONS

%	Percent
g	Gram
ml	Milliliter

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 - Project

PREVALENCE OF GASTROINTESTINAL PARASITES IN CAPTIVE BOVIDAE AT ZOO NEGARA

By

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Parasitic diseases constitute one of the major problems causing mortality in captive Bovidae. Thus a study on the prevalence of gastrointestinal parasites was investigated from various species of animals from the Bovidae family housed at Zoo Negara Malaysia. A total of 14 pooled fecal samples were collected randomly from 8 species from 5 genus which are *Bos* (6), *Hippotraginae* (2), *Tragelaphus* (2), *Oryx* (2), and *Kobus* (2). All samples were examined by direct wet mount preparation, formalin ethyl acetate concentration technique and permanent stains which are trichriome and giemsa. Intestinal parasites that were found in all species were *Strongylids* (21.4%), *Moniezia sp. (*14.9%), *Capillaria sp.* (7.1%),

Cryptosporidium spp. (7.1%) and *Entamoeba sp.* (7.1%). All samples that were positive for helminths or protozoa were asymptomatic animals with low parasitic loads. Monitoring the gastrointestinal parasite of wild Bovidae in captivity is imperative in assisting zoo management in the formulation and implementation of preventive and control measures against the spread of infectious parasitic diseases among these animals within the zoo or to humans.

Keyword: Gastrointestinal parasite, Bovidae, formalin ethyl acetate concentration, helminths, protozoa

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KELAZIMAN PARASIT GASTROUSUS DI DALAM BOVIDAE TAWANAN DI ZOO NEGARA

Oleh

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2016

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Penyakit parasit merupakan salah satu masalah paling besar yang menyebabkan kematian di kalangan haiwan keluarga bovidae dalam kurungan. Oleh itu, satu kajian berkenaan kelaziman parasit-parasit gastrousus telah dijalankan ke atas pelbagai spesies haiwan daripada keluarga Bovidae yang terdapat di Zoo Negara. Sebanyak 14 sampel tinja dikumpulkan secara rawak daripada 8 spesies dari 5 genus iaitu *Bos* (6), *Hippotraginae* (2), *Tragelaphus* (2), *Oryx* (2), dan *Kobus* (2). Semua sampel telah dikaji menggunakan kaedah penyediaan pelekap basah secara langsung, teknik kepekatan formalin etil asitat dan pewarna kekal iaitu trichriome dan giemsa. Parasit usus yang dijumpai di dalam semua

spesies adalah *Strongylids* (21.4%), *Moniezia sp. (*14.9%), *Capillaria sp.* (7.1%), *Cryptosporidium spp.* (7.1%) dan *Entamoeba sp.* (7.1%). Semua sampel yang didapati positif bagi helmin atau protozoa adalah haiwan asimptomatik dengan beban parasit yang rendah. Penyeliaan parasit bagi Bovidae liar adalah mustahak dalam tugas membantu pengurusan zoo di mana pengubalan dan pelaksanaan tindakan pencegahan dan kawalan penyebaran penyakit berjangkit parasit di kalangan haiwan di zoo ataupun ke atas manusia.

Kata-kata kunci : parasit gastrousus, bovidae, helmin,protozoa

1.0 INTRODUCTION

1.1 Gastrointestinal Parasitism in captive bovidae

Zoological gardens in Malaysia exhibit wild animals from Bovidae Family for aesthetic, conservation and educational purpose. However, parasitic diseases constitute one of the major problems causing even mortality in these animals while in captivity.(Varadharajan & Pythal, 1999) Unfortunately, there has been few detailed and comprehensive studies on the prevalence of the intestinal parasites in animals housed in zoological garden. Some studies have reported on the incidence of intestinal parasitic infections in hoofed animals in one particular zoological garden in Malaysia. (Lim *et al* , 2008)

However it was a pooled sampled from the hoofed aniamals. Inadequate information on diseases and parasites of zoo animals is a major limiting factor at zoological garden (Opara & Fagbemi, 2016). Therefore, this study pioneers examining and documenting the gastrointestinal parasites profile among Bovidae Family kept in Zoo Negara and for better understanding of the gastrointestinal parasite fauna of these animals and management of the animal's health by preventing spread of infectious parasitic diseases among the herd, within the zoo or humans.

Hence, this study was undertaken to fulfill the objective of to determine the gastrointestinal parasite population in Captive Animals of the Bovidae Family at Zoo Negara

For this research, the following hypothesis was proposed:

I. The population of gastrointestinal parasites in the family of Bovidae at Zoo Negara differs according to species.

2.0 LITERATURE REVIEW

2.1 Gastrointestinal parasites of wild ruminants

Gastrointestinal parasites (GIT), which are acquired through the process of foraging, represent one of the most pervasive challenges to the survival and reproductive capacities of their herbivore hosts (Coop *et al*., 1982). There have been few detailed and comprehensive studies on the prevalence of the intestinal parasites in animals housed in zoological garden. (Lim, Ngui, Shukri, Rohela, & Naim, 2008). Helminth infections were more common than protozoic infection in captive animals. (Khatun & Begum, 2014). Moreover, the common parasites identified in wild herbivore were strongyloids, *Moniezia sp*, coccidia and stomach worm (Khatun & Begum, 2014).

2.2 Epidemiology of gastrointestinal parasites

Under natural condition, wild animals live on vast areas leading to a low genetic resistance against parasitic infections because of low exposure. However, when herds of these wild animals are kept in captivity in zoological gardens, the problem of parasitic infections can aggravate and pose a serious threat to the endangered species, occasionally causing sudden and unexpected local declines in abundance.(Muoria, 2006)

Occurrence of parasites in animals housed in zoological gardens might vary according to the type of husbandry practices, disease prophylaxis and treatment administered. Intensive husbandry of animals produces conditions which facilitates the spread of parasites. The frequent use of antihelmintics often cause resistant strains to

evolve. Moreover, the nutritional status of captive animals can also enhance or diminish their resistance to disease (Mooney, 2016)

2.3 Detection Methods of Gastrointestinal Parasite

Diagnosing gastro-intestinal parasites of bovidae which are basically ruminants requires, the parasites or their eggs/larvae to be recovered from the digestive tract of the animal or from faecal material. These are subsequently identified and quantified. The samples are examined for the presence of adult worms or their larvae, before further analysis according to standard laboratory procedures (Soulsby, 1982 and MAFF, 1986) for helminth eggs and protozoan cysts.

Gross coprological examination by direct smear method is done to detect is used for the detection of trophozoites and cysts of protozoa, and eggs and larvae of helminthes (Urquhart *et al*, 2011). In this method a small amount of feces take on a microscope slide with drop of saline to the feces and mixed thoroughly. The type of liquid added depends on what want to accomplish with the technique. It is then covered with a cover slip. Slide is examined under 10X objective continued with 40X objective. It can be used in conjuction with McMaster technique to detect low number of eggs. (Urquhart *et al*, 2011). Besides this, formol ether sedimentation techniques can be used and eggs or oocysts identified using the light microscope at x40 objective.

2.4 Gastrointestinal Parasites in Domestic Ruminant

On the basis of taxonomic criteria, it has been estimated that about 20-40% of the parasites commonly recorded in wild ruminants are also found in domestic animals. (Woodford, 1986). Gastrointestinal parasitism in domestic ruminants are recognized asa major constraint to livestock production throughout the tropics and elsewhere (Githiori *et al.* 2004). They cause mortality in these animals (Sykes , 1994), affect growth in the animals (Kochapakdee *et al*. 1995) and lowered productivity (Perry and Randolf , 1999). Prevalence of gastrointestinal helminthes is reported to be ranging from 0.72% to 84.1 5 in domestic animals from various parts of the world. The risk fastors associated with the prevalence of helminthes would include age, sex, weather condition and husbandry or management practices (Miller *et al.* 1998, Raza *et al.* 2007)

3.0 MATERIALS AND METHODS

3.1 Study site

This study was conducted at Zoo Negara which is located at Ulu Klang, north-east of Kuala Lumpur. The park was officially opened on 14 November 1963 and has a total of over 5000 animals from 459 species of mammals, birds, reptiles, amphibians and fish coming from South East Asia, Asia, Africa and American continents. Over the years, the zoo has developed itself into an open concept zoo with over 90% of its animals kept in spacious exhibits with landscape befitting its nature. The bovidaes in the zoo were kept under the Savannah section and Mammal's section.

3.2 Animals and husbandry

The study covered 8 species from the family of Bovidae which can be categorized into 5 genuses. The animals which comes under the genus bos are as follows: Banteng (*Bos javanicus),* Ankole (*Bos taurus),* Malayan Gaur (*Bos gaurus hubbanki*), Indian gaur (*Bos gaurus gaurus).* Genus *Kobus* is represented by Red lechwe (*Kobus leche).* Genus *Hippotraginae* has the Sable antelope (*Hippotragus niger).* The genus *Oryx* is represented by Oryx (*Oryx dammah*) and lastly the genus *Tragelaphus* has Nyalas *(Tragelaphus angasii).* Most of the animals lived either singly or in a herd with a cement or sandy floor covered by wood shavings or surrounded by iron railing at night and will be placed in open-air exhibition area with sandy and grassy soil at day time. Animals within the facilities were cleaned daily with high-pressure pipe water.

However, the soil and sand facilities were not cleaned with water but, dung was removed daily except at savannah enclosure

3.3 Sample collection

A total of 14 faecal samples were collected randomly from various animal species mentioned earlier. The samples were collected twice from each species of animals listed in Table 1. Each pooled sample consist of 3 fecal samples. Collections of fecal samples were done early in the morning with the assistance of the zoo staff before the zoo is opened out for exhibition in the morning. Only fresh fecal samples were collected into plastic bag and kept in icebox with cold packs to keep the samples chilled and not frozen. All samples were processed immediately at Veterinary Parasitology Lab of Faculty of Veterinary Medicine, Universiti Putra Malaysia.

Genus	**Common name**	**Scientific Name**	**No. of animals**	**No. samples collected(pooled,1 =3 samples)**
Bos	Banteng	*Bos javanicus*	23	2
Bos	Ankole	*Bos taurus*	5	2
Bos	Malayan Gaur	*Bos gaurus hubbanki*	2	1
Bos	Indian Gaur	*Bos gaurus gaurus*	8	1
Kobus	Red letwe	*Kobus letche*	8	2

Hippotraginae	Sable antelope	*Hippotragus niger*	6	2
Oryx	Oryx	*Oryx dammah*	1	2
Tragelaphus	Nyalas	*Tragelaphus angasii*	5	2

Table 3.3: List of sampled animals with their scientific name and its population at Zoo Negara

3.4 Sample Processing

All samples were processed and examined by direct wet mount preparation, McMaster technique, formalin ethyl acetate concentration technique and permanent stains such as trichrome and giemsa stains. Direct wet mount was done by taking some fecal samples using wood applicator and mixing it with a drop of saline unto the glass slide. A drop of Lugos Iodine added before applying cover slip on top of it to be examined under the power of 10X objective under microscope. This method used for the detection of trophozoites and cysts of protozoa, and eggs and larvae of helminthes. (Soulsby, 1982)

Quantitative examination was done using Mcmaster technique where by 2g faeces was diluted with 30ml of concentrated salt solution. It was mixed thoroughly, filtered through fine mesh net. The solution is then withdrawn using a pipette and filled up into the Mcmaster chamber and it is viewed under microscope at a magnification of 10x objective. Eggs of helminthes and coccidial oocyst were to be identified and quantified using this method. The eggs per count is calculated as follow:

$$\frac{\text{eggs /oocyst}}{weight\ of\ faeces} \times \frac{original\ volume\ used}{2(0.15)ml}$$

The processing of sample is followed by formal ethyl-acetate sedimentation method when a gram of faeces is mixed into 10ml of 4% formalin and mixed well. It is then filtered and added with 2ml of diethyl ether which will absorb the debris and fatty components from the fecal material. The sample is centrifuged at 2000rpm for 5 minutes. A sediment will be obtained with the debris and formalin separated as shown in Figure 3.4. The sediment is obtained using a wood applicator and a fecal smear is made on 2 clean slides for each species. The smears will be fixed in methanol for 5 minutes and stained with 2 different stains, namely the trichrome and giemsa.

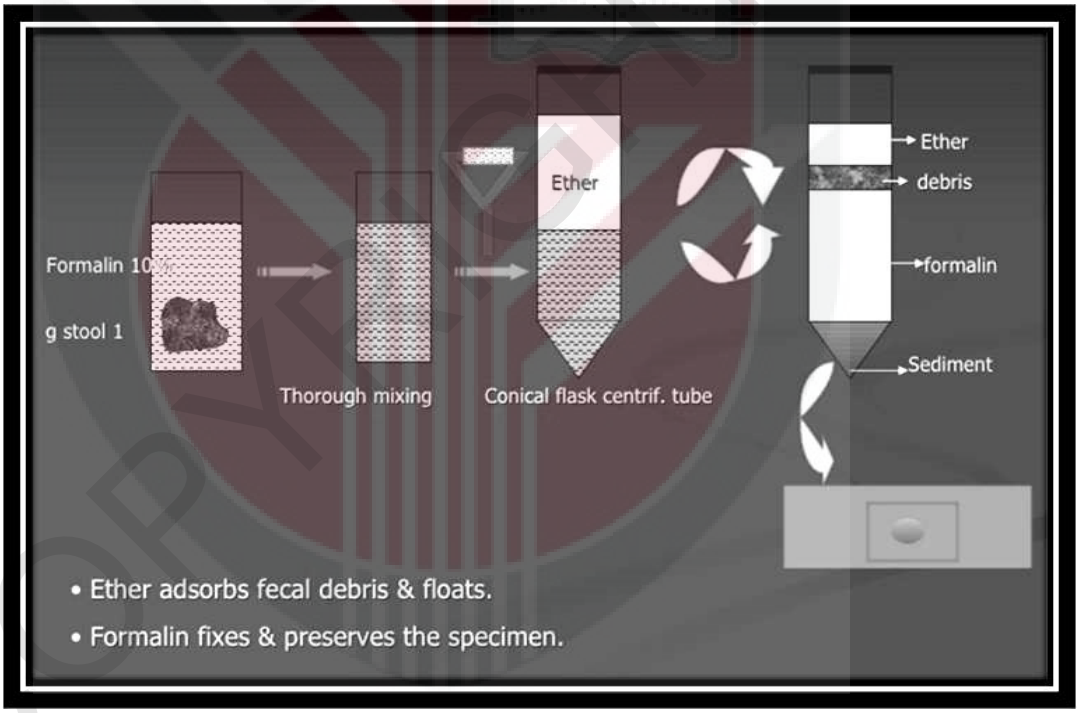

Figure 3.4: Formal-ether-Acetate sedimentation technique.

As for the trichrome staining, methanol fixed slides are now immersed into trichrome stain for 90 minutes and rinsed in acid alcohol for 10 seconds. It is then rinsed in 95% ethanol to rinse off excess stain. The slide is examined under low power objective and followed by high power objective to confirm the organism found.

In addition to this, giemsa staining is done by immersing the other methanol fixed fecal smear slide into giemsa stain for 30 minutes and excess is washed off after that. It is viewed under low objective power and then high objective power under the microscope.

4.0 RESULTS

4.1 Population of Gastrointestinal parasite

Non invasive study of wildlife parasites can instantly provide information on presence or nascence of parasitic prevalence (Gillespie TR, 2006)A total of 14 fecal samples of different species from the Bovidae family were examined for the presence of gastrointestinal parasites. Eggs of helminthes and protozoas were identified in this study in all the species tested except the Malayan Gaur, Indian Gaur and the Oryx. The overall parasite species that were found in the samples were as follows:

Parasites identified	
Helminths	**Protozoa**
Strongylids	*Cryptosporidium spp*
Capillaria sp.	*Entamoeba spp.*
Moniezia sp	

Table 4.1: Type of parasites identified in the fecal samples of captive Bovidae

4.2 Overall Prevalence

The overall prevalence of parasitic infection was 64.3% (9/14) with 50% (7/14) of helminth infections and 14.3% (2/14) of protozoic infections. Results indicated that the helminthes infection were more common than the protozoic infection in these captive bovidaes.

Species	No. Of samples examined	No. Of positive samples			Prevalence (%)		
		Protozoa	Helminth	Total	Protozoa	Helminth	Total
Banteng	**2**	**0**	**2**	**2**	**0**	**100**	**100**
Ankole	2	0	2	2	0	100	100
Malayan gaur	1	0	0	0	0	0	0
Indian gaur	1	0	0	0	0	0	0
Red letwe	2	2	0	2	100	0	100
Sable antelope	2	0	1	1	0	50	50
Oryx	2	0	2	2	0	100	100
Nyalas	2	0	2	2	0	100	100
Total	14	2	7	9	14.3	50	64.3

Table 4.2: Overall gastrointestinal parasitic infection in captive Bovidae at Zoo Negara

4.3 Prevalence of different gastrointestinal parasites in captive Bovidae at Zoo Negara

Each species of animals were infected with at least one type of intestinal parasite except the Malayan Gaur, Indian Gaur and the Oryx. The overall prevalence of strongylids are 21.4%(3/14), *Moniezia sp,* 14.9% (2/14), *Capillaria spp* 7.1% (1/14), *Crptosporidium spp* and *Entamoeba spp* are both recorded at 7.1% (1/14) respectively. The 50% of the bos genus were infected with 37.5% (3/8) strongylids and 12.5% (1/8) *Capillaria sp.*. The genus *Kobus* was infected with 50% (1/2) *Cryptosporidium spp* and 50% (1/2) *Entamoeba spp.* Meanwhile, the genus *Hippotraginae* is infected with 50% (1/2) *Moniezia sp.* and followed by the genus *Tragelaphus* which is infected with 50% (1/2) strongylids and 50% (1/2) *Moniezia spp.* .

Parasites identified	
Helminths	**Prevalence(%)(positive samples)**
Strongylids	21.4 (3/14)
Capillaria sp.	7.1 (1/14)
Moniezia sp	14.9 (2/14)
Cryptosporidium spp	7.1 (1/14)
Entamoeba spp.	7.1 (1/14)

Table 4.3.1: Overall Prevalence of different gastrointestinal parasites in captive Bovidae at Zoo Negara

Species of animals	**Parasites Identified**	**No. of positive samples(no of samples)**	**Prevalence (%)**
Banteng	Strongylids	1(2)	50
	Capillaria sp.	1(2)	50
Ankole	Strongylids	2(2)	100
Malayan gaur	-	0(1)	0
Indian Gaur	-	0(1)	0
Red letwe	*Cryptosporidium spp.*	1(2)	50
	Entamoeba spp.	1(2)	50
Sable antelope	*Moniezia spp.*	1(2)	50
Oryx	-	0(2)	0
Nyalas	Strongylid	1(2)	50
	Moniezia spp.	1(2)	50

Table 4.3.2: Prevalence of different gastrointestinal parasites in captive Bovidae at Zoo Negara

4.4 Intensity of Ova/cyst/larvae of different gastrointestinal parasites in captive Bovidae at Zoo Negara

As not enough samples of each species were available mean egg per gram of faeces (EPG), ova per gram of faeces (OPG), cyst per gram of faeces (CPG) and larvae per gram of faeces (LPG) were not calculated. So the results presented in Table 4.4 simple shows the lowest and the highest numbers found in any sample. The highest infection rate was found for strongylids with 200 EPG in Ankole cattle, followed by Moniezia with 100 EPG in Sable antelope.

Genus	**Species**	***Strongylids* e.p.g**	***Moniezia spp* e.p.g**	***Capillaria spp* e.p.g**	***Cryptosporidium spp* e.p.g**	**Entamoeba sp e.p.g**
Bos	Banteng*)*	50	-	50	-	-
	Ankole	200	-	-	-	-
	Malayan Gaur	-	-	-	-	-
	Indian gaur	-	-	-	-	-
Kobus	Red lechwe	-	-	-	50	50
Hippotragi nae	Sable antelope	-	100	-	-	-
Oryx	Oryx	-	-	-	-	-
Tragelaphlaus	Nyalas	50	50	-	-	-

Table 4.4: intensity of ova/cyst/larvae of gastrointestinal parasites in captive Bovidae at Zoo Negara

5.0 DISCUSSION

5.1 Prevalance of gastrointestinal parasite in captive bovidae at Zoo Negara

A vital point to be considered in this study is that the number of animals in the study was very low and the results, though are interesting, are statistically irrelevant and rather anecdotical. 64.3% of the captive Bovidaes at Zoo Negara were found to be positive for gastrointestinal parasites. Other authors reported higher (Opara *et al*, 2010) or lower prevalences (Lim, Ngui, Shukri, Rohela, & Mat Naim, 2008) ranging between 54.5% and 76.6%.

In all species of bovidaes, the prevalence of helminth infections was higher than the prevalence of protozoa except in Red letwe, an observation also confirmed in other studies((Lim, Ngui, Shukri, Rohela, & Mat Naim, 2008) where the helminth infection was much higher than the protozoic infection in captive bovidaes. The overall helminth infection was about 50% and protozoal infection was recorded at 14.3%. The higher prevalence of helminthes encountered in the survey can be explained by the favourable climatic conditions, which support the prolonged survival of infectious nematode larvae.

In this study, no gastrointestinal parasite were found in the Malayan Gaur, Indian Gaur and the Oryx. This could be due to the feeding management, deworming and sample size too. Epidemiological investigation on the deworming of the gaurs revealed that the Malayan Gaur and the Indian Gaur has been recently dewormed 2 months prior to this study. That explains the absence of gastrointestinal parasite eggs in these animals whereby the anthelminthics would have suppressed the egg production of the parasites in the animals.

The Banteng and the Red letwe were infected with mixed population of more than 1 parasite as compared to Ankole and the Sable Antelope which was infected with single parasite alone. This results differs from a study by (Lim, Ngui, Shukri, Rohela, & Mat Naim, 2008) which had mixed infection in the Ankole and Sable Antelope and the rest of the species solely infected by hookworms. The absence of infection in Oryx can be explained by the fact that it's the only animal of its species in the enclosure and doesn't come into contact with any other animals as well.

Strrongylids infection has the highest infection rate among these species with 50% of the Bos genus infected with it. This is a similar finding that was reported at Dublin Zoological Garden(Mooney, 2016).

6.0 CONCLUSION

In conclusion, this study indicated the importance of evaluation and monitoring the gastrointestinal parasitic level at zoological gardens. The present study found that the 64.3% of the captive Bovidaes are infected with gastrointestinal parasites and the population of parasites differ to some extent with mixed infection of more than one parasite population occurring in 25% of the Bovidaes tested. Thus, the results of this study suggest that even with high standards of husbandry and regular fecal examination by the Zoo Negara, there remains a detectable level of parasitic infection which can be assumed as healthy infection as the burden is still low. However, it could also suggest that there are rooms for anthelmintic resistance to occur. Therefore, if the standards were lowered or monitoring of the parasite level is not done regularly, a higher degree of infection could be expected.

7.0 RECOMMENDATIONS

It is recommended that Zoo Negara could adopt a more thorough practice of regular fecal screening in these animals. Currently, fecal floatation method is used to identify the parasite population and anthelmintics are given based on the level of parasite burden. Other methods like fecal sedimentation method coupled with McMaster technique could be adopted to help the management in monitoring the parasitic burden and also a study on anthelmintic resistance could be done too to further aid the zoological management to identify ways to deal with these gastrointestinal parasite infection problems efficiently.

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8.0 APPENDICES

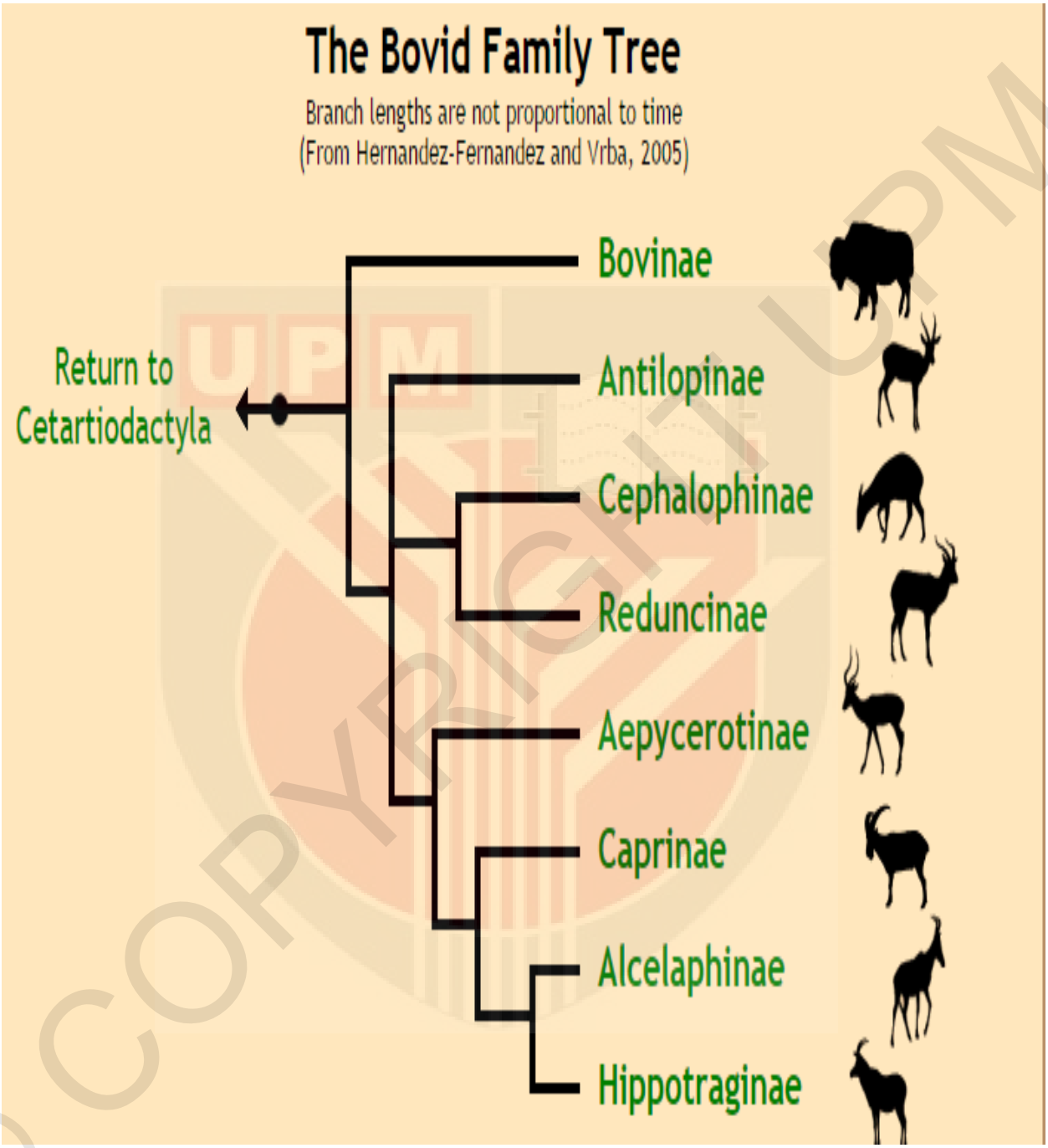

Figure 8.1: Breakdown of bovidae family

Figure 8.2: Gastrointestinal parasite population in captive bovidaes at Zoo Negara
From Top left: Strongylids, *Capillaria, Entamoeba spp*
From bottom left: *Cryptosporidium spp, Moniezia*