THE PREVALENCE OF *CRYPTOSPORIDIUM* AND *GIARDIA* INFECTING LONG-TAILED MACAQUES AND PROBOSCIS MONKEYS IN THE LOWER KINABATANGAN WILDLIFE SANCTUARY, SABAH, MALAYSIA

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ABSTRACT

The current study was aimed in determining the occurrence of these parasitic protozoans in two large populations of nonhuman primates in the Lower Kinabatangan Wildlife Sanctuary (LKWS), the long- tailed macaques and proboscis monkeys. The objectives of the study were to 1) determine the prevalence of *Cryptosporidium* and *Giardia* infection in long-tailed macaques and proboscis monkeys and 2) To determine the factors that were influencing the prevalence of *Cryptosporidium* and *Giardia* that were infecting both long-tailed macaques and proboscis monkeys in the LKWS. Fecal samples were collected noninvasively and the infection of both parasites was detected by using immunochromatographic test strips (CoproStrip™, Savyon Diagnostics, Ltd, Israel). PCR was used to clearly distinguish the donor of the samples since both primate species were always found close to one another. From the analysis, 44.71% (N=38) of tested samples were positive for *Cryptosporidium* spp. and only 17.64% (N=14) of samples were positive for *Giardia* spp. The result also showed a contrasting pattern in each host-parasite association, with *Giardia* spp. achieving higher but still moderate prevalence in long-tailed macaques (33.33%) and *Cryptosporidium* spp. achieving unexpectedly high prevalence in proboscis monkeys (87.5%). Among the six variables (host species, habitat types, average weekly rainfall, host abundance, primate diversity and river sections) host species was the most important factor that influences the prevalence of *Cryptosporidium* and *Giardia* infection. However, due to the limitation of the current study, I am not able to differentiate whether the current finding were due to (i) the two nonhuman primate species encounter each parasite at very different rates in the environment; (ii) each species has different resistance traits for each parasite; (iii) there is interspecies competition between parasites, where one parasite species inhibit (directly or indirectly) the establishment of other parasites in its host; or (iv) some other unknown reason. However, the current study did managed to achieve its aim and is successful in documenting the occurrence of *Cryptosporidium* and *Giardia* in nonhuman primates, and is the first to report in Sabah. For future studies, researchers need to focus on the zoonotic potential of these parasites.

ABSTRAK

KELAZIMAN CRYPTOSPORIDIUM DAN GIARDIA YANG MENJANGKITI KERA EKOR PANJANG DAN MONYET PROBOSCIS DI TEMPAT PERLINDUNGAN HIDUPAN LIAR KINABATANGAN, SABAH, MALAYSIA

Kajian semasa bertujuan untuk menentukan berlakunya protozoa parasit ini di dua populasi primata bukan manusia terbesar di Sanktuari Hidupan Liar Bawah Kinabatangan (LKWS) yang merupakan monyet ekor panjang dan monyet proboscis. Objektif kajian ini adalah untuk 1) menentukan kelaziman jangkitan Cryptosporidium dan Giardia dalam monyet ekor panjang dan monyet proboscis dan 2) Untuk menentukan faktor yang mempengaruhi kelaziman Cryptosporidium dan Giardia yang menjangkiti kedua-dua kera ekor panjang dan monyet proboscis di LKWS. Sampel tahi dikumpulkan secara tanpa ceroboh dan jangkitan parasit dikesan dengan menggunakan jalur ujian immunochromatographic (CoproStrip ™, Savyon Diagnostics, Ltd, Israel). PCR digunakan untuk membezakan penderma sampel kerana kedua-dua monyet ekor panjang dan monyet proboscis sentiasa ditemui berdekatan satu sama lain. Dari analisis didapati bahawa 44.71% (N = 38) sampel yang diuji adalah positif untuk Cryptosporidium spp. dan hanya 17.64% (N = 14) sampel adalah positif untuk Giardia spp. Hasil juga menunjukkan corak yang berbeza dalam setiap persatuan tuan-parasit, dengan Giardia spp. mencapai tahap yang lebih tinggi tetapi masih sederhana pada monyet ekor panjang (33.33%) dan Cryptosporidium spp. mencapai kelaziman tinggi dalam monyet proboscis (87.5%). Antara enam pembolehubah (spesies tuan rumah, jenis habitat, purata hujan mingguan, kelimpahan tuan rumah, kepelbagaian primata dan bahagian sungai), spesies tuan rumah adalah penting dalam mempengaruhi jangkitan Cryptosporidium dan Giardia. Walau bagaimanapun, disebabkan oleh had kajian, saya tidak dapat membezakan sama ada penemuan ini adalah disebabkan oleh (i) dua spesies primata menemui setiap parasit pada kadar yang berbeza dalam persekitaran; (ii) setiap spesies mempunyai ciri-ciri ketahanan berbeza untuk setiap parasit; (iii) ada persaingan interspis antara parasit, di mana satu spesies parasit menghalang (secara langsung atau tidak langsung) penubuhan parasit lain di dalam tuan rumah; atau (iv) beberapa sebab lain yang tidak diketahui. Walau bagaimanapun, kajian ini berjaya mencapai matlamat kajian dan berjaya mendokumentasikan jangkitan Cryptosporidium dan Giardia dalam primata bukan manusia, dan merupakan yang pertama melaporkan di Sabah. Untuk kajian masa depan, penyelidik perlu memberi tumpuan kepada potensi zoonotik parasit ini.

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%	Percentage
°C	Degree Celcius
g	Gram
h	Hour
mg	milligram
mg/ml	Milligram per mililiter
µl	Microliter
µl/ml	Microliter per mililiter
dH_2O	Distilled water
NHP	Nonhuman primate
ha	Hectare
km	kilometer
Km/h	Kilometer per hour
Cyt-b	*Cytochrome B*
ml	millilitre

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CHAPTER 1

INTRODUCTION

1.1 Study background

Parasites are organisms that rely on other living things known as the host, for shelter and sustenance (Moore, 2002). The two types of parasites are microparasites and macroparasites. Microparasites such as viruses, bacteria and protozoans are short-lived, but they are able to reproduce in copious amount inside their host (Combes, 2005). Macroparasites have longer life span but they need the host in order to spread their offsprings through the host defecation (Loudon and Sauther, 2013). Parasitism in wildlife is known to affect the host survival through pathological effects and reducing the host's physical condition (Hudson 1992; Boyce 1990).

Although parasite infection does not always results in mortality, severe parasite infection can produce detrimental effects to the host (O'Donnell, 1997). Severe infections can cause blood loss, abortion, damages to tissue and death (Chandra and Newberne, 1997; Despommier *et al.*, 1995), while less severe infection impairs the host ability to escape predators, foraging and competition for resources or mates (Packer *et al.*, 2003; Coop and Holmes, 1996; Dobson and Hudson, 1992; Hudson *et al.*, 1992). Since parasites and the disease that they cause are able to accelerate wildlife population declines, they are now a major threat in conservation biology (Thompson *et al.*, 2010; Mccallum and Dobson, 1995).

The primate taxon is an important conservation concern as disease outbreaks could possibly put some population into serious threat (Chapman *et al.*, 2005a). The

emergence of a new disease such as the novel flavivirus which was reported in India, could easily result in large scale mortality in wild nonhuman primates (NHPs) (Brack, 1987) and virus such as Ebola has been responsible in causing the death of chimpanzee population in Africa (Morell, 1995; Le Guenno *et al.*, 1995). However, with anthropogenic disturbance occurring globally, more natural habitats are being converted into human settlements and infrastructures, thus bringing humans closer to wildlife (Fahrig 2003). Habitat disturbance has been linked to the increase in the transmission of parasite infection in wildlife (Salzer *et al.*, 2007; Gillespie *et al.*, 2005, 2004). The alteration of parasite transmission in wild animals can adversely impact the survival of remnant populations of endangered species (Daszak *et al.*, 2000; Patz *et al.*, 2000). Habitat disturbance could also result in the spillover of wildlife parasites to livestock and eventually human (Ostfeld and Keesing 2013, 2012; Friggens and Beier 2010; Keesing *et al.* 2010).

Since many of the parasite infecting wild nonhuman primates are zoonotic (Pedersen, 2010; Wolfe *et al.*, 2007), they could potentially be the most serious spreader of cross-species disease transmission to humans, often with cataclysmic results such as the transmission of SIV-HIV (Keele *et al.*, 2006) and malaria (Liu *et al.*, 2010). Therefore, nonhuman primate parasite study is becoming increasingly important in order to have a better understanding of the factors that drive infectious disease (Tompkins, 2001; Anderson and May, 1978). Additionally, a better understanding on the role of parasites in wildlife could be useful in future conservation and management efforts (Altizer *et al.*, 2003) that would benefit both wildlife and the human race.

The current study was focused on investigating the occurrence of two diarrheal disease causing organisms namely *Cryptosporidium* and *Giardia* in two significantly large populations of nonhuman primates (long-tailed macaques and proboscis monkeys) in the Lower Kinabatangan Wildlife Sanctuary (LKWS), Sabah, Malaysia. Both *Cryptosporidium* and *Giardia* have been reported to cause mortality and morbidity in both developing and developed countries as they are easily dispersed through water supplies (Lim *et al.*, 2008; MaxKenzie *et al.*, 1995; Smith *et al.*, 1990). Similar to human, nonhuman primates can also be infected with *Cryptosporidium* and *Giardia*

(Karim *et al.*, 2014; Muriuki *et al.*, 1997). However, information on the infection of these parasites on wild nonhuman primates is scarce, especially in Southeast Asia. Therefore there is a need to investigate the current status of *Cryptosporidium* and *Giardia* in wildlife, as infectious diseases is also one of global conservation concerns which could cause drastic population decline.

1.2 Justification

The current study investigated the prevalence of *Cryptosporidium* and *Giardia* in nonhuman primate, two highly infectious protozoans capable of infecting both wildlife and human. Studies of nonhuman primate gastrointestinal parasites is not of a recent subject, many studies have been published since the 60`s (Kuntz and Myers, 1966; Myers and Kuntz, 1965), but starting from the 90`s more nonhuman primate parasite studies especially gastrointestinal parasites has been gaining a lot of attention, partly due to reports of nonhuman primate parasites infecting human (Gillespie *et al.* 2008; Chapman *et al.* 2005). However, many of these publications were studies originating mainly from a single continent which is Africa (Kooriyama *et al.*, 2012; Gillespie *et al.*, 2005, 2004; Hoagland and Schad, 1978). Little information is available when it comes to parasite infection in primates in Southeast Asia (Hopkins and Nunn, 2007).

Wildlife parasitism plays a key role in natural selection that affects host population growth (Nunn and Altizer, 2006). However, parasite infection is influenced by a variety of factors including environmental factors, host-parasite ecology and the physiology of both host and their parasites (Altizer *et al.,* 2006; Hudson *et al.,* 2002b; Loehle, 1995). Nonhuman primates living in disturbed habitats for instance, have higher chance of getting parasite infection as range overuse exposed them to their own fecal contamination (Parr *et al.*, 2013). While, rainy seasons have been reported to increase the prevalence of some parasites (e.g. protozoans, soil-transmitted helminths) allowing them to stay longer in the environment and spread further (Frias *et al.*, 2018; Jagai *et al.,* 2009). Moreover, host abundance and host diversity were also reported to significantly influence parasites prevalence (Keesing *et al.,* 2010; Morand and Poulin, 1998). Bigger population has higher chance for parasite infection and transmission,

especially among social animals (Freeland 1976). Host diversity on the other hand may reduce or amplify parasite prevalence (Keesing and Ostfeld, 2006).Understanding which factors influence parasitism in wild populations is crucial for both scientific and conservation purposes (Poirotte *et al.*, 2016).

In the current study, I hypothesized a similar prevalence in infection for both *Cryptosporidium* and *Giardia*, since both host species are often found next to each other. Also, both host species are using similar habitats in the LKWS, therefore I suspected that, similar variables such as habitats characteristics (natural VS disturbed), weekly rainfall, and habitat diversity and host abundance would have similar effects on the prevalence of both parasites in the region.

1.3 Aim

Parasite documentation is a crucial part in wildlife population monitoring, as the knowledge is useful for conservation efforts. However, reports on primate parasites are still lacking especially in Southeast Asia. This study aimed to identify the infection of *Cryptosporidium* and *Giardia in* two large population of primates (long- tailed macaques and proboscis monkeys) in the Lower Kinabatangan Wildlife Sanctuary.

1.4 Objectives

There are two main objectives of the current study:

I. To compare prevalence of *Cryptosporidium spp.* and *Giardia spp.* between two dominant primate populations (long tailed macaques and proboscis monkeys).

II. To identify intrinsic (species) and extrinsic (habitat type, average weekly rainfall, group abundance, primate diversity and river sections)variables that influence the prevalence of *Cryptosporidium* spp and *Giardia* spp in long-tailed macaques and proboscis monkeys.

CHAPTER 2

LITERATURE REVIEW

2.1 Primate

Nonhuman primates are known to be one of the most diverse groups in the animal kingdom, with approximately 500 species recorded worldwide (Mittermeier *et al.* 2013). They are the closest living evolutionary relatives of human and we share over 90% of our genes with old world monkeys and apes, in which some aspects of their morphology and physiology are also very similar to ours (Sibley *et al.*, 1990; Lovejoy, 1981). The distinguishing feature of the primate order is the size of their brain, which is relatively larger than most mammals in the animal kingdom (Pough *et al.*, 2005). Some other distinguishable characteristics of nonhuman primates includes the number of digit of their fingers which is similar to human (five digits) and although not exclusive for primate, most primate species have opposable thumbs (Pough *et al.,* 2005).

They are an amazing group of animals with the ability to adapt to a vast range of habitats, ranging from equatorial rain forests to water-scant desert regions (Mittermeier *et al.,* 2014). Moreover, nonhuman primates also occupy a variety of feeding niches in their respective ecosystems, as they can either be part time folivores, frugivores, omnivores, insectivores, gumnivores, or a combination of several of these categories (Rowe, 1996). They play important ecological functions as primary consumers and participate in the recycling of matter, nutrients, and energy in the ecosystem (Estrada and Coates-Estrada, 1993) and numerous species of plants are dependent on nonhuman primates for seed dispersal (Chapman, 1988; Garber, 1986;

Estrada and Coates-Estrada, 1984). They are widely distributed all over mainland Africa, Madagascar, Asia and the Neotropics (Estrada *et al.*, 2017).

2.1.1 Nonhuman Primates (NHP) of Borneo

Borneo, known as a center for biodiversity and endemism (Sodhi *et al.*, 2010), harbors 13 different species of nonhuman primates (Groves, 1993). The primates in Borneo include one hominid species (*Pongo pygmaeus*), two hylobatids (*Hylobates agilis* and *Hylobates muelleri*), two macaques (*Macaca fascicularis* and *Macaca nemestrina*), six colobines (*Presbytis Hosei*, *Presbytis fermoralis*, *Presbytis frontata*, *Presbytis rubicunda*, *Nasalis larvatus* and *Trachypithecus cristatus*), one tarsiid (*Tarsius bancanus*) and one species of slow loris (*Nycticebus menagensis*). Of the 13 listed species, the orangutan, agile gibbon, Bornean gibbon, banded leaf monkey, Bornean leaf monkey, white-fronted leaf monkey, maroon leaf monkey, and proboscis monkey are endemic to the island of Borneo (Groves, 2001).

Although Borneo is rich in biodiversity, many species are also highly threatened from intensive logging and large-scale conversion of forest to agricultural lands (Sodhi, Koh, Brook, & Ng, 2004). Therefore, the distribution of these nonhuman primates tends to be biased to old growth forests compared to disturbed habitats such as oil palm plantation or cleared forest (Bernard *et al.*, 2016). In general, species such as long-tailed macaques are very common and widely distributed in the lowland areas, whereas pig-tailed macaques are less common and often encountered in hill forests and adjacent lowlands (Payne *et al.,* 1985; Medway, 1970). Larger species such as the orangutan and gibbon are usually found in forest areas as they prefer natural, undisturbed habitats (Bernard *et al.,* 2016). On the other hands, the distribution of colobine monkeys varies between species. Proboscis monkeys and silvered leaf monkeys are usually found in peat swamp forest, freshwater swamps and mangrove swamps, while maroon langurs are mostly found in tropical wet evergreen forest (Grove, 2001).

(i) Proboscis monkey

Proboscis monkey (*Nasalis larvatus*) is a primate species belonging to the Colobinae family and is endemic to the island of Borneo (Yeager, 1989). As such, there are only two states in Malaysia where the species can be found: Sabah and Sarawak. They mostly inhabit lowland areas, and are usually found along coastal forests, mangroves, riverine and fresh water swamp forests (Sha *et al.*, 2008). Proboscis monkeys most distinguished features are their enlarged fore stomach and reduced thumb (Napier, 1985).

Proboscis monkeys exhibit sexual dimorphism in which the females weigh approximately 10 kilograms, while males may be twice the weight of females (Schultz, 1942; Allen and Coolidge, 1940). Although most proboscis monkeys have large nose, the male in particular has larger nose compared to the females which may exceed 10.2cm in length (Ellis, 1986). They are primarily folivorous, although seasonal availability of foods may affect their food preferences (Boonratana, 2000). In fact, proboscis monkeys have been recorded to travel farther in search of better quality foods, and may stay in the same area during the fruiting or flowering season, suggesting a preference for higher quality foods over lower quality food such as young leaves (Boonratana, 2000).

(ii) Long- tailed macaque

Long-tailed macaque (*Macaca fascicularis*) or crab-eating macaque is the most successful of the nonhuman primates when it comes to adapting in human modified landscape (Murayama and Eudey, 2004; Fooden, 1995). The different subspecies of long tailed macaques can be distinguished by the color of their pelage which range from blackish to brownish colored (Fooden, 1995) and the species have been occasionally seen to utilize tools while hunting for foods (Malaivijitnond *et al.*, 2007). Long- tailed macaque has wide geographic distribution range and is not an endemic species to the island of Borneo (Grove, 2001). They can be found all over Southeast Asia, including islands of Malaysia, Philippines and Indonesia (Fooden, 1995). Long-tailed macaque habitats are quite similar to those of proboscis monkeys, as they too occupy mangrove forests, swamp forests, riverbanks and seashores (Fooden, 1995). Thus, throughout much of their respective ranges in Borneo, these two species live in sympatry.

Food variations consumed by the long tailed macaque reflect ecological plasticity allowing them to adapt to changes in food abundance and availability (Suryoboto and Widayati, 2014). In general, long-tailed macaques prefer to consume fruits and are thus generally classified as frugivores in the literature (Berenstain, 1986; Lucas and Corlett, 1991). However, they also consume other plant materials, ranging from seeds to flowering plants and insects, if the primary sources of foods are no longer available, in the case of forest fire (Berenstain, 1986). Since they are able to adapt within human settlements, they also engaged in crop raiding and often seen to exploit food waste, therefore branded as a pest species (Twigg, 2008).