UNIVERSITI PUTRA MALAYSIA

A PRELIMINARY SURVEY ON ENDOPARASITES IN THE FAECES OF WILD ASIAN ELEPHANTS (Elephas maximus) AT BELUM-TEMENGGOR FOREST COMPLEX, PERAK

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "A Preliminary Survey on Endoparasites in the Faeces of Wild Asian Elephants (*Elephas Maximus*) At Belum-Temenggor Forest Complex, Perak." by Nur Husna Abdul Rahman and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4901 -Projek.

SATU TINJAUAN AWAL TERHADAP ENDOPARASIT DI DALAM TINJA GAJAH LIAR (*Elephas maximus*) DI HUTAN KOMPLEKS BELUM-TEMENGGOR, PERAK

Oleh

Nur Husna Abdul Rahman

2020

Penyelia: Dr. Tengku Rinalfi Putra Tengku Azizan

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Di Malaysia, kes gajah liar memasuki kawasan penempatan manusia adalah semakin meningkat. Gajah-gajah liar ini berpotensi untuk membawa cacing zoonotik yang menjadi semakin membimbangkan. Satu tinjauan awal dilaksanakan untuk memastikan peratus keberadaan endoparasit di dalam tinja gajah liar Malaysia (*Elelphas maximus)* di Hutan Kompleks Belum-Temenggor, Perak. Sampel tinja daripada gajah liar dikumpul daripada tiga kawasan terpilih; Sira Gajah, Sira Papan and Sungai Tiang. Kawasan sasaran ini adalah kawasan garam semulajadi dimana gajah-gajah seringkali melawat untuk proses ‘geophagy’ (memakan tanah). Sampel tinja dianalisa menggunakan modifikasi khas pengapungan kaedah McMaster, pemendapan tinja dan kaedah Baermann. Identifikasi spesies untuk parasit nematod dan trematod dilakukan secara morfologi. Sejumlah 13 sampel tinja (Sira Gajah = 7,

Sira Papan = 6 and Sungai Tiang = 1) telah dianalisa. Peratusan tinggi untuk keberadaan endoparasit dijumpai daripada sample tinja; 13 (100%). Larva nematod dijumpai di dalam semua sampel 13 (100%), dimana telur endoparasit sebanyak 12 (92%); kedua-dua nematod dan trematod berkongsi peratus yang sama dengan 8 (61%). Untuk telur nematod, 5 (38%) positif dengan strongyle, diikuti dengan *Ascaris* sp.; 5 (38%) dan *Strongyloides* sp. dengan 1 (7%) peratus. Untuk telur trematode, 8 (46%) positif untuk *Paramphistomum* sp., diikuti oleh *Fasciola* sp., dengan 4 (30%) peratus. Spesies baru dilapor yang dijumpai dalam gajah liar di Hutan Kompleks Belum-Temenggor, Perak di dalam penyelidikan ini adalah *Ascaris* sp. dan *Strongyloides* sp.

Kata kunci: *endoparasit, geophagy, zoonotik, Hutan Kompleks Belum-Temenggor*.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901- Project.

A PRELIMINARY SURVEY ON ENDOPARASITES IN THE FAECES OF WILD ASIAN ELEPHANTS *(Elephas maximus)* AT BELUM-TEMENGGOR FOREST COMPLEX, PERAK

by

Nur Husna Abdul Rahman
2020

Supervisor: Dr. Tengku Rinalfi Putra Tengku Azizan
Co-supervisor: Dr Nur Mahiza Md Isa

The cases of wild elephants entering human settlement are increasing in Malaysia. The potential of these elephants carrying zoonotic endoparasite is becoming a growing concern. A preliminary survey was carried out to determine the percentage of presence of endoparasites in the faeces of the wild Malaysian Elephant (*Elephas maximus*) at Belum-Temenggor Forest Complex, Perak. Faecal samples from the wild Elephants were collected from three selected areas; Sira Gajah, Sira Papan, and Sungai Tiang. These targeted areas are the natural salt lick areas where elephants frequently visit for geophagy (eating soil). The faecal sample was analysed by using a specially modified McMaster floatation method, faecal sedimentation method and Baermann method. Species identification of nematode and trematode parasite were identified morphologically. A total of 13 faecal samples (Sira Gajah = 7, Sira Papan

= 6, and Sungai Tiang = 1) were analysed. A high percentage of endoparasite was found from the faecal samples; 13 (100%). Nematode larvae were found in all samples 13 (100%) while endoparasite ova were found in 12 (92%); both nematode ova and trematode ova share the same percentage with 8 (61%). For nematode ova; 5 (38%) were positive with strongyle, followed by *Acsaris* sp.; 5 (38%) and *Strongyloides* sp. with 1 (7%) percentage. For trematode ova, 8 (46%) were positive for *Paramphistomum* sp., followed by *Fasciola* sp., with 4 (30%). New species that were reported to be found in wild elephants from Belum-Temenggor Forest Complex, Perak in this study were *Ascaris* sp. and *Strongyloides* sp.

Keywords: *endoparasite, geophagy, zoonotic, Belum-Temenggor Forest Complex.*

1.0 INTRODUCTION

Elephants are the biggest land mammal known to date. In the Peninsular of Malaysia, only one species of Asian Elephants can be found which is the *Elephas maximus* hirsutus. It has been recorded within the IUCN Red List of the species as Endangered (Choudhury et al., for the IUCN, 2008). In 1972, the status of elephants in Malaysia was protected species, but it has been elevated to totally protected species in 2010 (Saaban et al., 2011).

1.1 HUMAN-ELEPHANT CONFLICT

The decrease of elephants in Malaysia is because of the loss and fragmentation of forests in their original habitat. Thus, the elephants ended up fragmented and prone to infringe on the human plantation to look for food, water, and mates. Between 2006 and 2011, the most commonly reported human-elephant conflict was crop-raiding and followed by elephants wandered into the plantation and enter villages (Saaban et al., 2011). To add to their destructive behavior, elephants found to harbour different types of endoparasite that can become a health problem to both humans and domesticated animals (Horak et al., 1988). In 2015, Thailand elephant was found to have potentially zoonotic endoparasite; *Oesophagostomum aculeatum* that also infects their non-human primates (Phuphisut et al., 2015).

1.2 ELEPHANTS IN BELUM-TEMENGGOR FOREST COMPLEX, PERAK

Elephants from Belum-Temenggor Forest Complex (BTFC) consist of the local elephants and the translocated elephants from other states of Peninsular Malaysia. Over the years, there is an increase in elephant crop raids in BTFC, reported from all settlements in the landscape (Lim et al., 2017). The risk of transmission of zoonotic helminths from the elephants to humans or domesticated animals in the area has not yet been investigated. Moreover, the studies on wild elephant helminths in the BTFC is limited. There is only one study by Wong (2018) wherein 13 elephants, including both translocated and local, were found to have 100% presence for strongyles, *Paramphistomum* sp., and *Fasciola* sp. in their faecal sample. If there are potential zoonotic helminths, all of the faeces could infect humans and domesticated animals through a contaminated environment. However, in Wong's study, they did not use floatation method and sedimentation method to identify the endoparasites in faeces as they focus more on analysing the faecal glucocorticoid. Thus, this study is conducted as a preliminary assessment of the percentage of endoparasites in the faeces of wild elephants in the BTFC by using floatation, sedimentation and Baermann method with the following objectives.

1. To obtain a list of common endoparasite found in the faeces of Wild Asian Elephants in BTFC.
2. To calculate the percentage of endoparasite of the faeces of Wild Asian Elephants in BTFC.

2.0 LITERATURE REVIEW

2.1 ELEPHANTS IN LITERATURE

Elephants are divided mainly into two species: the African elephants (*Loxodonta africana*) and Asian elephants (*Elephas maximus*). For *Loxodonta*, there are two subspecies; *Loxodonta africana* africana (the bush African elephant) and *Loxodonta africana* cyclotis (the forest African elephant). On the other hand, Asian elephants are divided into five subspecies, the Sumatran Asian elephant (*Elephas maximus* sumatranus), the mainland Asian elephant (*Elephas maximus* hirsutus), the Sri Lankan Asian elephant (*Elephas maximus* maximus) (Fowler & Mikota, 2006), and the Borneo Asian elephant (*Elephas maximus* borneensis) (Sukumar, 2006). Asian elephants are scattered in India, Nepal, Bhutan, Bangladesh, Myanmar, Thailand, Peninsular Malaysia, Sabah, Kalimantan, Cambodia, Laos, Vietnam, China, and the islands of Sumatra (Indonesia) and Sri Lanka (Santiapillai & Jackson, 1990; Sukumar, 2003; Sukumar, 2006). In peninsular Malaysia, it is evaluated that approximately 1223-1677 elephant individuals are dispersed broadly from the state of Kedah in the north to Johor in the south, and from Negeri Sembilan in the west to Terengganu in the east (Saaban et al, 2011). Although there was no estimated number of elephants individuals reported in Belum-Temenggor (Saaban et al., 2011), Hing (2017) found that 55 adults elephant visited 'Sira Gajah' a salt lick area at the south of Temenggor in the span of one year (October 2012-October 2013) (Lim et al., 2017).

2.1.1 ELEPHANT CONSERVATION

Elephants are important seed dispersers in the ecosystem, with the most frugivorous being the African forest elephants followed by Asian and African savannah elephants (Campos-Arceiz & Blake, 2011). Harich et al. (2016) studied the seed dispersal potential of Asian elephants and found 6253 ingested seeds from the dung of five elephants over the entire course of the feeding trials. Moreover, McConkey et al. (2018) reported that elephants are the most effective disperser of the megafaunal fruit *Platymitra macrocarpa* because of their large body size. Other than that, elephants contribute to the self-thinning of the forest by trampling and eating fast growing plants (reducing light, water, and space among tree) that allows slow-growing trees to grow. Eventually, the forest biomass increased for carbon stock, thus less carbon emission to the environment (Berzaghi, 2019).

The decline of the number of elephants could trigger cascading impacts in the general system functioning through changes in the territory and trophic structures, leading to changes or extinction of other animal species (Wolf et al., 2013; Bello et al., 2015; Malhi et al., 2016; Harich et al., 2016). In Malaysia, the number of elephants is declining due to the loss and fragmentation of forests. Their numbers have been drastically reduced from the states of Selangor, Melaka, Negeri Sembilan, Perlis, Penang, Kedah, and Johor (Ning et al, 2016). For the past 40 years, Malaysia has addressed the elephant-human conflict by practicing translocation of elephants from conflict areas to protected areas at the Royal Belum State Park, Taman Negara, and Endau Rompin. On the year of 2011, the project of 'Management & Ecology of Malaysian Elephants' (MEME) was introduced and applied GPS-satellite tracking, camera-traps, and non-invasive

molecular tools to bring an evidence-based approach to the conservation of Malaysian elephants (Hing et al., 2016). They also stated that parasites have a complicated relationship with the host's immune system and can tell us approximately the environment that supports the intermediate hosts (Hing et al., 2016) as it can be an effective, practical, and non-invasive warning system for the health of the individual (Macrogliese, 2015).

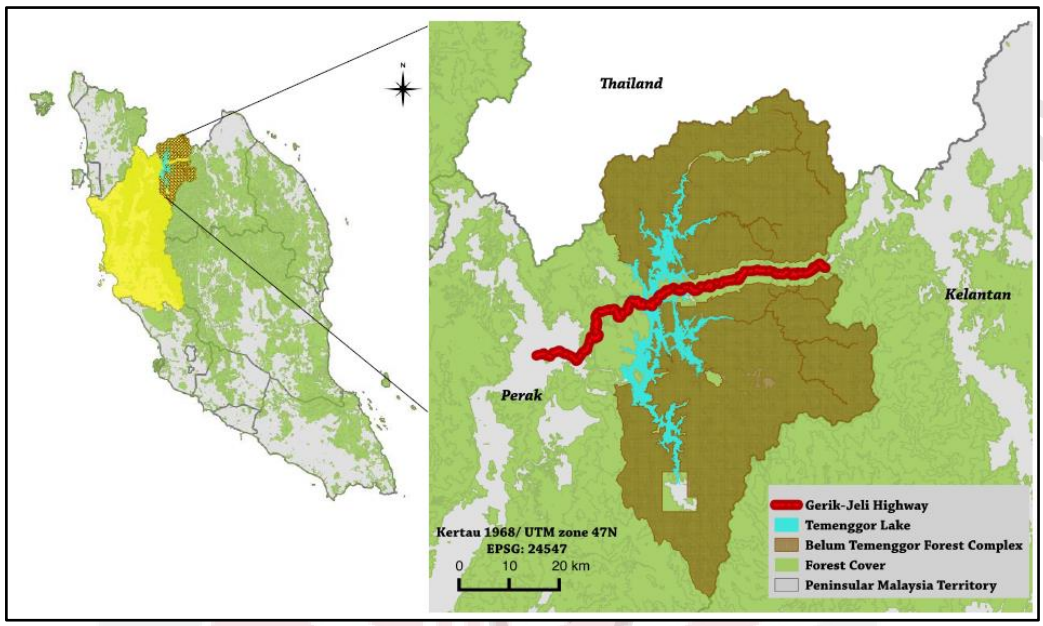
Few studies on the relationship between elephant stress and endoparasite burden were reviewed. The study by Wong (2018) was unable to detect a downstream effect of potential stress from elephant translocation on immunity from the use of parasite egg because it may be confounded by the medical, social, and environmental factors as they were not able to detect any signs of immunosuppression. Hing (2012) in her study 'A Survey of Endoparasites in Endangered Bornean Elephants *Elephas Maximus* borneensis in Continuous and Fragmented Habitat' concluded that parasite generalisations should be avoided and we should consider the complexion of their life cycles (host, agent, and environmental factors) that influence the infection dynamics of different type of parasite.

2.1.2 ELEPHANT GEOPHAGY

Mammalian herbivore and omnivore population have reported eating soil deposits or waterholes (geophagy) from a desirable area (natural mineral licks) that includes elephants (Klaus et al., 1998). Elephants consume soil that is rich in minerals containing sodium, calcium, and phosphorus (Mercy, 2009) to supplement the mineral deficiency in the body (Fowler & Mikota, 2006). They burrow out the material by beating the soil with their fore toes where the lick increases in size by sideways extension, following digging at many points on the perimeter while depositing their dungs in large amounts on and near the salt licks (Weir, 1969). In BTFC, it was estimated that there are 60 salt lick locations where it has become spots for tourists to catch a glimpse of wildlife in their natural habitat including elephants (Abdullah et al., 2011) which will further discussed in 2.4.

2.1.3 SALT LICK IN BELUM-TEMENGGOR FOREST COMPLEX

Figure 1: Belum-Temenggor Forest Complex map



As shown in Figure 1, Belum-Temenggor Forest Complex (BTFC) is arranged within the north of Perak (highlighted in yellow), near to the border of Thailand. Sira Gajah is in the south-west of Temenggor Lake. BTFC is a critical territory for wild elephants (*Elephas maximus* indicus), Malayan tiger (*Panthera tigris* jacksoni), gaur (*Bos gaurus*), and numerous others.(Clements et al., 2010; Hedges et al., 2015; Rayan and Linkie, 2015; Wong, 2018). The BTFC is a hill dipterocarp and upper dipterocarp forest that is located in the north-west of Peninsular Malaysia. It covers the Royal Belum State Park (1,175km^2), Temenggor Forest Reserve (1,489km^2), state land (131km^2), innate towns, manors, lakes, waterways, and 49 dams and this site is separated by the Gerik-Jeli East-West interstate approximately 121km in length. (Malaysian Nature Society, 2009; Clements et al., 2010; Rayan & Linkie, 2015; Wong, 2018). The location is the second largest reserve for wild elephants in Peninsular Malaysia and there are

self-evident signs that the elephants regularly visit salt licks to expend soil minerals by the presence of dungs that they deposited during the digging of salt lick activity (Ning, 2017).

Sira Gajah is one of the most famous salt licks to be visited by tourists which is located at the Temenggor side of the BTFC (Chuan et al., 2017). To get there, it takes roughly 1 hour by speedboat from Banding Island and 15 minutes of trekking into the forest. Ning (2017) observed that 55 wild elephants visited Sira Gajah, throughout the year from October 2012 until October 2013 by using camera traps with the most frequent in December 2012 and less frequent in February 2013.

In Belum, the famous salt lick area was Sira Rambai, Kejar, Atap, Batu, Papan, and Kuak (Abdullah et al., 2011). Traveling to Sira Papan requires a boat from Banding Island to Sungai Papan campsite and trekking around 15 to 30 minutes. Nor Liyana et al. (2016) spotted two elephants at Sira Papan from August 2014 to November 2014 through camera traps.

Meanwhile, Sira Kuak is an area of 92m^2 and is located the closest to the river of access. The river, called Sungai Tiang located 8 km from the jetty at the Lower Belum, Complex. According to Lazarus et al. (2019), five elephants were observed by camera traps that were set in Sungai Tiang within 6 months of the duration of the study. Thus, this study chooses Sira Gajah from Temenggor, Sira Papan, and Sungai Tiang from Belum as the sampling location because of its accessibility and the availability of elephant faeces.

2.1.4 ELEPHANT FAECAL SAMPLING

According to Chame (2003), elephant faeces is big and cylindrical with a length of 15 to 20cm. The faecal circumference of Asian elephants in Malaysia ranging from 18 to 57 cm and 31 to 52 cm for male and female elephants respectively (Karuppannan et al., 2019). Faecal sample that obtained from wild elephants can be classified according to the stages of dung decay based on the 'S system' as described by Hedges and Lawson (2006) as in the table below.

Table 1: Stages of decay by Hedges and Lawson (2006)

Stage	Condition of dung pile
S1	Very fresh and intact bolus. Looks moist with odour.
S2	Fresh and intact bolus. Looks dry with no odour.
S3	Some disintegrated bolus with some recognisable bolus.
S4	Completely disintegrated bolus
S5	Completely decayed

According to the study of 'Influence of Asian Elephant Dung Decay on DNA Recovery' by Karuppannan et al. (2019), the faecal bolus from Kuala Gandah captive elephant took 5 days to reach the S3 stage although no environmental factors were taken into account. Thus, in this study, the faecal bolus S1 is considered as less than 24 hours old, S2 as 2 to 5 days old, and S3 onwards more than 5 days old. A study of 'Effects of Fecal Collection and Storage Factors on Strongylid Egg Counts in Horses' by Nielsen (2010) stated that faecal samples collected from the ground could be used for faecal egg count analysis if they are less than 12 hours old. However, obtaining a fresh faecal sample from wild elephants can be very challenging. Wong (2018) followed the wild elephants in her study by utilizing GPS tracking logs to estimate the time of the elephant within

the area before collecting the faecal samples. Similarly, Hing et al. (2013) tracked the elephants on foot and wait until the elephants leave the area to a safe distance for faecal collection. Due to lack of time and equipment, this study focuses on elephant dung that is present at salt lick areas in the BTFC at the time of collection.

Lysndale et al. (2015) found that faecal sample that was taken from the edge and the centre has no significant difference as parasite egg densities are uniform within the faeces of Asian elephants. However, as advised by Mikota (personal communication) in Hing et al. (2013) study, the sample should be taken from the core of the bolus to prevent contamination from soil nematodes and from different sizes of bolus to avoid duplication and reduce the reliability of prevalence estimation. To maintain the faecal egg count throughout transportation time to the laboratory for faecal analysis, numerous storage methods were used in past studies. For example, faecal sample was collected in pre-prepared polyethylene specimen containers containing 95% ethanol (Hing et al., 2013) and storage in zip-lock bags which then frozen at -20°C (Wong, 2018). Lynsdale et al. (2015) discovered that storing Asian elephant faeces in fixative solution decreases the faecal egg count because the fixative changes the egg morphology and causes rupture that can reduce the floatability and visibility of eggs. Hence, the faecal sample should be stored in an anaerobic condition such as sealed zip-locked bags at 4 to 6°C for about seven days without a significant reduction in egg recovery (Lynsdale et al., 2015; Nielsen et al., 2010). Also, refrigerating the samples up to 120 hours (5 days) did not affect the quantitative egg counts, while freezing the samples abundantly declines the fecal egg count for the first 12 hours (Nielsen et al., 2010).

2.2 ENDOPARASITES IN ELEPHANTS

The most common problem of Asian elephants that cause morbidity were injuries, endo- and ectoparasitism, and gastrointestinal disease respectively (Miller et al., 2015). In severely infected elephants, endoparasite leads to gastrointestinal disease. For example, diarrhoea and constipation are common clinical signs for trematode infestation and nematodes cause poor food utilization and diarrhea. During the rainy seasons, paramphistomiasis usually occurs with clinical signs of anorexia, severe diarrhoea, and high mortality in young elephants (Fowler & Mikota, 2006). These parasitic infections can lead to death in the elephants and can become a source of infection for domestic animals, and vice-versa (Abhijith et al., 2018). Fowler and Mikota (2016) also stated that healthy animal can be a reservoir of a wide range of endoparasites without showing any clinical signs unless there is an upset of the equilibrium between the host and parasite. The death of elephants by endoparasites can happen when the elephants are stressed or immunocompromised because of habitat loss due to deforestation (Hing et al., 2013).

2.2.1 WILD ELEPHANTS ENDOPARASITE

Wild elephants can be very aggressive (Ree, 2012) as it is very challenging for researchers and veterinarians to determine their health status by physical-contact examination. Therefore, collecting faecal samples of elephants from afar and calculating the endoparasitic load is one of the stress and health indicators of wild elephants for wildlife conservation (Marcogliese, 2005). Even though elephant study is crucial, there are limited studies on endoparasite mainly on wild elephants (McLean et al., 2012). According to Fowler and Mikota (2006), elephants can harbour species of strongyles for example *Murshidia* sp., *Quilonia* sp., *Amira* sp., *Decrusia* sp., *Equinurbia* sp., *Choniangium* sp., *Bathmostomum* sp., *Grammocephalus* sp., and *Parabronema* sp. In addition, cestode tapeworms by *Anoplocephala* sp. and trematode flukes like *Fasciola* sp. and *Pseudodiscus* sp. (Fowler & Mikota, 2006).

Research by Chandrasekharan et al. (2009) found 21 different species of helminths under the family of strongyles, trematodes, and cestodes in both wild and captive elephants (*Elephas maximus* indicus) at Kerala, India. A more recent study from south Wayanad forest division, Kerala, India has observed that 55 wild elephants were positive of propagules and nematode larvae. The highest prevalence was a combination of *Strongyloides* sp. and *Strongyle* type egg with 58.1%. A small percentage contributed by *Anoplocephala* tapeworm species in only 3.6% (Abhijith et al., 2018). Another study from Mudumalai and Anamalai Wildlife Sanctuary, India by Vimalraj et al. (2015) found similar species of endoparasite as Abhijith (2018) but with a higher prevalence of *Anoplocephala* tapeworm species with 42%.

In Sri Lanka, a study by Abeysekara, et al. (2018) had compared the prevalence of the gastrointestinal parasite in the wild, semi-captive, and captive Asian elephant (*Elephas maximus* maximus*)*. The wild elephants have the highest prevalence of gastrointestinal parasites compared to semi-captive and captive overall. The helminths that contributed in descending order were Strongyles, *Strongyloides* sp. *Paramphistomum* sp. and *Fasciola* sp. The quantitative method of parasite analysis was run by using the McMaster method. For the qualitative analysis, they carried out direct saline and iodine mounts, modified salt flotation, Sheather's modified sucrose flotation method, and sedimentation techniques (Abeysekara et al., 2018).

In East Malaysia, Hing et al. (2013) compared the prevalence and parasitic load of wild Bornean elephants (*Elephas maximus* borneensis) at two places of fragmented habitat and continuous habitat at Lower Kinabatangan Wildlife Sanctuary and Tabin Wildlife Sanctuary respectively. The prevalence of *Fasciola* species is the most followed by strongyles and *Anoplocephala* tapeworms. Hing et al. (2013) also observed that there is a presence of mixed parasite infection in an individual elephant with a variety of prevalence percentages similar to the studies by Abhijith et al. (2018) and Vimalraj et al. (2015). All faecal samples from this study were positive for at least one parasite (Hing et al., 2015). In Peninsular of Malaysia, Wong (2018) collected faecal sample from 13 wild Asian elephants at Belum-Temengor Forest Complex and Kenyir Forest Complex where all elephants were prevalent on nematode (strongyles) and trematode (*Paramphistomum* sp. and *Fasciola* sp.). The result of Hing et al. (2013) and Wong (2018) study cannot be compared due to different parasitological methods.

2.2.2 FAECAL SAMPLE PROCESSING

McMaster floatation method was used to analyse the faecal sample of wild elephants followed by counting the eggs using a light microscope (Chandrasekharan, et al., 2009; Vimalraj et al., 2015; Abhijith, 2018; Abeysekara et al., 2018; Hing et al., 2013).

The implementation the centrifugation technique before McMaster to obtain a more reliable and precise result (Lynsdale et al., 2015). Roepstorff and Nansen (1998) method that includes the centrifugation method has the most sensitivity and reliability when detecting helminth eggs because the faecal suspension was sufficiently clear for examination as demonstrated by Vadlejch et al., 2011. Hing et al. (2013) uses the standard protocol of the McMaster technique (special modification) by MAFF (1986) to increase the sensitivity from 50 egg per gram to 10 egg per gram. McMaster floatation technique is more sensitive towards detecting nematodes and cestodes ova because they have a low specific gravity that enable them to float on high specific gravity solution such as concentrated salt or sugar solution (Alvarado-Villalobos et al., 2017).

Meanwhile, trematode ova has a higher specific gravity that allows them to sink in a low specific gravity solution such as tap water or formalin-ether solution (Alvarado-Villalobos et al., 2017). A study by Abhjith (2018), reported that there was no significant difference between sedimentation and floatation technique, however, isolation of heavy trematode egg is better in sedimentation technique. Rizwar et al. (2018) and King'ori et al. (2020) both detected trematode ova from faecal sedimentation by mixing the faecal sample with water. Rizwar et al. (2018) mixed 100 samples with 100ml of water in a beaker which then left for

60 minutes, and then, the supernatant is removed and the process is repeated several times until the solution becomes clear. Meanwhile, King'ori et al. (2020), used 4g of sample which then mixed with 45ml of tap water in a centrifuge tube where it is left for 30 minutes. Then, the supernatant is removed and the process is repeated until there is a clear suspension.

Larvae of the nematodes in the faeces can be harvested by using the Baermann method. Thurber et al. (2011) mixed 8g of faeces with 2g of vermiculite in a covered plastic cup with plastic wrap. The incubation time was 14 days to obtain the L3 larvae which then harvested by using the Baermann technique where they used a hollow-stemmed disposable champagne glass. Faecal sample is wrapped in cheesecloth and suspended in warm water then left overnight. In 24 hours, the larvae migrate out of the faeces and it swim to the bottom of the glass where it is harvested and stored in 1.5ml of 100% ethanol (Thurber et al., 2011). In a study of 'Developing a Practical and Reliable Protocol to Assess Nematode Infections in Asian Elephants' by Abeysinghe et al., (2012), stated that the Baermann method is better to calculate the nematode load in elephants due to its reliability and ease although it took more time to process the samples (7 days incubation period) (Abeysinghe et al., 2012).

2.2.3 RISK OF ENDOPARASITE TRANSMISSION FROM ELEPHANT TO DOMESTICATED ANIMALS AND HUMANS

The risk of endoparasite transmission from wild elephants to humans and domestic animals occurs when the elephants enter human settlements or farms near the elephant habitats. Elephants will enter the new environment and transmission of endoparasite can occur by the faecal-oral route (Fowler & Mikota, 2006). The faeces with endoparasites ova or larvae that are deposited in a certain area could contaminate surrounding soil, water or crops (Betson et al., 2020). Transmission of endoparasites to humans and domestic animals happens by skin penetration and consumption of forage or crops on these contaminated areas (Betson et al., 2020).

Five orang Asli settlements with a reported human population of 6864 in 2016 resides in the BTFC. In a study by Lim and Campos-Arceiz (2019), from the year 2011 to 2016, the human settlers suffered a total of RM948,570 damage with 401 complaints of elephant raiding in Perak. All of the settlements reported that there is some degree of crop-raiding by the elephants in the area (Lim et al., 2017). The risk of transmission of endoparasite to these human population is high due to the frequent elephant raiding.

However, there is limited study on the potential zoonotic endoparasites from elephants. A study by Phuphisut et al. (2015) discovered that the elephants in Salakpra Wildlife Sanctuary, Kanchanaburi, Thailand had a strongyle, *Oesophagostomum aculatum*. The identification of *O. aculatum* was made by running a DNA sequencing to a positive faecal sample and comparing it with the DNA sequences of related nematodes. It was also detected in their Japanese

macaque, a non-human primate, which may then potentially transmit the *O. aculatum* to humans in this wild elephant interrupted area. Although there is yet evidence of transmission of *O. aculatum* from elephants to humans or domestic animals, further observation and study should be done to observe the potential risk (Phuphisut et al., 2015).

Moreover, the risk of transmission of endoparasite from elephants to domestic animals is more likely to happen than from elephants to humans. *Fasciola* sp. and *Paramphistomum* sp., a species of trematodes was reported to infect both elephants and ruminants (Wong, 2018; Najib et al., 2020; Tan et al., 2017). Other than that, some parasites can infect a wide range of hosts including the elephants and domestic animals such as *Toxocara* sp. and *Schistosoma* sp. (Ziegler & Macpherson, 2019; Brant et al., 2013; Gordon et al., 2019; Betson et al., 2020).

3.0 MATERIALS AND METHODOLOGY

3.1 SAMPLE COLLECTION

Elephant faecal sample was collected at Sira Gajah, Sira Papan and Sungai Tiang, Belum-Temengor Forest Complex, Perak. Dung piles that are present in the area were identified as fresh fecal samples according to the colour, consistency, and presence of insect activity. S1 faecal sample is collected and it was taken from different sizes of bolus to reduce duplication of the same sample. By using a spatula, core samples was obtained from the centre of the bolus. The fresh samples are then sealed in a labeled zip-lock bags and stored in a cooler box for a maximum of 8 hours and to be transferred to a fridge (4 to 6°C). The faecal sample must be analysed within seven days (Nielsen et al., 2010).

3.2 SAMPLE PREPARATION

The faecal sample and tap water were mixed thoroughly. Then, for about 30 seconds, the contents were then sieved and were delicately agitated over a beaker. After that, the contents were transferred into a centrifuge tube and then centrifuged (1500 rpm for 5 minutes). The faecal sediment is obtained.

3.3 SAMPLE PROCESSING

3.3.1 McMaster method (special modification) by MAFF,1986

4.5g of faecal sediment was weighed and saturated salt is added to the solution to make a total of 45ml volume in a beaker (ratio of salt to tap water 350g: 1000ml, with estimated SPG of 1.33). 0.5ml of the solute was transferred into a double-chambered McMaster slide. Before pipetting into the second chamber, the solute was mixed again. The slides were then left for about 5 minutes before observing under a light microscope (brand of the microscope). The numbers of eggs were counted from both chambers inside and outside the marked grid to obtain faecal egg count (FEC). The egg per gram (EPG) was calculated by multiplying the total number of eggs by 10.

3.3.2 Faecal sedimentation technique

4.5g of faecal sediment is weigh and mixed with tap water to make a total volume of 45ml in a centrifuge tube. The solution is stirred until became slurry and sieved then left for 30 minutes. The process is repeated two to three times until the suspension is clear. Then, one drop of sediment is put in a petri dish along with a few drops of tap water. The trematode ova was observed under dissecting microscope.

3.3.3 Baermann method

30 g of faecal material was put in the centre of the cheesecloth. Then, a pouch is formed containing the faecal material by holding the four corners of the cheesecloth together and moulding the cloth around the faecal material. A stick is pushed under the pouch tie so it can be suspended. The funnel is filled with lukewarm water until all faecal material is covered. Then, the apparatus is left overnight to obtain the sediment. The lukewarm water is poured out and the sediment is transferred into a microcentrifuge tube and centrifuged for 2 minutes with 1000rpm. A drop of sediment was put on a glass slide, with a drop of iodine. Coverslip was gently placed over and the slide is observed under a light microscope.

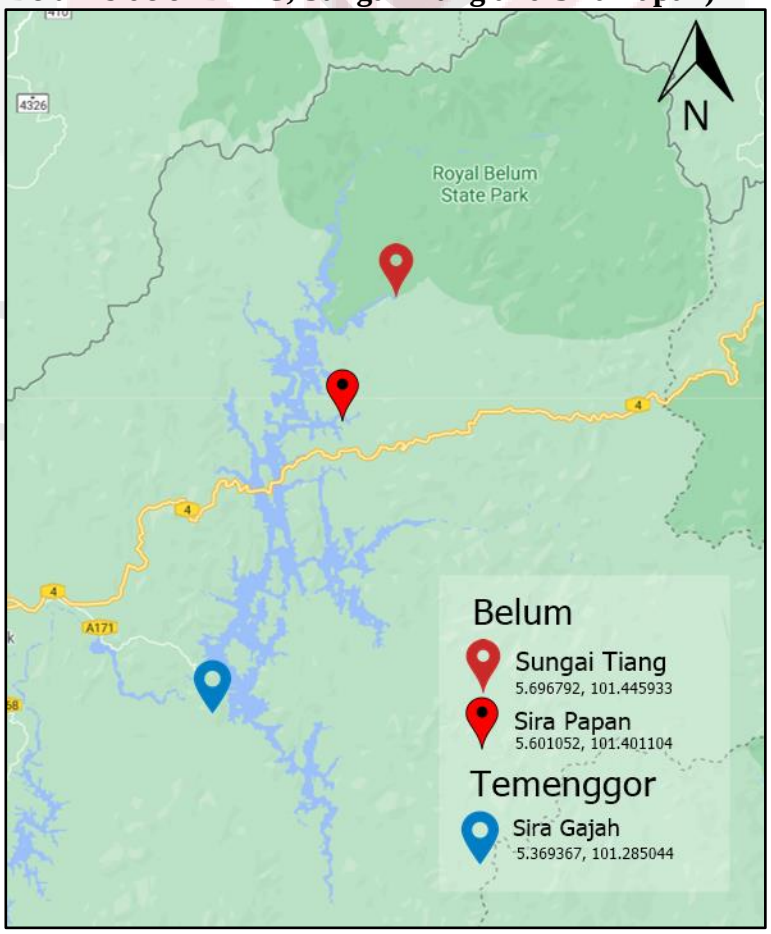
3.4 ENDOPARASITE INDENTIFICATION

Endoparasites were identified based on egg morphology and morphometry and parasitology textbook of Veterinary Clinical Parasitology 8th edition by Zajac and Conboy (2011). The nematode larvae were described by observing the shape of the head (bullet-shaped, rounded, squared, or uneven tip), the presence of protective sheath (with or without), the larvae tail (notched or not notched), the sheath tail (absence, presence, long, medium or short), and filamentous tail (absence, presence, long, medium or short) (Van Wyk & Mayhew, 2013; The RVC/FAO Guide to Veterinary Diagnostic Parasitology; Lloyd, 2020).

4.0 RESULTS

A total of 13 faecal samples were collected from 2 elephant herds in the Belum-Temenggor Forest Complex, Perak from Temenggor herd at Sira Gajah with seven samples and Belum herd at Sira Papan with five samples, and Sungai Tiang with one sample. The location is showed in Figure 2. However, during sampling, no S1 faeces were collected: only S2 and S3 faeces were collected from all the areas. S2 faeces were collected from Sira Papan while S3 faeces were collected from Sira Gajah and Sungai Tiang. The condition of faecal bolus was described in 2.1.5 (Table 1).

Figure 2: Map of the sample collection (Temenggor side of BTFC, Sira gajah and Belum side of BTFC, Sungai Tiang and Sira Papan)

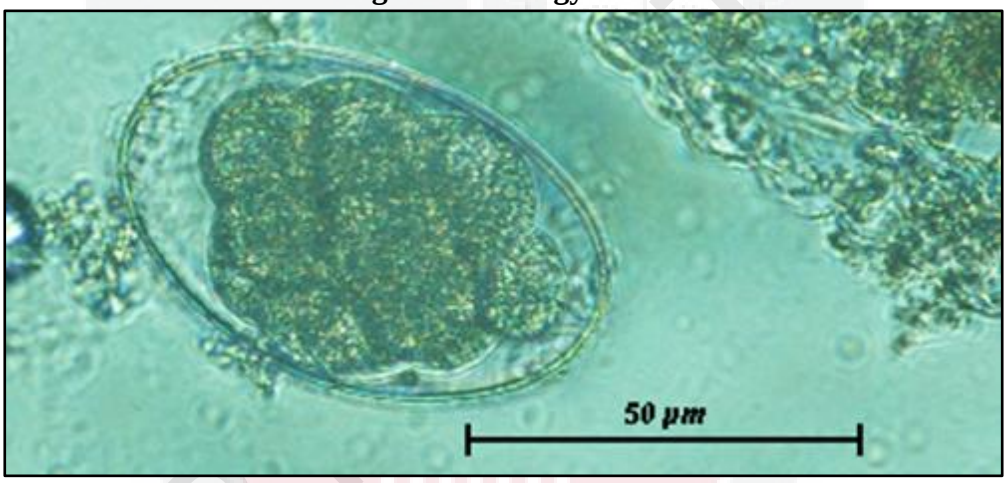


4.1 THE ENDOPARASITES IN THE ELEPHANTS FAECES IN BTFC

In this study, nematode and trematode ova were identified. The nematodes include *Strongyles, Ascaris* sp. and *Strongyloides* sp. while trematodes represented by *Fasciola* sp. and *Paramphistomum* sp. For nematode larvae, four types were identified which is Type 1, Type 2, Type 3 and Type 4.

4.1.1 Strongyles

Figure 3: Strongyle ova



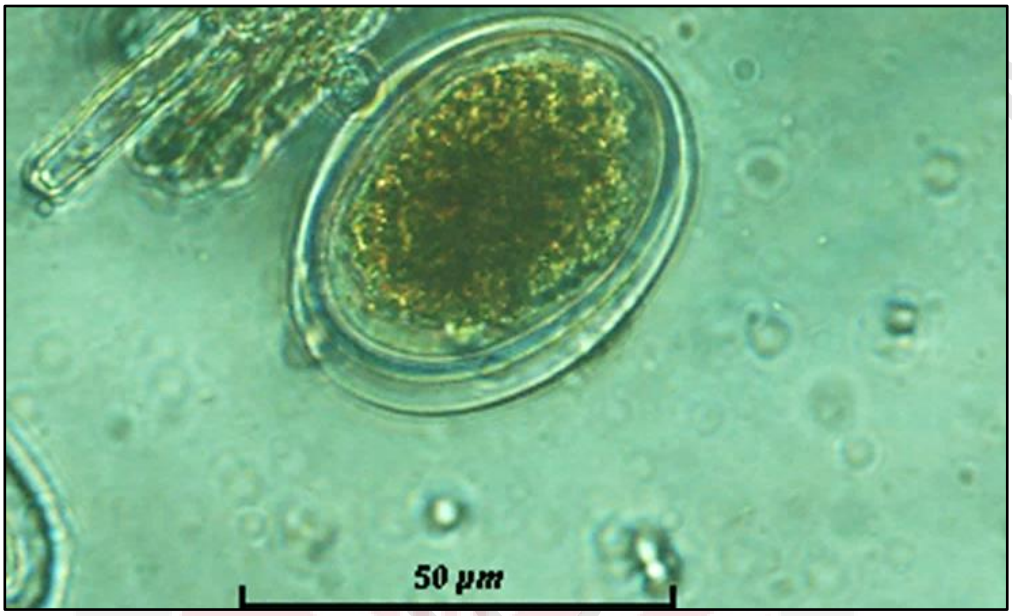
Distinctive characteristics of strongyles ova are: they have typical oval-shaped, thin smooth walls, and the presence of blastomeres as shown in Figure 3. The strongyle that was found in this study (n=5) has a length of 60 to 65 µm and a width of 30 to 40 µm. Table 2 shows individual size for strongyles found in this study.

Table 2: Size of strongyles found in this study

Number	Length (µm)	Width (µm)
01	61	31
02	63	35
03	61	33
04	64	36
05	65	40

4.1.2 *Ascaris* sp.

Figure 4: *Ascaris* sp. ova



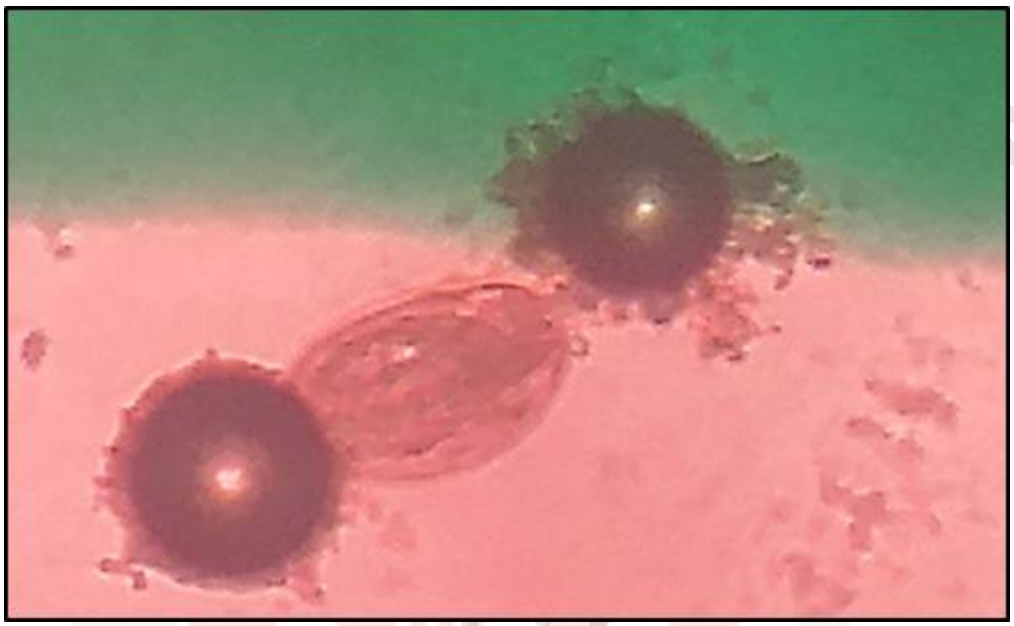
The distinctive characteristics of *Ascaris* sp. ova are: they have thick shell with double-layered, roundish in shape, and presence of embryo as shown in figure 4. *Ascaris* sp. ova that was found in this study (n=5) has a length of 46 to 52 µm and a width of 33 to 44 µm. Table 3 shows individual size for *Ascaris* sp. found in this study.

Table 3: Size of *Ascaris* sp. found in this study

Number	Length (µm)	Width (µm)
01	47	35
02	46	39
03	51	44
04	50	43
05	52	43

4.1.3 *Strongyloides* sp.

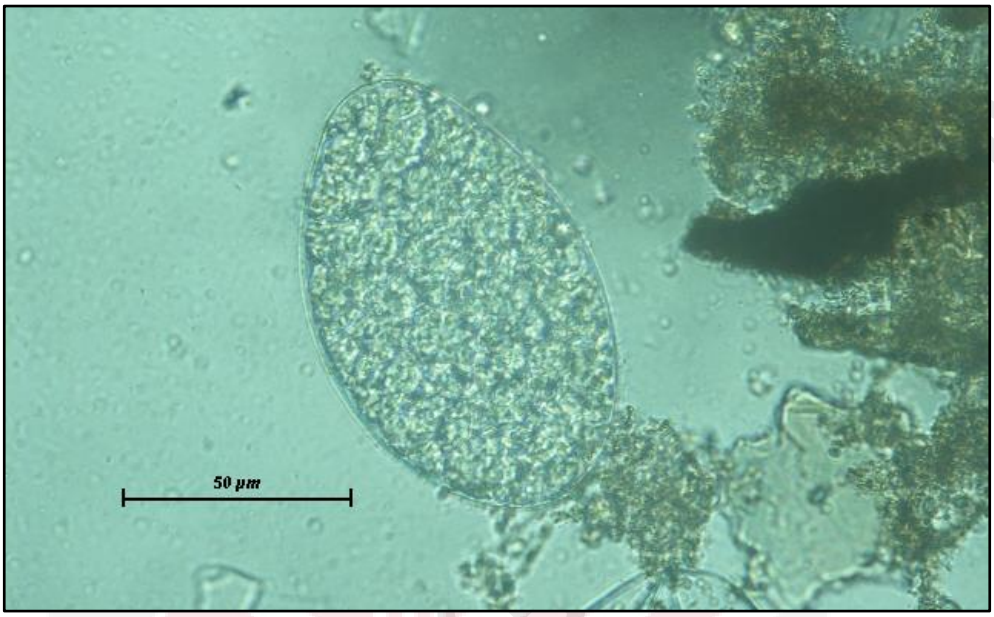
Figure 5: *Strongyloides* sp. ova



The distinctive characteristics of *Strongyloides* sp. ova are: they have a thin wall and contained larvae. *Strongyloides* sp. ova that was found in this study (n=1) and unfortunately the length and width were do not recorded in this study.

4.1.4 *Paramphistomum* sp.

Figure 6: *Paramphistomum* sp. ova



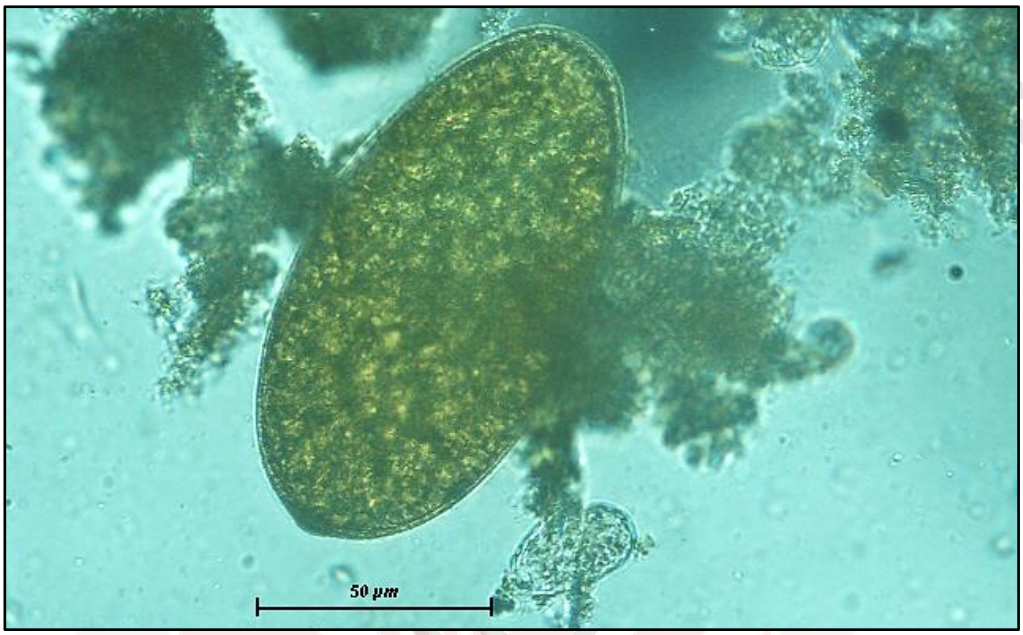
The distinctive characteristics of *Paramphistomum* sp. ova are: they are large, ovoid, operculated, and clear as shown in Figure 6. *Paramphistomum* sp. ova that was found in this study (n=6) has a length of 85 to 136 µm and a width of 47 to 73 µm. The colour is clear and transparent. Table 4 shows individual size for *Paramphistomum* sp. found in this study.

Table 4: Size of *Paramphistomum* sp. found in this study

Number	Length (µm)	Width (µm)
01	136	73
02	106	71
03	91	47
04	85	48
05	127	71
06	132	67

4.1.5 *Fasciola* sp.

Figure 7: *Fasciola* sp. ova



The distinctive characteristics of *Fasciola* sp. ova are large, ovoid, operculated, and have golden/yellow hue as shown in Figure 7. *Fasciola* sp. ova that was found in this study (n=5) has a length of 111 to 121 µm and a width of 59 to 60 µm. The colour is golden-yellow. Table 5 shows individual size for *Fasciola* sp. found in this study.

Table 5: Size of *Fasciola* sp. found in this study

Number	Length (µm)	Width (µm)
01	111	59
02	121	59
03	120	60
04	116	60
05	118	60

4.1.6 Nematode larvae (Type 1, Type 2, Type 3 and Type 4)

Figure 8: Nematode larvae Type 1

Figure 9: Nematode larvae Type 2

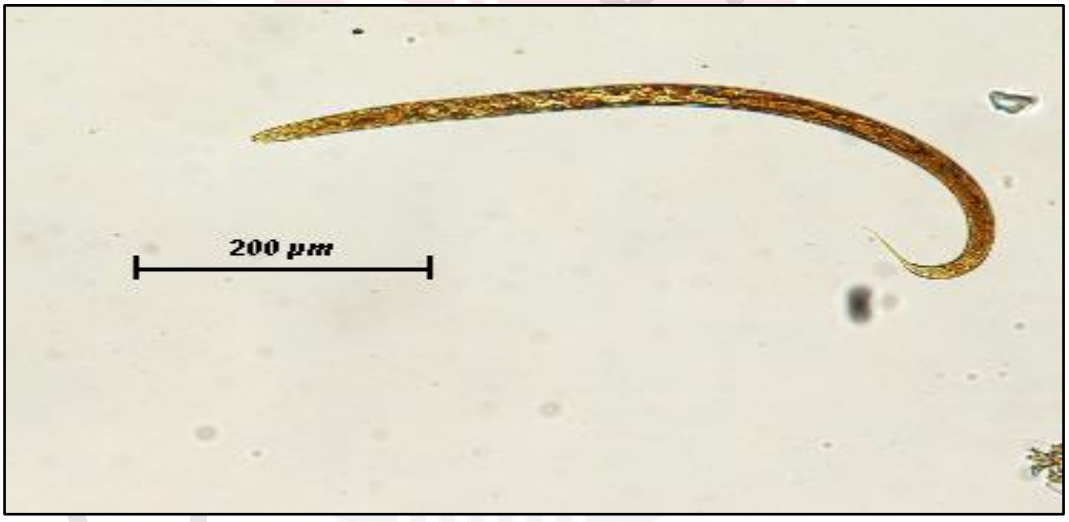


Figure 10 Nematode larvae Type 3

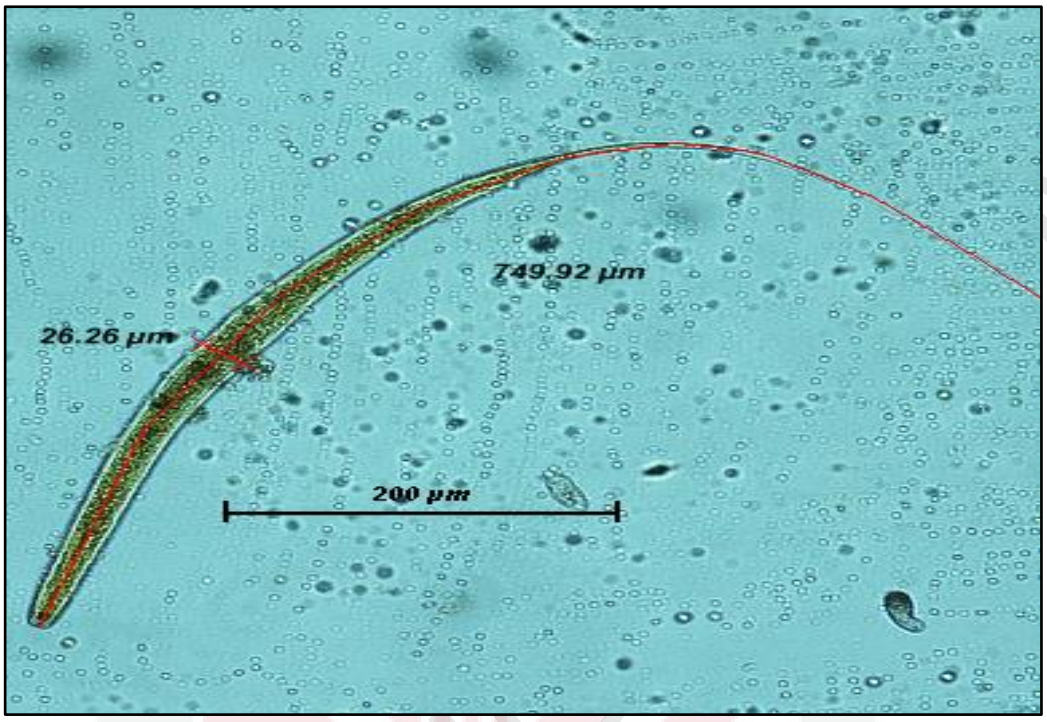


Figure 11 Nematode larvae Type 4

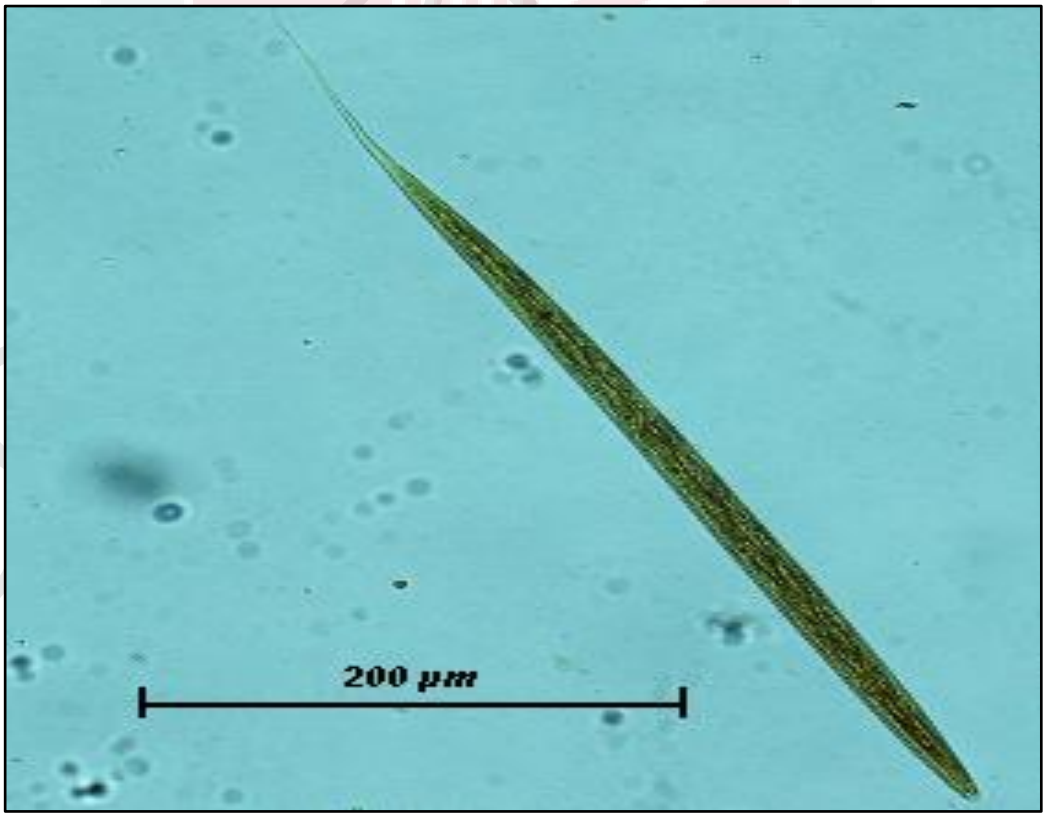


Table 6: Description of nematode larvae found in this study

Larvae	Description				
	Protective sheath	Head	Tail		
			Larvae	Sheath	Filament
Type 1	Presence	Bullet	Not notched	Medium	Long
Type 2	Absence	Bullet	Not notched	None	Short
Type 3	Absence	Squared	Not notched	None	Long
Type 4	Presence	Bullet	Not notched	Short	Long

The description of the nematode larvae found in this study was recorded in Table 6. The distinctive characteristics of these larvae through the presence of protective sheath, the shape of the head and the tail anatomy indicates that there were four different types of nematodes that was observed in this study, where type 1 that was found (n=12) to have a length of 647 to 706 µm and a width of 18 to 22 µm. Meanwhile, type 2 larvae (n=7) has a length of 655 to 689 µm and a width of 20 to 23 µm. The type 3 larvae that were observed (n=5) have a length of 730 to 749 µm and a width of 24 to 26 µm and type 4 larvae (n=4) has a length of 630 to 690 µm and a width of 25 to 29 µm. The individual width and length for all types of larvae were recorded in Table 7.

Table 7: Size of *nematode larvae (Type 1, Type 2, Type 3, Type 4)* found in this study

Larvae type	Number	Length (μm)	Width (μm)
Type 1	01	647	21
	02	649	21
	03	666	20
	04	698	19
	05	706	18
	06	701	18
	07	655	21
	08	667	19
	09	679	20
	10	696	22
	11	700	20
	12	706	21
Type 2	01	655	20
	02	657	21
	03	656	20
	04	671	23
	05	675	21
Type 3	01	730	24
	02	744	25
	03	735	26
	04	736	24
	05	748	24
Type 4	01	630	25
	02	690	28
	03	654	23
	04	688	29

4.2 PERCENTAGE OF ENDOPARASITE IN ELEPHANT FAECES OF BTFC

Both trematode and nematode ova were found with 61.5% (8/13) percentage. For nematodes, strongyle was the most common with 38.5% (5/13) followed by *Ascaris* sp. with 30.7% (4/13) and the least with *Strongyloides* sp. with 7.6% (1/13). Meanwhile, for trematodes, *Paramphistomum* sp. was found with 46.2% (6/13) and *Fasciola* sp. with 30.7% (4/13) in all samples. Nematode larvae however were found in all samples with 100% (13/13).

In Temenggor herd, Strongyles, *Ascaris* sp., and *Paramphistomum* sp. was found with 57.1% (4/7) followed by *Fasciola* sp. with 28.5% (2/7) and *Strongyloides* sp. with 14.2% (1/7).

In the Belum herd, *Fasciola* sp. was found the most common with 50% (3/6) followed by *Paramphistomum* sp. with 33.3% (2/6). Strongyles and *Ascaris* sp. were found the least with 16.6% (1/6). Table 8 shows the percentage of endoparasite ova and larva found from the elephant faeces of the Temenggor herd and Belum herd.

Table 8: The percentage of endoparasite ova and larvae in the elephant faeces in BTFC

Types of endoparasite	Temenggor herd	Belum herd	Total
Strongyle	57.1% (4/7)	16.6% (1/6)	**38.5% (5/13)**
Ascaris sp.	57.1% (4/7)	16.6% (1/6)	**30.7% (4/13)**
Strongyloides sp.	14.2% (1/7)	0% (0/6)	**7.6% (1/13)**
Paramphistomum sp.	57.1% (4/7)	33.3% (2/6)	**46.2% (6/13)**
Fasciola sp.	28.5% (2/7)	50% (3/6)	**30.7% (4/13)**
Nematode larvae	100% (7/7)	100% (6/6)	**100% (13/13)**

According to Table 8, 100% of the samples (13/13) were positive for nematode larvae, however, the types of larvae can be found in different percentages for both herds. For type 1 nematode larvae, 92.3% (12/13) of the samples were positive, followed by type 2 nematode larvae with 53.8 (7/13), type 4 nematode larvae with 38.5% (5/13) and the least were Type 3 nematode larvae with 30.7% (4/13).

In the Temenggor herd, Type 1 nematode larvae can be found in all samples followed by Type 2 larvae with only 57.1% (7/13) and Type 3 larvae with the same percentage. There were no Type 4 nematode larvae that were observed in the Temenggor herd.

In the Belum herd, 83.3% (5/6) of the samples were found to have Type 1 nematode larvae. The same percentage can be observed with Type 4 nematode larvae and the least with 50% (3/6) of the samples for Type 2 nematode larvae. There were no Type 3 nematode larvae that were observed in the Belum herd. Table 9 shows the percentage of each types of nematode larvae presence in the elephant faeces.

Table 9: Percentage of nematode larvae types in elephant faeces in BTFC

Nematode larvae	Temenggor herd	Belum herd	Total
Type 1	100% (7/7)	83.3% (5/6)	**92.3% (12/13)**
Type 2	57.1% (4/7)	50% (3/6)	**53.8 (7/13)**
Type 3	57.1% (4/7)	0% (0/6)	**30.7% (4/13)**
Type 4	0% (0/7)	83.3% (5/6)	**38.5% (5/13)**

5.0 DISCUSSION

5.1 ENDOPARASITES

The BTFC wild elephants were found to have nematode and trematode endoparasites. No cestode endoparasite were detected in any three of the methods used. These findings are similar with Wong (2018) study, where no cestodes were found from the wild elephants of BTFC. However, the percentage of endoparasites found in this study was lesser compared to Wong (2018) where they found 100% of nematode and trematode ova in all faecal samples. This may be due to the conditions of the faecal samples where she used fresh faecal samples. In this study we use S2 (2 to 5 days old) and S3 (more than 5 days old) faecal samples.

Three nematodes that was found in this study were the strongyles, *Ascaris* sp. and *Strongyloides* sp. Strongyles has been reported in numerous studies of the wild Asian elephant endoparasites (Wong et al., 2018; Phuphisut, 2015; Shahi & Gairhe, 2019; Abeysekara et al., 2018; Hing et al., 2013; Abhijith et al., 2018). The prevalence of strongyles found in other studies was ranging from 55.6% to 100% (Wong et al., 2018; Phuphisut, 2015; Shahi & Gairhe, 2019; Abeysekara et al., 2018; Hing et al., 2013). In this study, strongyle was the most common nematode present with 38.5% of the faecal samples. In Malaysia, it has been reported that other wild animals especially the non-human primates in the wild, urban, and captive populations was reported having a prevalence of 26.9% for strongyle *Oesophagostomum* spp. (Adrus et al., 2018). In Thailand, *Oesophagostomum aculatum* was found in both of their wild Asian elephants and non-human primates which may impose a zoonotic potential (Phuphisut et al,

2015). The identification of *Oesophagostomum aculatum* was done by running a DNA sequencing to a positive faecal sample and comparing it with the DNA sequences of related nematodes. Although the zoonotic potential of the strongyle in this study cannot be determined because no DNA sequencing was done, there is still a probability of our wild elephants and non-human primates share the same species of strongyles as demonstrated in Phuphisut (2015).

Ascaris sp. has not been reported in the wild elephants of BTFC. Only two studies reported *Ascaris* sp. from the faecal sample of wild elephants that has a prevalence of 37.8% in Sri Lanka and 2.3% in Thailand (Phuphisut et al., 2015; Abeysekara et al, 2018). In this study, the presence of *Ascaris* sp. in the faecal sample was 30.7%. Fowler and Mikota (2006) stated that *Toxocara elephantis* was the only genus of Ascarids from Asian elephants and the occurrence is rare. In non-human primates, Adrus et al., (2018) reported that *Ascaris* sp. was the most common nematodes found in Malaysia, with 49.7% of prevalence. However, there is no past reports that the same species of *Ascaris* sp. could infect both wild elephant and non-human primates in Asia unlike *Oesophagostomum* sp.

Another species of nematodes that were found present in the faecal sample of wild elephants in BTFC, was the *Strongyloides* sp. This species was only reported from three studies in Asia which include from India with a prevalence of 52.73%, Nepal with 44.74% and Sri Lanka with 31.1% (Shahi & Gairhe , 2019; Abeysekara et al., 2018; Abhijith et al., 2018). In this study, only 7.6% of the faecal sample was present with *Strongyloides* sp. Fowler and Mikota (2006) mentioned that, detecting *Strongyloides* spp. in faecal samples is difficult because

the eggs hatch into larvae very quickly. Even though 23.4% of the non-human primates in Malaysia is prevalent with *Strongyloides* sp. (Adrus et al., 2018), there are no reports that the non-human primates and Asian elephants share the same species of *Strongyloides* sp. same like *Ascaris* sp. The possibilities of these animals sharing same species of *Strongyloides* sp. and *Ascaris* sp. is unknown. However, non-human primates share a wide range of endoparasite with humans that includes strongyles, *Strongyloides* sp. and *Ascaris* sp. (Adrus et al., 2018).

Two trematodes that were found in this study were *Paramphistomum* sp. and *Fasciola* sp. To complete their life cycle, they depend on the presence of snail as intermediate hosts and aquatic environments (Fowler & Mikota, 2006). *Paramphistomum* sp. were reported from Wong et al., (2018), Shahi & Gairhe (2019) and Abeysekara et al. (2018). In BTFC from Wong's study, *Paramphistomum* sp. was found in all samples, Nepal with 28.95% prevalence, and Sri Lanka with 6.7% prevalence (Wong et al., 2018; Shahi & Gairhe, 2019; Abeysekara et al., 2018). In this study, 46.2% of the faecal sample was positive for this parasite. *Paramphistomum* sp. can be found in Malaysian goats with 4.5% prevalence, while the cattle and buffaloes in Terengganu were prevalent with 18% (Tan et al., 2017; Khadijah et al., 2017). There were no reports on the same *Paramphistomum* species found in the wild elephants and ruminants in Asia.

Fasciola sp. were more common to be found in wild Asian elephants compared to *Paramphistomum* sp. that includes elephant from BTFC with 100% in all samples, Sabah with 70.2% prevalence, Nepal with 39.7% prevalence, and Sri Lanka with 20% prevalence (Wong et al., 2018; Shahi & Gairhe, 2019;

Abeysekara et al., 2018; Hing et al., 2013). In this study, 30.7% of the samples were positive for *Fasciola* sp. In a case study by Caple et al. (1978), two out of nine elephants that were allowed to graze in the clearing of Pahang forest has developed fascioliasis with clinical signs of submandibular and ventral oedema. *Fasciola jacksoni* were obtained from the bile duct of an elephant during post-mortem and six of them were found to have *Fasciola jacksoni* eggs from the faecal sample with a width of 60 to 72 µm and a length of 108 to 132 µm (Caple et al., 1978). In Perak, the prevalence of *Fasciola* sp. from ruminants were reported in cattle with 7.46% and buffaloes with 7.69% (Najib et al., 2020). In Sri Lanka, *F. jacksoni* from elephants and *F. magna* from deer has the least divergence in their DNA and higher divergence with *Fasciola* sp. from ruminants (Rajapakse et al., 2019). No study has reported that the same species of *Fasciola* sp. can be found in wild elephants and ruminants or other domesticated animals. There is limited study done on observing nematode larvae in elephants. The identification of species by observing the larvae could not be done because there was no key to the identification of nematode larvae in elephants unlike in other domesticated animals such as ruminants and horses (Van Wyk & Mayhew, 2013; The RVC/FAO Guide to Veterinary Diagnostic Parasitology; Lloyd, 2020). In African elephants from Namibia, the identification of nematode larvae was done by observing the length where *Murshidia* sp. around 700 µm, *Quilonia* sp. approximately 800 µm, and *Khalilia* sp. approximately 1000 µm (Thurber et al., 2011). In Indonesia, the nematode larvae from elephants were only identified as *Strongylus* sp. by observing the tail anatomy where it has a bent tail and sharp like a needle at the posterior part (Juniar et al., 2015).

5.2 SAMPLE CONDITION

In this study, lack of samples available during sampling time may be due to the weather condition as it was raining a week before and on the day of sampling. When it rains, the mineral salt from the soil will leach out and making it easily accessible for the elephants, thus there was less elephant visit on the salt lick area (Matsubayashi et al., 2006). All faecal sample that is available was collected and the age of faecal samples was done by observing the conditions from the Hedges and Lawson (2006) faecal decay stages as described in 2.1.4.

5.3 RELATIONSHIP BETWEEN SAMPLE CONDITION AND PERCENTAGE OF ENDOPARASITE

To obtain the best result of endoparasite burden and identification, a fresh faecal sample should be used because in one to two days, nematode ova will hatch in the environment to become larvae and in 10 to 12 days, trematode ova matures to become miracidium (Fowler & Mikota, 2006). The parasite count was remarkably reduced in this study because all of the faecal samples were more than two days, thus, the endoparasitic burden was not studied. In addition, the percentage of presence of endoparasite in all the faecal samples is greatly affected and it might not be an accurate presentation of the presence of endoparasites. In Wong (2018), the faecal sample aged less than 8 hours and she found that all samples were positive for nematode and trematode ova. While, in this study, only 61% of all faecal samples were positive for nematode and trematode ova. While, all faecal samples were positive for nematode larvae because they took one week to migrate from the faeces to climb on vegetations (Fowler & Mikota, 2006).

5.4 SAMPLE PROCESSING METHODS

In this study, *Strongyloides* sp. and *Fasciola* sp. were only found in the Baermann method and not from either McMaster floatation technique or faecal sedimentation. The difference between these methods was the amount of faecal sample that was used in the Baermann method was larger (30g) than in McMaster floatation and sedimentation technique (4.5g). The parasite egg might be reduced in number because of the age of faecal sample that made the detection of parasites becomes less sensitive. Moreover, the distribution of endoparasites eggs in elephant faeces is not uniform due to the weight of the bolus, which approximately between 1.0 kg to 2.5kg and it contained a lot of fibrous material (Fowler & Mikota, 2006). It is recommended by Fowler and Mikota (2006), that a larger faecal sample should be used to obtain more accurate count. This is contradicting with Lynsdale et al. (2015) that parasite abundance can be estimated when it is collected from anywhere in the faeces of Asian elephants.

6.0 CONCLUSION

In conclusion, from the result of this study, 61% of nematode and trematode ova and 100% of nematode larvae shows there is a high percentage of endoparasites in the faeces of wild elephants from the BTFC. The transmission of these parasites to the environment and contaminate the area is high whenever the elephants enter the human population or domesticated animals area through the faecal-oral route. Two new species which were the *Strongyloides* sp. and *Ascaris* sp. that was found from BTFC wild elephants show that these elephants can harbour more unreported species of endoparasites. However, the zoonotic potential of these parasites remains unknown.

7.0 RECOMMENDATION

For future studies, bigger sample size should be used for better representation of the population of elephants in BTFC by increasing the visitation to the sampling area. At the same time, fresh faecal sample (S1) can be collected to obtain more accurate result. We can also calculate the endoparasitic burden of the elephants if we can obtain the fresh faecal samples.

Moreover, the adjustments of parasitology methods should be done by increasing the amount of faecal samples in the McMaster floatation technique and faecal sedimentation technique to obtain a better and more accurate result.

Species identification of the nematode larvae can be done by using DNA sequencing and comparing it to the DNA of nematodes from other species especially the non-human primates for potential zoonotic endoparasites.

In addition, we recommend repeating this study in the elephants of the captive population to compare the prevalence and types of endoparasite.

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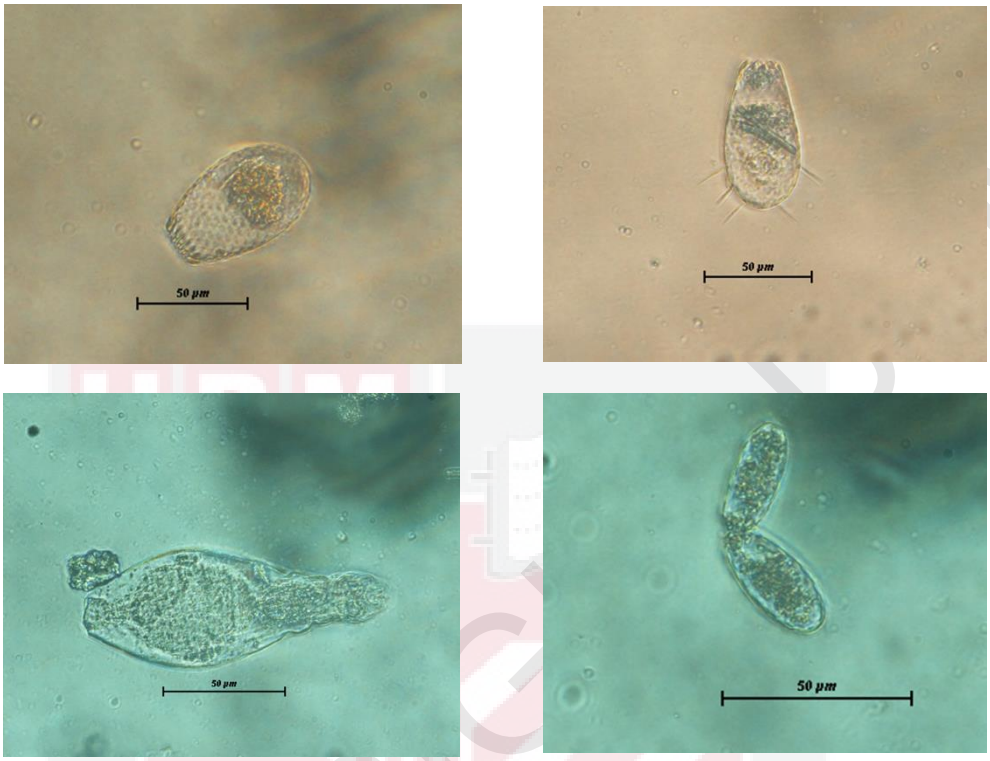
9.0 APPENDIX

A. Expert from Excel worksheet results

Herd	Sample ID	Strongyle	*Strongyloides* sp.	*Ascaris* sp.	*Paramhistomum* sp.	*Fasciola* sp.
	TG01	+	+	+	+	+
	TG02	+			+	
	TG03	+				+
	TG04	+		+		
	TG05			+		
	TG06			+	+	
Temenggor	TG07				+	
	BPA01			+		
	BPB02					+
	BPB03					+
	BP04				+	+
	BP05	+			+	
Belum	BT06					

Herd	Sample ID	Type 1 nematode larvae	Type 2 nematode larvae	Type 3 nematode larvae	Type 4 nematode larvae
	TG01	+	+	+	
	TG02	+		+	
	TG03	+	+	+	
	TG04	+			
	TG05	+	+		
	TG06	+			
Temenggor	TG07	+	+	+	
	BPA01	+	+		+
	BPB02	+			+
	BPB03	+	+		+
	BP04		+		+
	BP05	+			
Belum	BT06	+			

B. Unknown organism



C. Unknown ova