GASTRO-INTESTINAL PARASITES OF ASIAN ELEPHANT (*Elephas maximus* ***Linnaeus*****, 1758) IN AND AROUND CHITWAN NATIONAL PARK, NEPAL**

Sabina Shrestha

T.U. Registration No.: 5-2-37-495-2010

T.U. Examination Roll. No.: 144 /071

Batch: 2071

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Ecology.

Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

March, 2018

DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Date: **…….** **……**

Sabina Shrestha

T.U. Registration No.: 5-2-37-495-2010

T.U. Examination Roll No: 144 /071

Batch: 2071

TRIBHUVAN UNIVERSITY
☎ 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Institute of Science and Technology

Ref.No.:

RECOMMENDATION

This is to recommend that the thesis entitled **"Gastro-intestinal parasites of Asian elephant (***Elephas maximus* **Linnaeus, 1758) in and around Chitwan National Park, Nepal"** has been carried out by Ms. Sabina Shrestha for the partial fulfillment of Master's Degree of Science in Zoology with special paper Parasitology. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

Date: **…….**

……
Supervisor
Dr. Mahendra Maharjan
Associate Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

TRIBHUVAN UNIVERSITY
☎ 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Institute of Science and Technology
Ref.No.:

LETTER OF APPROVAL

On the recommendation of supervisor **" Associate Prof. Dr. Mahendra Maharjan"** this thesis submitted by Ms. Sabina Shrestha entitled **"Gastro-intestinal parasites of Asian elephant (***Elephas maximus* **Linnaeus, 1758) in and around Chitwan National Park, Nepal"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Parasitology.

Date: **…….**

……

Prof. Dr. Ranjana Gupta
Head of Department
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu,
Nepal

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.
Institute of Science and Technology
Ref.No.:

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ms. Sabina Shrestha entitled **"Gastro-intestinal parasites of Asian elephant (***Elephas maximus* **Linnaeus, 1758) in and around Chitwan National Park, Nepal"** has been approved as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Parasitology.

EVALUATION COMMITTEE

…….

(Supervisor)
Dr. Mahendra Maharjan
Associate Professor
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

…….

(Head of Department)
Prof. Dr. Ranjana Gupta
Central Department of Zoology
Tribhuvan University
Kirtipur, Kathmandu, Nepal

……...

External Examiner

……...

Internal Examiner

Date: …….

ACKNOWLEDGEMENTS

I would like to express my eminent gratitude to my respected academic supervisor Dr. Mahendra Maharjan, Associate Professor, Central Department of Zoology, Tribhuvan University. Without his continuous guidance and encouragement, this dissertation would have been a herculean task. Similarly, I would like to express my special gratitude to Prof. Dr. Ranjana Gupta, Head of Dept., Central Department of Zoology, Tribhuvan University for her co-operation and support to carry out my thesis work.

This work would not have been possible without the support and permission of Department of National park and Wildlife Conservation (DNPWC) and Chitwan National Park. So, I am thankful to DNPWC and Chitwan National Park for granting immense help for conducting my research work. My gratefulness goes also to Mr. Abhinaya Pathak, Assistance Conservation officer, Chitwan National Park for his continuous support and co-ordination. I am indebted to Mr. Ramkaji Shrestha, for his kind and appreciative support, cooperation during my fieldwork. I would like to give my heartily thanks to the staffs of elephant breeding centre, Khorsar, Sauraha for great support during sample collection.

Special thanks go to all the staffs of Tiger Tops Tharu Lodge, Temple Tiger Lodge and Machang villa, who supported my work and contribute their valuable time on providing the samples which made me easier to complete my task.

My gratefulness goes also to all the staffs of Central Department of Zoology, TU for their continuous motivation, aspiration and cooperation and the facilities during my thesis work. My special thanks also goes to my supportive and co-operative friends Ms. Prashamsa Paudel, Mr. Sanskar Neupane and Mr. Prabin Baral, Ms. Karuna Khadka for their support and insights as well as knowledge which helped me to find and fill gaps in my dissertation.

Lastly, but definitely not least I would like to express my special thanks to my family and all my family members for their inspiration and generous support.

Sabina Shrestha

rozasabina201159@gmail.com

CONTENTS

LIST OF TABLES

Table **Title of Table** **Pages**

LIST OF FIGURES

Figure Title of figures Pages

LIST OF PHOTOGRAPHS

LIST OF ABBREVIATIONS

cm	-	Centimeter
CNP	-	Chitwan National Park
DNPWC	-	Department of National Park and Wildlife Conservation
EEHV	-	Elephant Endotheliotropic Herpes Viruses
GIT	-	Gastrointestinal tract
IUCN	-	International Union for Conservation of Nature
g	-	Gram
P value	-	Probability Value
sp.	-	Species
µm	-	Micrometer
UK	-	United Kingdom
USA	-	United State of America
WWF	-	World Wildlife Fund

ABSTRACT

Asian elephant is an endangered species in Nepal and its numbers are declining day by day. Present study was conducted to assess the prevalence of gastrointestinal parasites in Asian elephant in and around the Chitwan National Park. A total of 20 dung samples from wild elephants were collected by opportunistically from Chitwan National Park whereas as a total of 80 dung samples were collected from 40 captive/domestic elephants by the duplicate sampling technique with interval of 15 days in the month of May 2017. The collected dung samples were preserved in 2.5% potassium dichromate and microscopically examined using the direct smear method and concentration methods. Prevalence of protozoan and helminthes parasites was found to be 90% and 57% in wild and captive/domestic elephants respectively. In both wild and captive/domestic elephants, one protozoan parasite was reported: *Eimeria* sp. with 15% and 7.5% respectively. Among the helminthes parasites, one genus of cestode was reported from wild elephants: *Anoplocephala* sp. (10%) but was not found in captive/domestic elephant. Eight different genera of nematodes were identified in wild elephants (*Ascaris* sp. (45%), *Strongyloide* sp. (85%), *Haemonchus* sp. (25%), *Trichostrongylus* sp. (10%), *Dromeostrongylus* sp. (30%), *Chabertia* sp. (15%), *Bunostomum* sp. (10%), and *Nematodirus* sp. (10%) whereas four different genera of nematodes were identified in captive/domestic elephants (*Ascaris* sp. (27.5%), *Strongyloide* sp. (52.5%), *Haemonchus* sp. (7.5%), *Bunostomum* sp. (2.5%). No trematode was reported from the both wild and captive/domestic elephants. Location wise analysis suggested the higher prevalence of gastrointestinal parasite in dung samples collected from Chure area (30%) where as it was found higher from the Khorsar breeding center (15%) and Tiger Tops Tharu Lodge (15%) in captive/ domestic elephant. Mixed infection was reported in wild elephant with double (35%), triple (40%) and multiple infections (15%) but no single infection. However, in case of captive/domestic elephant's single infection (32.50%) was reported with some mixed infection such double (17.50%) and multiple infections (7.50%). Intensity of parasites suggested that the light infection is prevalent than that of heavy infection in both cases. Comparing the morphologically similar parasites, the statistical analysis showed that there was significant difference in the prevalence of parasites among the wild and captive/domestic elephants (χ^2= 5.0931, P-value= 0.02). However, the parasites such as *Dromeostrongylus* sp., *Nematodirus* sp., *Chabertia* sp., *Trichostrongylus* sp. and *Anoplocephala* sp. were only observed in wild elephants.

1. INTRODUCTION

1.1 Background

Ecosystem is a community of living organism in conjunction with the non-living components of their environment interacting as a system. Every animal in an ecosystem is equally important. Some of the species has already been extinct and some has been listed as critically endangered, vulnerable, rare, threaten, protected etc. in both global and national status. There are different reasons for loss of animals from their natural habitat like habitat loss, natural disaster, low land food quality etc.

Nepal, with the variation in its climate and topography from tropical to artic has a notable feature of wildlife and vegetation diversity. In 2037 B.S, Government of Nepal established National Parks and Wildlife Reserve for the conservation and proper utilization of the wildlife and vegetation. There are 10 National parks, three wildlife reserves, one hunting reserves and six conservation Areas as declared by Nepal government. Among these 10 national parks, Chitwan National Park is first national park of Nepal, which was established in 1973. It is considered as home to varieties of wildlife and in which elephant is one of them. The vegetation of this national park is a moist deciduous vegetation type mainly characterized by Sal forest, which roughly covers 70% of park and the remaining vegetation types, includes grassland, riverine forest and Sal with Chir pine *Pinus roxburghii*. The grasslands are mainly located in the floodplains of rivers and form a diverse and complex community with over 50 different types of grasses including the elephant grass (*Saccharum* sp.) which is renowned for its immense height and can grow up to 8 meter in height.

It has been found that Asia elephant have been less focused to the endangered status than African elephants but actually the population of Asian elephant is in more endangered (Cheeran and Poole, 2009). Asian elephants which once used to be abundant throughout the lowland forests of Nepal are increasing under the risk of extinction due to rapid human population growth and subsequent resource demands including poaching (Kharel, 2001). In order to avoid the extinction of such endangered species, Nepal government have been trying to address various policies like including the elephant in the list of protected species in National Parks and Wildlife Conservation Act 1973 (NPWCA 2029), conducting Elephant conservation Action Plans (2008) and more importantly, adopting the Terai Arc landscape level conservation program. At the same time, considering the equal importance of the domestic or captive elephants Nepal government has established an elephant breeding facility at Chitwan National Park (Pradhan *et al*., 2011).

In Nepal, the majorities of private elephants are in and around CNP and are mainly used for patrolling and research purpose whereas the elephants are also mostly used for forest excursions and entertainment of tourists in buffer zones of parks and reserves (Pradhan *et al*., 2011).

1.2 Asian Elephant (*Elephasmaximus* Linnaeus, 1758).

At the present context, two species of the Elephants have been recorded i.e. African elephant (*Loxodonta africana* Anonymous, 1827) and Asian Elephant (*Elephas maximus* Linnaeus, 1758). Asian elephants are of five strains and they are Indian, Burmese, Ceylonese, Sumatran and Malaysian (Cheeran, 2009). Asian elephant (*Elephas maximus)*

is a largest terrestrial animal in Asia (Ahmed and Doley, 2017), which has been enlisted as an endangered in the List of Threatened species (IUCN, 2008).

1.3 Characteristics of Asian elephant

Elephants are fascinating animal with their various peculiarities (Cheeran, 2009). According to Chungath (2009), the Asian elephants are smaller than African elephant. They weigh about 5000kg with height of 10-11 ft. They have skin color grey and may be masked with soil because of dusting and wallowing. They have unique long featured trunk touching on the ground with one finger like process at the tip of it and smooth compared to African elephant. They have greatly developed sense of smell. The tusk can be found in tusker only and is short with stout. The Asian elephants have broad ear but is small in size compared to African and have excellent hearing power but it have poor eyesight. The back of the Asian elephant is unbroken convex and have twin domed forehead. They used to have five nails on forefoot and four in hind foots usually.

1.4. Population status

In Asia, there are about 35000- 40,000 wild elephants in 13 countries, which include Nepal, India, Bhutan, Bangladesh, Sri Lanka, Burma, Thailand, Kampuchea, Loas, Vietnam, South China, Indonesia and Malaysia (DNPWC, 2008). It has been estimated that the population of the wild elephants in Nepal is 107-145 individuals (Pradhan *et al*., 2011). The resident wild elephants are believed to occur in four-isolated populations that are known as Eastern (7-15), Central (25-30), Western (60-80) and Far western (15-20) populations (Shrestha and Gairhe, 2006). In Nepal, the history of captive elephant management was only recorded after 1903(Kharel, 2001). At present, the population of domestic elephant in Nepal is estimated to be 215.Out of which 16.66% male's ad 83.3% females have been recorded and the majority of the captive population is found in and around the CNP (Gairhe, 2012).

1.5. Ecology and behavior of Asian elephant

According to Baskaran *et al*. (2010) Asian elephants are well adapted to live in an adverse habitat by exploiting a wide spectrum of plant species. They show two peaks in feeding, one in the morning and other at evening. They used to rest more at around the midday rather than other time. They engaged themselves in activities such as mud bath, sunbath, salt licking. They are mega herbivores, which can consume up to 150 kg of plant matter per day (Samansiri *et al*., 2007). According to Pradhan *et al.* (2008), elephants consumes large amount of floodplain grass *Saccharum spontaneum*, particularly during monsoon season. According to Cheeran (2009), elephants feeds on all three tiers of plant life like grass, bush and canopy and have clean feeding habits as they used to pull out a bunch of grass and dust mud and dirt against their legs before eating it and can drink 200-250 liters of water per day. They love to swim in water and wallowing in marsh. The gestation period in elephant is 21 months. At its birth, the calf weighs 80-90 kg and 90-100cms in height. They defecate about 15-20 times a day and urinate 10-15 times a day. They can travel extensively, walking long distance in wild for food, shelter and water and do not prefer to confine in single place for long time. Elephants are gregarious in nature and used

to have matriarchal group with female as the leader of a herd whereas males are found to have less attachment to the herd. Defending strategy of elephant is one of their interesting behavior and they can never be domesticated completely as they have always desire to get back to their natural habitat.

1.6. Threats

The greatest threat for the elephant range in Nepal is constant threat of habitat loss and fragmentation to smaller area due to the increasing human population (DNPWC, 2008). Another most important threat for elephant is Human elephant conflict in which the fertile land with intensive agriculture of Terai next to elephant habitat seem as the fatal attraction to the elephant (Pradhan *et. al*., 2011). Illegal capturing of the wild elephant and their taming causes severe disruption of wild herd, cruelty and high death rate with a great number of captured babies dying in the captured and taming process so it is also considered as a serious threat to Asian elephant (Jayewardene, 2014).

1.7. Parasitic infection in Asian elephant

Parasite is an organism that lives on or in a host and gets its food from or at the expense of its host**.** They can live throughout the body but prefer the intestinal wall (Coop and Holmes, 1996; Coop and Kyriazakis, 1999). Wild and domestic animals share the common grazing land in most of the cases (Walker, 1995) therefore an animal infected with the different gastrointestinal parasites can get transfer to other animals (Mawdsley *et. al.,* 1995; Nunn *et. al.,* 2011). The frequent occurrence of diseases has been one of the major factors for the fact of decline in populations of some species of wild and domesticated mammals (Shrestha, 2003; Wolfe *et. al.,* 2004; Morgan *et. al.,* 2006).

The elephants face conflict with humans, habitat destruction and fragmentation, disease and other challenges across their ranges, they seems to be susceptible to various infectious and non-infectious disease. The infectious and parasitic disease includes tetanus (*Clostridium tetani*), tuberculosis (*Mycobacteriu tuberculosis*), hemorrhage septicemia (*Pasturllamultocida*), salmonellosis (*Salmonella* sp.), anthrax (*Bacillus anthracis*), rabies, elephantpox, foot and mouth disease, Elephant endotheliotropic herpes virus (EEHV) infection, mycosis, surra, piroplasmosis, 'bots', toxoplasmosis, helminthiasis and ectoparasitism (Fowler and Mikota, 2006; Firyal and Nureen, 2007; Chandrasekharan et al, 2009; Gairhe 2012). At least 85% cases of suspected EEHV (Elephant Endotheliotropic Herpes Virus) hemorrhagic disease have been recorded in elephant worldwide and the acute form of this disease has had an 80-90% fatality rate (Latimer et al., 2011). Mandal et al., (2013) on his recent study about the health status of Asia elephants (governmental owned captive elephants) in CNP, have recorded infectious diseases like Tuberculosis as the major to cause death in elephant and similarly prevalence of gastrointestinal helminthosis with 19.2% where as other non-infectious disease like insect bite, eye disorder, skin disorder digestive disorder etc. were also recorded. Elephant tuberculosis is considered as chronic disease that affects captive elephant. In Nepal, it was first identified from Chitwan National Park in 2002 (Gairhe, 2002) and there are some records of mortality of elephants in Nepal (2002-2009) because of tuberculosis (DNPWC, 2011).

1.8. Objectives

1.8.1 General objectives

To study the gastro-intestinal parasites of Asian elephants (*Elephas maximus*, Linnaus, 1758) in and around Chitwan National Park.

1.8.2 Specific objectives

- To determine the prevalence of gastro-intestinal parasites in wild elephant.
- To determine the prevalence of gastrointestinal parasites in captive/domestic elephant.
- To compare the prevalence of gastrointestinal parasites of wild and captive/domestic elephant.

1.9. Significance of the study

In global context, several of researches studies on the gastrointestinal parasites of elephants have been done. But in national context, very few research works have been done in captive/domestic as well as wild elephants in CNP (Karki, 2008; Mandal and Khadka, 2013; Devkota *et. al.*, 2014; Pandit *et al.*, 2015). Such kind of study in wild context has not been done in detail yet. Some of the parasites of elephants reported in previous studies are common to them.

However, Parasites and their effect involved in elephants should be highly considered. This study provide the overview on the gastrointestinal parasites in Asian elephants in and around the Chitwan National Park with some strong recommendation for the management and welfare of CNP including health aspect of the elephants and also provides guideline data for the further action plans worldwide.

2. LITERATURE REVIEW

Elephants are the most recognized charismatic mega fauna and studies on them have great appeal to scientists as well as the whole world (Sukumar, 2003). Asian Elephant are found to be mostly susceptible to gastro-intestinal parasitic infestation in the wild (Watve, 1995; Dharmarajan, 2000; Vidya and Sukumar, 2002) but in captivity as it is often confined to small enclosures (Vanitha, 2007) and in moist unhygienic condition it may have high susceptibility to parasitic disease (Dhungel *et al*., 1990; Chandrasekharan *et al*., 1995; Suresh *et al*., 2001). Parasitic infection have been also found to be associated with mortality in African elephants though research on the parasitic fauna of this species is limited (Vitovc *et al.*, 1984; Obanda *et al*., 2011) whereas there have been more research work in parasites of Asian elephants (Lei *et al*., 2012). Although the survey to study the parasite prevalence and load in Asian elephant are seldom reported in literature, collating data in large amount from captive Asian elephants (Fowler and Mikota., 2006), there have been relatively very few studies carried out on the parasites of the free ranging wild animal species which has led to a lack of baseline parasitological data (Hing *et al*., 2013).

2.1. Global scenario of parasites in elephant

It have been believed that the elephants were originated in Africa in late Eocene epoch but today, the Asian elephant (*Elephas maximus*), African bush elephant (*Loxodonta africana)* and African forest elephant (*Loxodonta africana cyclotis)* are the only species that have survived (Fowler and Miktoa, 2006). Some major researches regarding parasites in elephant have been carried out from different national parks, wildlife reserves, zoo and zoological gardens of different countries.

In case of American continent, the first captive elephant was imported as single animal in New York from India in the late 1700s (Goodwin, 1951). There are 78 zoos in North America, which hold 286 elephants of which 147 African and 139 Asian elephants have been recorded (Cohn, 2006). Some of the major researches regarding Endotheliotropic herpes viruses have been carried out in different zoos of North America. Richman *et. al*. (2000) described the presence of highly fatal disease caused by novel Endotheliotropic herpes viruses in nine Asian and two African elephants of North American Zoo by clinical and pathological findings. There have been eight confirmed death of captive Asian elephants in North America due to herpes virus since 1985 (Schmitt *et. al.*, 2000). Likewise, the bacterial disease Tuberculosis (*Mycobacterium tuberculosis*) has also been a disease of concern that may exacerbate the decline in the population of elephant in North America (Wiese, 1997). Mikota *et. al.* (2000) reported 3.3% prevalence of *Mycobacterium tuberculosis* isolated from 539 elephants in North America in between August 1995 and May 2000. In similar way, 40 elephants representing over 12% of the Asian elephants in United States were also diagnosed with Tuberculosis in between 1996 and 2007 (Mikton, 2008). However, the research work in regards to the gastro-intestinal parasites have been lacking in this continent.

Similarly, in case of African continent there are two distinct types of African elephants, often considered as two species: savannah elephants *Loxodonta africana* (Blumenbach,

1797) and forest elephants *L. cyclotis* (Matschie, 1900) (Maisels *et. al.*, 2013). Some of the research works regarding disease Tuberculosis have been done in elephants in Africa. The first case of fatal disease Tuberculosis in a wild African elephant was reported in Tsavo East National Park, Kenya (Obanda *et. al.*, 2013) and for the first time Rosen *et. al.* (2017) studied to assess the TB seroprevalence and risk factors in working African elephants in Zimbabwe and found six of 35 total elephants to be seropositive. Similarly, some of the major researches regarding the intestinal parasites of elephants have been done in different national parks of Africa. Kinsella *et. al.* (2004); Fowler and Mikota (2006); Thurber *et. al.* (2011) found the nematode frequently in African elephants. Kinsella *et. al*. (2004) reported Schistosome, *Bivitllobilharzia* sp., nematode Strongylids and ciliates in the faceal sample of six African forest elephants from the national park of Republic of Congo and Central African Republic. Fowler and Mikton, (2006) found Coccidian infection to be common in African elephants. Similarly, Lofty *et. al.* (2008) have considered *Protofasciola robusta*, the sole member of the Protofasciolinae as the elephant specific intestinal fluke. Obanda *et. al*. (2011) reported hookworms and trematode, *Protofasciola robusta* in the intestine of the free ranging African elephants in Kenya. Mbaya et al. (2013) examined 274 African elephants of the Chad Basin National Park, Nigeria of which 36.86% were found to be infected particularly with *Strongyloide* sp., *Coccidia* sp., and *Strongyle* sp. Brant *et. al*. (2013), reported Schistosome eggs from the dung samples of the wild forest elephants from the Central African Republic. Similarly, Baines *et. al*. (2015) analyzed a total of 458 feceal samples of the wild elephants from Okavango Delta, Botswana and reported the prevalence of Coccidian oocytes (51%), nematode (77%) and trematode eggs (27%) which was consistent with that of intestinal fluke *Protofasciola robusta*.

In case of European continents, there have been some research works in Tuberculosis in captive elephants. Ghilmetti *et. al.* (2017) investigated the presence of *Mycobacterium tuberculosis* in all three Asian elephants which were wild born in Indonesia and moved to Europe, Swiss zoo. Similarly, some of the research works regarding the Endotheliotropic Herpes virus (EEHV) on the captive elephants have been carried out in different countries of Europe. However, at least three Asian elephants calves have been lost due to EEHV in Switzerland earlier, 5 out 7 Asian elephants were reported as Shedder of the particular virus (Ackermann *et. al.*, 2017). Kendall *et. al.* (2016) reported 29.6% of fatalities in Asian elephants born in captivity in United Kingdom and Ireland by EEHV Hemorrhagic disease. Similarly, few research works have been done regarding intestinal parasites in elephants. Delgado *et. al. (*2003) analyzed feceas from 34 species of ruminants at Lisbon Zoo and reported 3.6% of crysptosporidium infection. Fagiolini *et al.* (2010) investigated in the mammals housed in two of the main Italian zoological gardens and diagnosed prevalence of *Strongyles* in 16.7% of Proboscideans.

In case of Australian continent, the research work regarding the Tuberculosis in a captive Asian elephants have been reported from Taronga zoo, Australia (Vogelnest *et. al.*, 2015). Similarly, Seeman and Finnie (1987) reported the death of the captive African elephant at Western plain zoos, Dubbo, in central New South Wales due to acute Myocarditis, probably by viral etiology. Hence, these are some evidences that the African and Asian

elephants have been in different Zoos and Sanctuaries of this continent but very few research works regarding the disease and parasites in them have been reported.

In case of Asian Continent, wild Asian elephants are found in 13 Asian countries (Kempf and Santiapillai, 2000) and today about 15000 Asian elephants are in captivity comprising one-third of the global population (Fernando, 2012). Some of the research works regarding the Tuberculosis (*Mycobacterum tuberculosis*) have been carried out in different national parks and zoos of different countries. Verma *et. al.* (2012) recorded the evidence of higher prevalence of asymptomatic *M.tuberculosis* in captive Asian elephant, India. Perera *et. al.* (2014) reported first confirmed case of fatal TB in a wild Asian elephant from Srilanka. Likewise, four Asian elephants in Thailand were also confirmed with *M. tuberculosis* (Angkawanish *et. al.,* 2010). Likewise, some research works regarding Endotheliotropic herpes viruses (EEHV) have been carried out in different free ranging as well as captive elephants in different countries. Reid *et. al.* (2006) carried out the first PCR- confirmed test in wild caught 3-year-old elephant, found dead in Cambodian elephant sanctuary. Similarly, Barman *et. al.* (2017) reported the EEHV with an association of mortality in a captive elephants of Assam, India.

However, some major research works regarding the intestinal parasites in elephants have been carried out in different wild and captive condition in different countries of Asia. Chandrasekhara (1992) recorded 91.27% Strongylosis in captive elephants, maintained by the forest department. Huang (1981); Chandrasekharan *et al*. (1982) and Dutta and Bordoloi (1989) identified Amphistomosis. Futhermore Chandrasekharan (1992) reported Amphistomiasis (17.8%) in the captive elephants, Kerala. Gaur *et al.* (1979); Chandraskharan *et al*. (1982); Dutta and Bordoloi (1989); Roa *et al*. (1992); Cheeran (1999) reported high incidence of *Strongyle* infection in captive elephants. Similarly, Suresh *et al*. (2001) reported 63.64% and 87.5% *Strongylosis* at S.V Diary Farm, Tirupati and Nehru respeectively. Vidya and Sukumar (2002) analysed 320 samples of Asian elephants in Nilgiris, southern India and reported 86.8% of at least one parasite propagule, and 84.4% of at least one *Strongyle* eggs whereas *Anoplocephala* were found in two individual's samples with mixed infections recorded in 11.9% of the samples. Saseendran *et al.* (2004) examined 99 samples of captive elephant in Kerala, out of which 17.7% were positive for parasitic infection among which Strongyles (10.10%) *and* Amphistomes (7.07%) were reported. Similarly, Jani (2008) studied the prevalence of gastrointestinal parasites in 40 Indian elephants kept in different temples of Gujarat, where 62.5% of elephants were reported to have parasitic prevalence with high prevalence of *Fasciolias* sp. (15%) in single infection followed by *Paramphistomum* sp. (10%), *Strongyloides* sp. (5%), *Oesophagostomum* sp. (5%), *Mursidia* sp. (2.5%), *Ascaris* sp. (2.5%) and the prevalence of other double infection was also recorded. Arunachalam *et. al*. (2007) reported 36% prevalence of *Strongyle* sp. among domestic Asian elephants in Tamil, Nadu. Vanitha *et al.* (2011) further more evaluated the prevalence of parasites in faecal samples collected from 115 captive elephants of Tamil, Nadu and reported Strongyles sp (37%). Nishanth *et al*. (2012) investigated the incidence of endoparasitism in 75 dung samples (each of 25 dung samples) from free ranging elephants of Tamil Nadu state at Mudumalai, Anamalai and Sathyamangalam forest and reported 82.66% of samples positive with gastrointestinal parasites containing Strongyle egg (21.33%),

Strongyloides sp. (25.33%), *Anoplocephala* sp.(13.33%), *Bivitellobilharzia* sp.(2.6%). Ahmed and Doley (2017) screened 50 Asian elephants of Tripura for the prevalence of parasitic infestation of which 68% of samples were revealed positive out of which, the incidence of *Fasciola* sp. was found to be highest (44.12%) followed by *Paramphistomum* sp. (20.59%), Ascaris sp. (14.71%), Strongyle sp. (14.71%) and of *Oesophagostomum* sp. (5.89%).

Hing *et al*. (2013) were the first to do the parasitiological survey of Endangered Asian Bornean elephants and reported the prevalence of parasites in all 104 dung samples with *Fasicola* (70.2%), *Strongyles* (66.3%) followed by *Anoplocephala* (50.0%) and majority of the samples (65.4%) were revealed with more than one phylum of endoparasites in two key range in Sabah, Malaysia. Bhoyar *et al*. (2014) coprologically examined four female Asian elephants of Rambo international circus camped at Bidar, Karnataka state of India and reported each of *Schistosome* ova, *Strongyle* ova and Coccidian oocysts with mild prevalence. Gurelli and Ito (2014) first reported seven ciliated genera consisting 36 species of intestinal ciliates from the dung of three Asian elephants living in Sasali National Park, Turkey. Lynsdale et al. (2015) commonly reported Strongyle and Strongyloides type of nematode eggs with suspected *Paramphistomum* eggs occasionally in faecal sample of Asian semi- captive population of working elephants located in the two area of Sagaing region, northern Myanmar, Katha. Phuphisut *et. al.* (2016) microscopically examined the faceal samples of Asian wild elephants of the Salakpra Wildlife Sanctuary, Kancanburi, Thailand and reported higher prevalence of Strongylids (93.0%) followed by trichurids (2.3%) and ascarids (2.3%). Futhermore, Abeysinghe *et. al.* (2017) reported the prevalence and abundance of gastrointestinal parasites strongyle in captive elephant of Pinnawela Elephant Orphanage (38%), privately owned (90%) and in wild elephants (100%) in Srilanka.

2.2. National scenario of parasites in elephant

Asian elephants, which once were abundance throughout the lowland of Nepal, are increasingly under the risk of extirpation due to rapid human population growth and subsequent resource demands including poaching (Kharel, 2001). Wild elephants in Nepal are confined in protected area mainly Suklaphata wildlife Reserve, Bardia National Park, Chitwan National Park and Parsa Wildlife Reserve and nearby forests areas (DNPWC, 2011). The Asian elephants are susceptible to various infectious diseases but there are very few documentation found in the disease and parasite condition of elephants in Nepal (Karki, 2008). In Nepal, Tuberculosis (TB) was first identified from Chitwan National Park in 2002 (Gairhe, 2002) and have the record of death of seven captive elephants in the period of seven years (2002-2009) because of tuberculosis (DNPWC, 2011). Mandal et al. (2013) on his recent study about the health status of Asia elephants (governmental owned captive elephants) in CNP, have recorded infectious diseases like Tuberculosis as the major cause for the mortality in elephant with the prevalence of gastrointestinal helminthosis (19.2%) and other non-infectious disease like insect bite, eye disorder, skin disorder digestive disorder etc. Similarly, Pandit *et. al.* (2015) have reported, out of 35 captive elephants from buffer zone of CNP, Sauraha, 63% of elephants were negative with Tuberculosis and 23% positive with treatment ongoing, 11% have

completed the TB treatment before one year and 3% have completed the TB treatment before 2 years. Pandit *et. al.* **(**2014**)** revealed zero EEHV infection in the elephants of Chitwan National Park but have suggested the need of regular checkup of the disease.

However, Karki (2008) was the first to conduct the study in order determine the prevalence of the parasitic infection among captive elephants from different wildlife reserve parks of Nepal such as Suklaphanta, Bardiya, Sauraha and Koshi tappu by collecting the faceal samples from a total of 20 elephants and 100% prevalence of parasitic infestation was reported with 6 samples positive with *Fasciola jacksoni*, 2 samples with *Paramphistome*, 15 samples revealed the presence of egg and larvae of *Oesophagostomum* spp and *Chabertia* spp followed by 18 samples positive for *Scishistosomes* spp and each of one sample positive for *Dicrocoelium* spp and *Monezia* spp. Mandal and Khadka (2013) recorded 19.2% cases of gastrointestinal helminthosis in government owned captive elephants at CNP. Devkota *et al*. (2014) searched for the Schistosomes in and around CNP of Southern Nepal as the digenetic fauna of Nepal were poorly known and collected 2 dung samples from wild elephants and 22 samples from Captive elephants in CNP of which 1 of the two dung sample from wild and 1 of 22 sample from the captive elephant was found to be positive for Schistosome (*Bivitellobiharzia nairi*). Pandit *et. al*. (2015) conducted study in the private elephant stable located around the buffer zone of CNP, Sauraha, Nepal in monsoon season and among 35 elephants 9% of elephants has the infestation of *Fascioloide magna* and 23% of elephant have the infestation of *Strongyloides westeri*.

3. MATERIALS AND METHODS

3.1. Study Area

Nestled at the foot of the Himalayas, Chitwan National Park is the last surviving example of natural ecosystem of terai region. It was formerly known as Royal Chitwan National park but after peoples' revolution it has been addressed as Chitwan national park, which was established in 1973 A.D. It is located in subtropical inner terai lowland of south-central terai (27°30' 0.65" north and 84°44' 05 41" east) of Nepal and covers an area of 932 sq.km. It is formerly extended over the foothills and four districts Chitwan, Parsa, Makwanpur and Nawalparasi. The core area of this national park lies between the Narayani and Rapti rivers to the north. Rheu River and Nepal-India International border in the south over the sumeswar and Churia hills and from Dawney hill west of Narayani and borders with Parsa wildlife reserve to east. It have sub-tropical monsoon climate, with high humidity throughout a year. The parks headquarter is at Kasara, Chitwan.

The park consists of tropical and sub-tropical forests with 70% of the area cover with Sal (*Shorea robusta*) forests and 20% of area cover with grassland. There are more than 50 different types of grasses, including the elephant grass (*Saccharum* sp.), renowned for its immense height as it can grow up to 8m heights. CNP is considered as home to different varieties of wildlife as it harbors 31% of mammals, 61% of birds, 34% of amphibians and reptiles, 34% of fishes recorded in Nepal. Due to its unique biological resource of outstanding universal value, UNESCO designated CNP as world heritage in 1984. Only 9 years after the establishment, CNP witnessed its first wild elephant from Parsa in 1984. The endangered fauna found in CNP are elephants, one-horned rhino, Gaur, Royal Bengal tiger, four-horned antelope, Pangolin, Golden monitor lizard, python, Giant hornbill, Black stork, white stork, Bengal florican, Lesser florican etc (Bhusal, 2007).

The elephants are found rearing around the CNP by the Governmental forest department as the captive one. Khosar breeding centre is unique center, which was established in 1985 for the captive breeding of domesticated elephant trapping for domestication located at 1-hour distance from Sauraha. There are about 20 elephants in breeding center. Similarly, Sauraha sector, Hattishar also rear about 11 elephants for the governmental purpose. There are many private hotels around the CNP, which used to keep elephants for tourism purpose as domestic elephant such as Tiger Tops Tharu Lodge, Temple Tiger Lodge and Machang country villa. They are situated in Nawalparasi district around the CNP area.

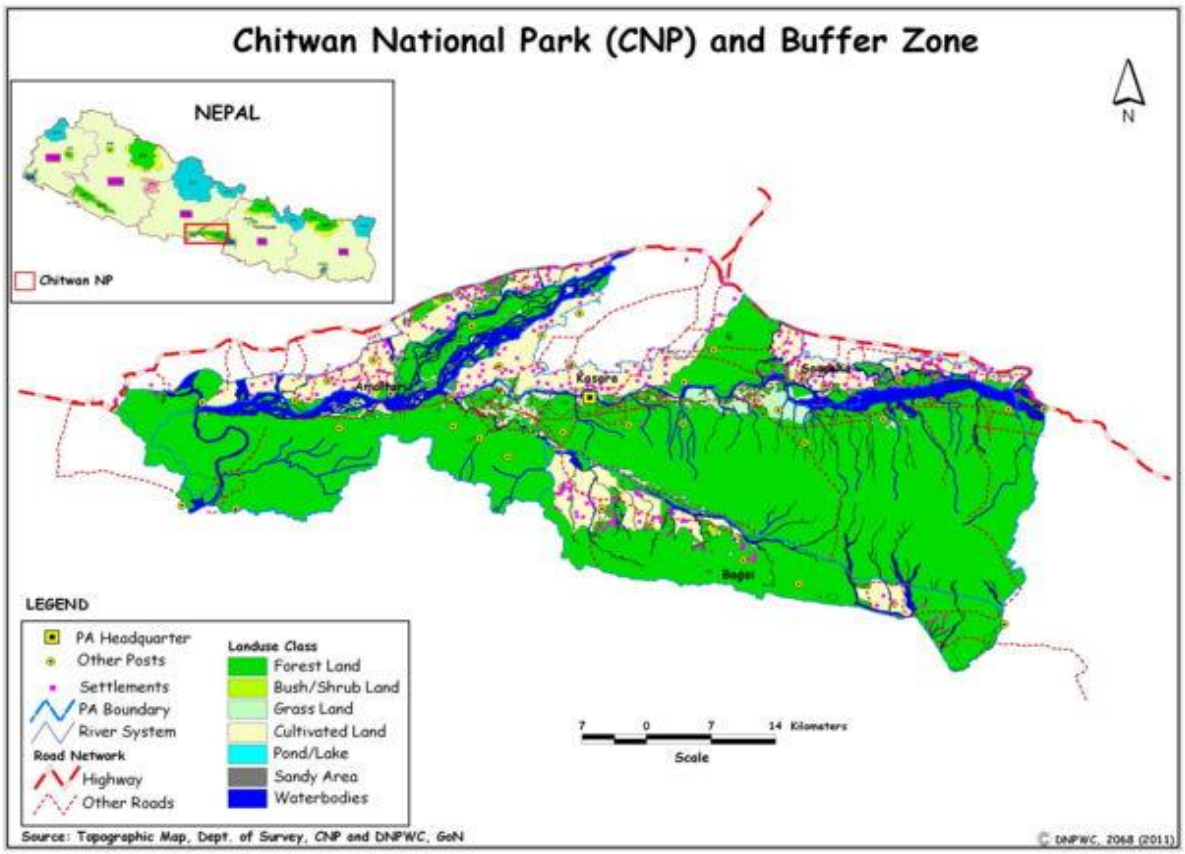


Figure 1. Map of Chitwan National Park, Nepal. (Source: DNPWC, 2011)

3.2. Materials

3.2.1. Materials for field

Potassium dichromate solution, Gloves and Masks, Map, Camera, Vial or Zipper bag and Binocular.

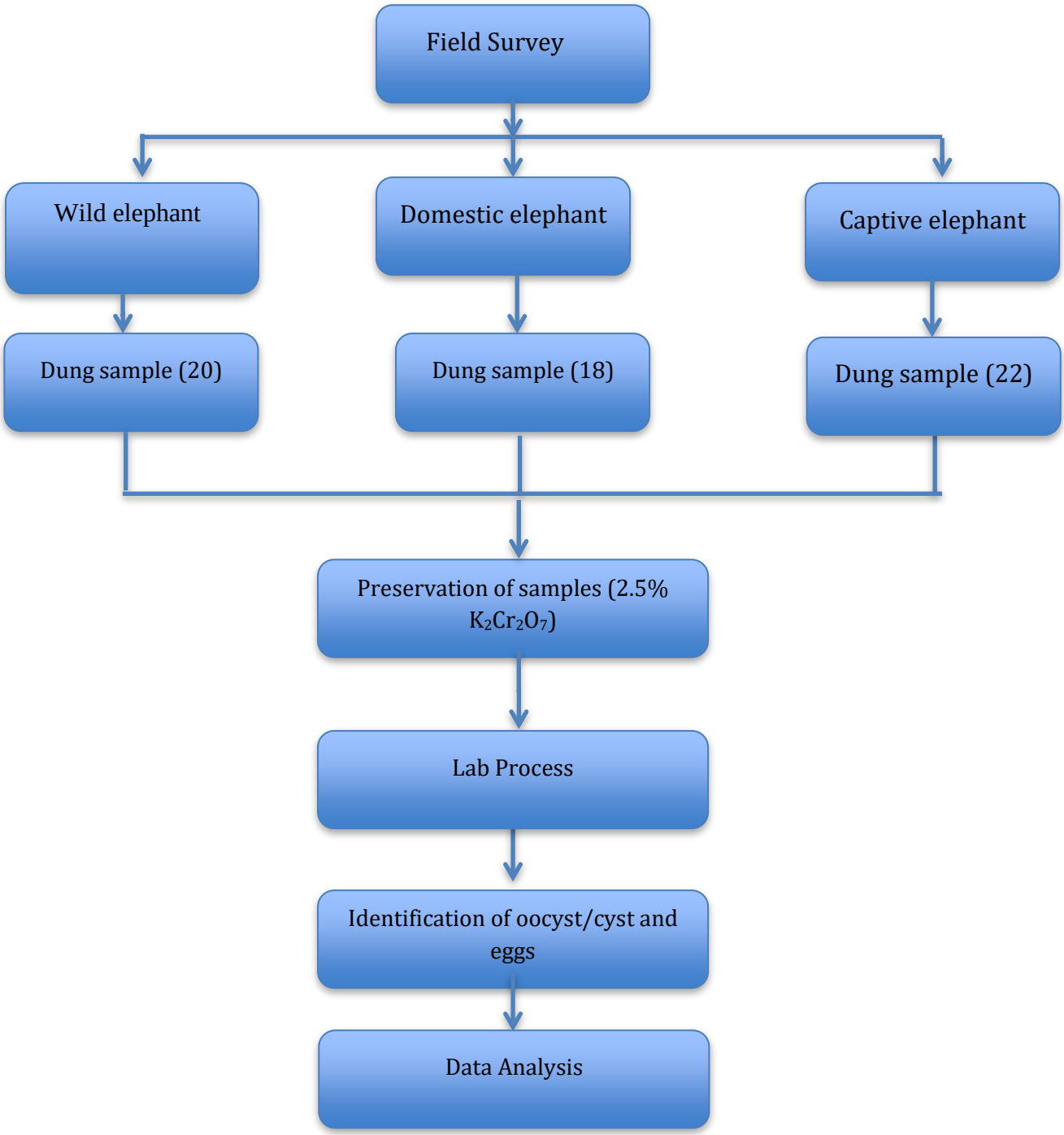
3.2.2. Materials for laboratory

Centrifugal tube, Centrifuge machine, Dropper, Strainer, Pipette, Petridis, Electronic microscope, Test tube, Test tube stand, Vials, Glass rod, Cover slip, Needle, Camera, Slide, Gloves and masks.

3.2.3. Chemicals

- Potassium dichromate solution
- Zinc sulphate solution ($Znso_4$)
- Sodium chloride (NaCl)
- Lugol's Iodine solution
- Methylene blue

3.3. Study design



3.3.1. Field survey

A pilot survey was conducted initially to know the habitat and condition of elephants in Chitwan National Park. Necessary information about wild and captive/ domesticated elephant was collected by discussion with park authorities, security personnel and local people. Finally CNP was selected as the study area to carry out this research.

3.3.2. Sample size

The present study was designed to assess the gastrointestinal parasitic infection in Asian elephant in and around Chitwan National Park. The study includes:

Approximately 20 dung samples of wild elephants were collected opportunistically within the National park within three months period.

Approximately 40 captive/domestic elephants were selected for the examination; among which 22 elephants were governmental captive elephants and 18 elephants were domestic elephants from three private lodges. So, a total of 80 dung samples were collected in duplicate sampling method from 40 captive/ domestic elephants with an interval of 15 days.

3.3.3. Identification of dung

Elephants are very poor digesters of their food so over 50% of what they eat, comes straight out the other side. It is also considered as the vehicle for seed dispersal. Since elephants dung is just fibrous with undigested seeds and nuts, moist and mostly a bit light yellow color in case of domestic because of their feeding of hay and in wild, it is a bit darkish green color because of their wild habitat.

3.3.4. Collection of samples

A total of 20 dung samples from free ranging greater wild elephant were collected opportunistically from different locations of CNP and a total of 80 dung samples from 40 captive/domestic elephants were collected by duplicate sampling method within the interval of 15 days in the month of May 2017. About 50 g of freshly voided dung sample was collected in individually labeled vials and these samples were properly sealed, labeled with date, time and place.

3.3.5. Preservation of samples

The collected dung samples were immediately preserved in 50 ml vials with 2.5% potassium dichromate that helps in maintaining the morphology of protozoan parasites and preventing the further development of some helminthic eggs and larvae. Thus preserved samples were transported to the laboratory of Central Department Of Zoology, TU, Kirtipur.

3.3.6. Laboratory process

The preserved samples were processed for the microscopic examination. The ova/oocytes/cysts and larvae of different parasites were identified according to the morphology and quantitative estimation by using direct smear method, concentration method (floatation and sedimentation) and Stoll's counting technique to determine mix infection and intensity of parasites (Soulsby, 1982).

3.3.6.1. Iodine wet mount

Small quantity (about 2 mg) of dung was mixed in a drop of Lugol's Iodine solution on a clean slide. Any grass fiber or particles were removed and cover with coverslip. The smear was examined under microscope at 10X and 40X (Soulsby 1982).

3.3.6.2. Concentration method

Eggs/cysts are often low in number in dung that they are difficult to be detected in direct smears or mounts. Therefore, this procedure was performed which includes floatation and sedimentation techniques Soulsby 1982).

3.3.6.2.1. Differential floatation technique

Nematode and cestode eggs present in faeces are detected through this technique. This technique ensures that the eggs float in the floatation liquid that helps to identify the eggs. Approximately, 5gm. of dung sample was taken in a beaker and 20 ml of water was added then the sample was mixed lightly with the help of mortar and pestle. The solution was then filtered by tea strainer. The filtrate solution was poured into centrifuge tube of 15 ml and was centrifuged at 2000 rpm for 5-minutes.the tube's water was replaced with saturated sodium chloride solution and was again centrifuged.

After centrifuge more saturated sodium chloride solution was added to develop convex surface at the top of the tube and one drop of methylene blue (to stain) where a cover slip was placed for a few minutes. Then the cover slip was removed and placed on slide and examined at 10X and 40X. Photographs of cyst and eggs were taken and identified based on egg's color, shape and size.

3.3.6.2.2. Sedimentation technique

This technique is used for the detection of trematode eggs. It provides good results as the eggs of the trematodes are a bit heavier than other, where sediments of centrifuged contents were taken for eggs detection.

Saturated salt solution was removed gently from the test tube after examining the floatation portion and the sediment was poured into the watch glass and stirred gently to mix it. One drop of mixture was taken to prepare a second slide. The specimen was stained with iodine wet mount solutions.

In this way, three slides were prepared from one sample (one from iodine wet mount, one from floatation and one from sedimentation) and examined under 10X and 40X magnification of microscope to detect eggs of helminthes and trophozoites or cysts of gastro-intestinal parasites.

3.3.7 Data analysis

Since, the study was mainly focused on prevalence and identification of different gastrointestinal parasites, the statistical significance of parasites was calculated by R-program software new version (3.4.1) where the descriptive statistics was expressed as proportion with 95% confidence interval (CI). For Chi-square Test, result was expressed in percentage with P-value and significance was determined when $P<0.05$.

Photograph 1. Wild elephant visiting the Khorsar breeding centre

Photograph 2. Elephant of Khorsar breeding centre being ready for safari for sample collection

Photograph 3. Jungle walk for the sample collection in Nandantal

Photograph 4. Visit to Kasara office, Chitwan

Photograph 5. Sample collection from the elephant of Khorsar breeding centre

Photograph 6. Sample collection from the wild elephant while Jungle walk in Nandan tal

Photograph 7. Stirring and filtration of dung sample in laboratory

Photograph 8. Showing the process of flotation in laboratory

Photograph 9. Showing the process of centrifugation of dung sample in centrifugal machine in laboratory

Photograph 10. Microscopically examination of eggs of parasite

4. RESULTS

Wild elephants are the free ranging elephants found roaming around the dense forest of the Chitwan Nation Park (CNP) whereas, captive/domestic elephants are the one which are reared by the different Governmental forest department for security as well as patrolling and in private resorts for tourism operations. So, both wild and captive/domestic elephants were selected for the examination of gastrointestinal parasites in the elephants in and around the Chitwan National park.

4.1. Gastro-intestinal parasites in wild elephants

A total of 20 samples of wild elephants were collected by using opportunistic sampling technique from the Chitwan National Park and were examined by using direct smear and concentration methods for gastro-intestinal parasites. Out of total samples examined, 90% of samples were found positive for different types of protozoan and helminthes parasites.

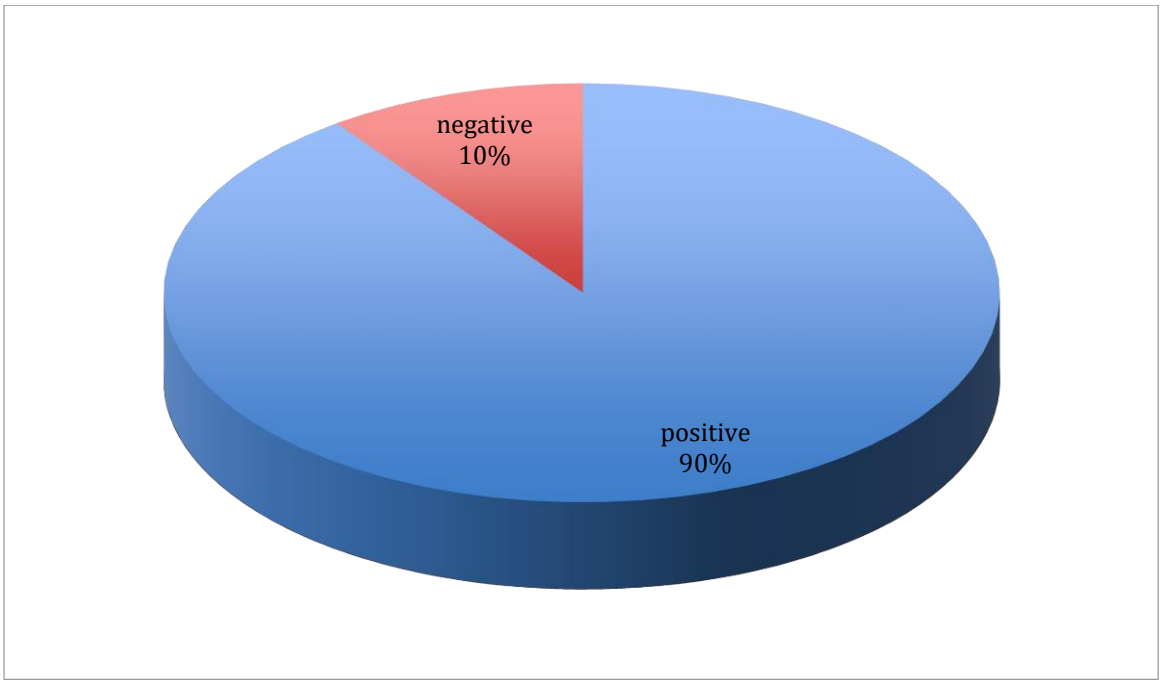


Figure 2: Prevalence of gastro-intestinal parasites in wild elephants

Prevalence of gastro-intestinal parasites in wild elephants

4.1.1. Protozoan parasite

Protozoans are the microscopic, one celled organism that can be free living or parasitic in nature, can be responsible for protozoan disease in animals too. Hence, Wild elephants were found infected by protozoan parasite belonging to the genus *Eimeria* (15%).

4.1.2. Helminth parasite

Among the helminth parasites, Trematode parasites were not observed from wild elephants. But they were found highly infected with nematode parasites belonging to eight different genera. Among them *Strongyloide* sp. (85%) showed the highest prevalence followed by *Ascaris* sp. (45%), *Dromeostrongylus* sp. (30%), *Haemonchus* sp. (25%), *Chabertia* sp. (15%) and each of 10% prevalence by *Nematodirus* sp.

Bunostomum sp. and *Trichostrongylus* sp. Similary, one genus of Cestode parasite was found infecting the wild elephants i.e. *Anoplocephala* sp. (10%).

Table 1: Prevalence of gastro-intestinal parasites in wild elephants

S.N	**Class**	**Genus**	**Percentag of occurrence (N=20)**	**$\chi 2$**	**P-value**
1.	Protozoa	*Eimeria*	15% (3)	-	-
2.	Cestode	*Anoplocephala*	10%(2)	-	-
3.	Nematode	*Strongyloide*	85%(17)	44.98	$1.37e^{-07}$
		Ascaris	45%(8)		
		Dromeostrogylus	30%(6)		
		Haemonchus	25%(5)		
		Chabertia	15%(3)		
		Nematodirus	10%(2)		
		Bunostomum	10%(2)		
		Trichostrongylus	10%(2)		

Statistically, there was significant difference in the prevalence of nematode parasites in wild elephant (χ^2=44.98, P-value= $1.37e^{-07}$ i.e. P<0.05). Similarly, there was significant difference in the prevalence of protozoa, nematode and cestode in wild elephant (χ^2=108.42, P-value= $2.2e^{-16}$).

4.1.3. Location wise prevalence of gastro-intestinal parasites in wild elephants

Table 2: Location wise prevalence of gastro-intestinal parasites in wild elephants

S.N	Location	Percentage of positive sample collected (N=20)	Parasites recorded	Probable elephants
1.	Chure area (N=8)	30%	1. *Eimeria* sp. 2. *Ascaris* sp. 3. *Strongyloide* sp. 4. *Haemunchus* sp. 5. *Dromeostrongylus* sp. 6. *Trichostrongylus* sp. 7. *Bunostomum* sp. 8. *Chabertia* sp.	Ronaldo, Govinda or other wild elephants.
2.	Tiger tops area (N=4)	20%	1. *Strongyloide* sp. 2. *Haemunchus* sp. 3. *Dromeostrongylus* sp. 4. *Chabertia* sp. 5. *Trichostrongylus* sp. 6. *Anoplocephala* sp.	Dhurbe, Ronaldo or other wild elephant
3.	Temple tiger area (N=2)	10%	1. *Eimeria* sp. 2. *Ascaris* sp. 3. *Strongyloide* sp. 4. *Haemunchus* sp.	Dhurbe or other wild elephant
4.	Khorsar area (N=2)	10%	1. *Ascaris* sp. 2. *Strongyloide* sp. 3. *Haemunchus* sp.	Ronaldo or Govinda
5.	Magartole (N=2)	10%	1. *Eimeria* sp. 2. *Ascaris* sp. 3. *Strongyloide* sp.	Ronaldo,
6.	Nandan tal (N=2)	10%	1. *Strongyloide* sp. 2. *Nematodirus* sp.	Ronaldo, Govinda or other wild elephants

From the present observation, 30% of positive sample with maximum eight different types of parasitic genera were recorded from the Chure area such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Dromeostrongylus* sp., *Haemonchus* sp.,*Trichostrongylus* sp., *Chabertia* sp., *Bunostomum* sp. followed by 20% of positive sample with six different types of genera was encountered from Tiger tops area such as *Strongyloide* sp., *Haemonchus* sp., *Dromeostrongylus* sp., *Chabertia* sp., *Trichostrongylus* sp., *Anoplocephala* sp. 10% of positive sample with four different types of parasitic genera was encountered from Temple tiger area such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Haemonchus* sp.. 10% of positive sample with three different types genera was reported from Khorsar area such as *Ascaris* sp.,*Strongyloide* sp.,*Haemunchus* sp.. 10% of positive sample with three different types of parasitic genera was reported from Magartole such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Haemonchus* sp. and followed by 10% of positive sample with two different genera of parasite such as *Strongyloide* sp. and *Nematodirus* sp. was encountered.

And from the above observation, Ronaldo which is one of a common wild elephant roaming around Chure, Tigertops, Magartole Nandantal and mainly the khorsar area whereas Govinda, one the known wild elephant of CNP which is found to be roaming around Chure, Nandantal and khorsar area and Dhurbe, which is also one of the known wild elephant of CNP roaming around the Tiger tops and Temple tiger area of CNP were supposed to be infected by the four different genera of parasites such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., and *Haemonchus* sp. Whereas *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Dromeostrongylus* sp., *Haemonchus* sp., *Trichostrongylus* sp. ,*Chabertia* sp., *Bunostomum* sp. *Nematodirus* sp. and last but not the least *Anoplocephala* sp. were found supposed to be infecting the other wild elephants which roam around the CNP area.

4.1. 4. Mixed infection in wild elephants

In the present study, the rate of mixed infection was also observed. Mixed infection was found in all the positive samples of wild elephants. Four types of infection was categorized such as single, double, triple and multiple infections. The occurrence of triple infection was found to be highest (40%) which is the mixed infection of three different genera of parasite such as each of 25% prevalence rate of *Strongyloide* sp., *Ascaris* sp. with *Haemonchus* sp., *Strongyloide* sp., *Ascaris* sp. with *Eimeria* sp. and *Strongyloide* sp., *Haemonchus* sp. with *Dromeostrongylus* sp. and each of 12.5% prevalence rate of *Strongyloide* sp., *Bunostomum* sp. with *Dromeostrongylus* sp. and *Strongyloide* sp.,*Chabertia* sp.with *Trichostrongylus* sp. was reported followed by 35% double infection with the mixed infection of two different genera of parasites with maximum prevalence rate of 29% each of *Strongyloide* sp. with *Ascaris* sp. and *Strongyloide* sp. with *Nematodirus* sp.and each of 14% prevalence rate of *Strongyloide* sp.,with *Haemunchus* sp., *Ascaris* sp. with *Eimeria* sp. *and Strongyloide* sp. with *Anoplocephala* sp.. Multiple infection (15%)with the mixed infection of four or more than four different genera of parasite and each of 10% prevalence rate of *Strongyloide* sp.,*Bunostomum* sp.,*Dromeostrongylus* sp., *Bunostomum* sp. with *Ascaris* sp., *Strongyloide* sp.,*Chabertia* sp. *Anoplocephala* sp. with *Dromeostrongylus* sp. and *Strongyloide* sp.,*Chabertia* sp.,*Ascaris* sp.with *Dromeostrongylus* sp. was reported. But no single inection was encountered.

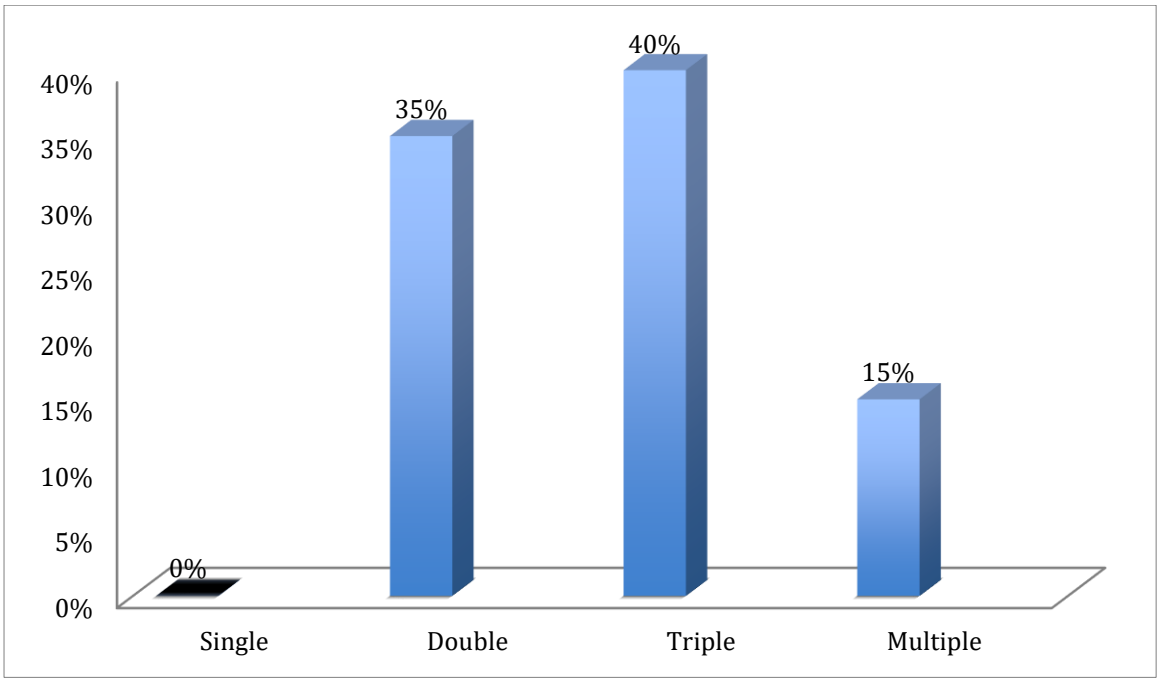


Figure 3: Mixed infection in wild elephants

4.1.5. Intensity of the parasitic eggs/cysts in wild elephants

Heavy parasitic infection was considered in those samples, which have six or more ova or oocyst observed per field. Maximum two samples of wild elephants showed the heavily infected with *Strongyloide* sp. (12%). Maximum two samples showed moderate intensity of *Strongyloide* sp. (12%) followed each of one samples with *Dromeostrongylus* sp. (17%) and *Ascaris* sp, (12.5%) which considered 4-6 ova/oocyst per field. And maximum four samples showed mild intensity of *Strongyloide* sp. (23%) followed by each of two samples with *Dromeostrongylus* sp. (33%), *Haemonchus* sp. (40%) and each of one samples with *Ascaris* sp. (12.5%), *Eimeria* sp. (33%), *Chabertia* sp. (33%) and *Bunostomum* sp. (50%) which considered 2-4 ova/oocyst per field and < ova/oocyst observed per field considered as light infection; maximum nine samples each showed the light intensity of *Strongyloide* sp. (53%) followed by six samples each with *Ascaris* sp. (75%),three sample each with *Dromeostrongylus* sp.(50%), each of two samples with *Eimeria* sp. (67%),*Anoplocephala* sp. (100%), *Haemonchus* sp. (40%), *Nematodirus* sp. (100%), *Trichostrongylus* sp. (100%), *Chabertia* sp. (67%) and one sample with *Bunostomum* sp. (50%).

Table 3: Intensity of the parasitic eggs/cysts in wild elephants

S.N	**Class**	**Genus**	**Light (+)**	**Mild (++)**	**Moderate (+++)**	**Heavy (++++)**
1.	Protozoa	*Eimeria*	2 (67%)	1 (33%)	-	-
2.	Cestode	*Anoplocephala*	2 (100%)	-	-	-
3.	Nematode	*Strongyloide*	9 (53%)	4 (23%)	2 (12%)	2 (12%)
		Ascaris	6 (75%)	1 (12.5%)	1 (1.5%)	-
		Dromeostrogylus	3 (50%)	2 (33%)	1(17%)	-
		Haemonchus	2 (40%)	2 (40%)	1 (20%)	-
		Chabertia	2 (67%)	1 (33%)		
		Nematodirus	2 (100%)			
		Bunostomum	1 (50%)	1 (50%)		
		Trichostrongylus	2 (100%)			

4.2. Gastro-intestinal parasites of captive/domestic elephants

Out of 40 captive/domestic elephants, 80 fresh faecal samples were collected by duplicate sampling technique from Chitwan and were examined by using direct smear and concentration methods for gastro-intestinal parasites. Among which 23 elephants were found positive for either protozoan or helminthes parasites i.e. 57%.

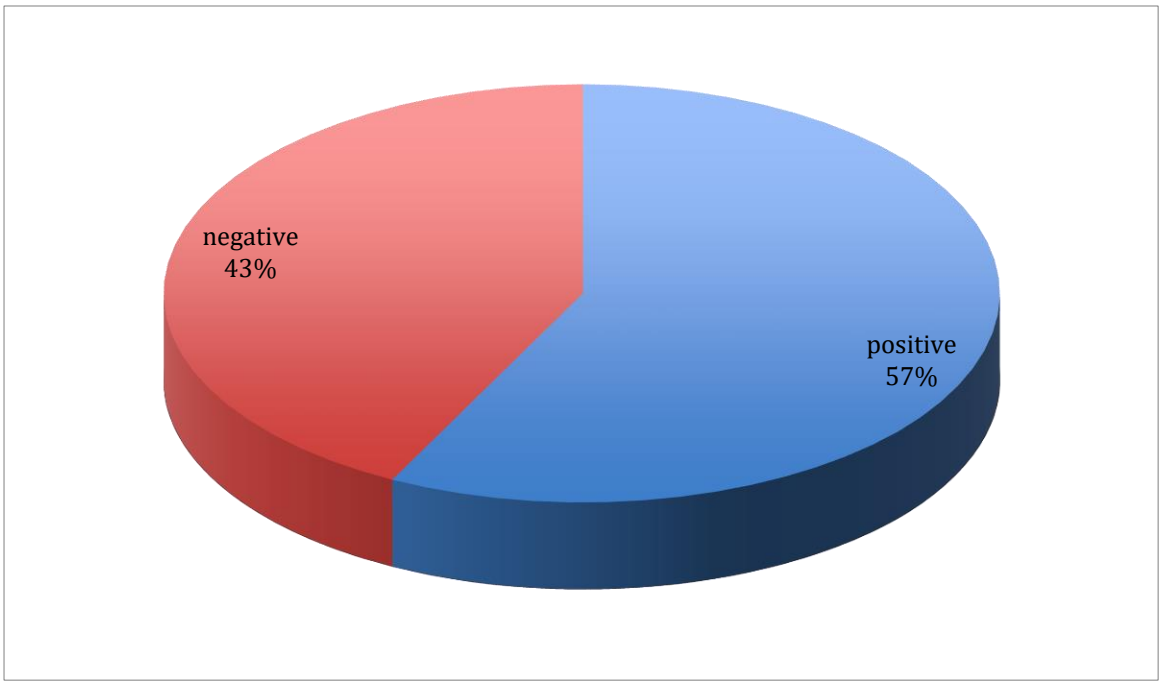


Figure 4: Prevalence of gastro-intestinal parasites in captive/domestic elephants

Prevalence of gastro-intestinal parasites in Captive/Domestic elephants

4.2.1. Protozoan parasite

The result revealed that domestic elephant were infected with minimum protozoan parasite, which is reported to be *Eimeria* sp. (15%). The Protozoan parasites are supposed to cause different protozoan diseases in elephants.

4.2.2. Helminthes parasite

From the present study among the helminth parasites, Trematode and Cestode parasites were not observed from domestic elephants. But they were found highly infected with nematode parasites belonging to four different genera. Among which *Strongyloide* sp. (52.5%) was found to be significantly infecting the domestic elephants followed by *Ascaris* sp. (27.5%), *Haemonchus* (7.5%), *Eimeria* (7.5%) and *Bunostomum* (2.5%).

Table 4: Prevalence of gastro-intestinal parasites in captive/domestic elephants

S.N	Class	Genus	Percentage of occurrence (N=40)	χ^2	P-value
1.	Protozoa	*Eimeria*	3 (7.5%)		
2.	Nematode	*Strongyloide*	21 (52.5%)	35.556	$9.29e^{-08}$
		Ascaris	11 (27.5%)		
		Haemonchus	3 (7.5%)		
		Bunostomum	1(2.5%)		

Statistically, there was significant difference in the prevalence of nematode parasites in captive/domestic elephants (χ^2=35.55, P-value = $9.29e^{-08}$ i.e. P<0.05) similarly there was significant difference in the prevalence of protozoa and nematode in captive/domestic elephants (χ^2=52.513, P-value= 4.27 e^{-13}).

4.2.3. Location wise prevalence of gastro-intestinal parasites in captive/domestic elephants

In the present observation below, 40 captive/domestic elephants were selected from five different sites of Chitwan (Khorsar breeding centre, Sauraha Sector Hattisar, Machang country villa, Tiger Tops, Temple Tiger) among which 5% of positive samples with maximum five different genera of the parasites were reported from the elephants of Machang country villa such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Haemonchus* sp., and *Bunostomum* sp., followed by 10% positive samples with four different genera of parasites were reported from the elephants of Temple Tiger such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Haemonchus* sp.,. Each of 15% positive sample from Khorsar breeding center and Tiger Tops with two different genera of parasites such as *Strongyloide* sp. *and Ascaris* sp.12.5% of positive sample with two different parasites genera such as *Strongyloide* sp. and *Ascaris* sp. were reported.

Table 5: Location wise prevalence of gastro-intestinal parasites in captive/domestic elephants

S.N	Location	Percentage of positive sample (N=40)	Parasites recorded
1.	Khosar breeding center (N=12)	15%	*I.* *Ascaris* sp. *II.* *Strongyloide* sp.
2.	Sauraha Sector, Hattisar (N=10)	12.5%	*I.* *Ascaris* sp. *II.* *Strongyloide* sp.
3.	Machang country villa (N=2)	5%	*I.* *Eimeria* sp. *II.* *Ascaris* sp. *III.* *Strongyloide* sp. *IV.* *Haemonchus* sp. *V.* *Bunostomum* sp.
4.	Tiger Tops Tharu Lodge (N=11)	15%	*I.* *Ascaris* sp. *II.* *Strongyloide* sp.
5.	Temple Tiger Lodge (N=5)	10%	*I.* *Eimeria* sp. *II.* *Ascaris* sp. *III.* *Strongyloide* sp. *IV.* *Haemonchus* sp.

4.2. 4. Mixed infection in captive/domestic elephants

During the study, different types of parasitic infections were encountered in domestic elephants. The single infection (32.50%) was found to be highest with the infection by single parasite such as *Strongyloide* (92.3%) and *Ascaris* (7.7%), which is followed by the Double infection (17.50%) with the highest prevalence of *Ascaris* with *Strongyloide* (85.7%), followed by *Eimeria* with *Ascaris* (14.3%) and Multiple infection(7.50%) with highest prevalence by *Eimeria, Ascaris, Haemonchus* with *Strongyloide* (67%) and *Ascaris, Strongyloide, Haemonchus* with *Bunostomum* (33%) but no triple infection was encountered.

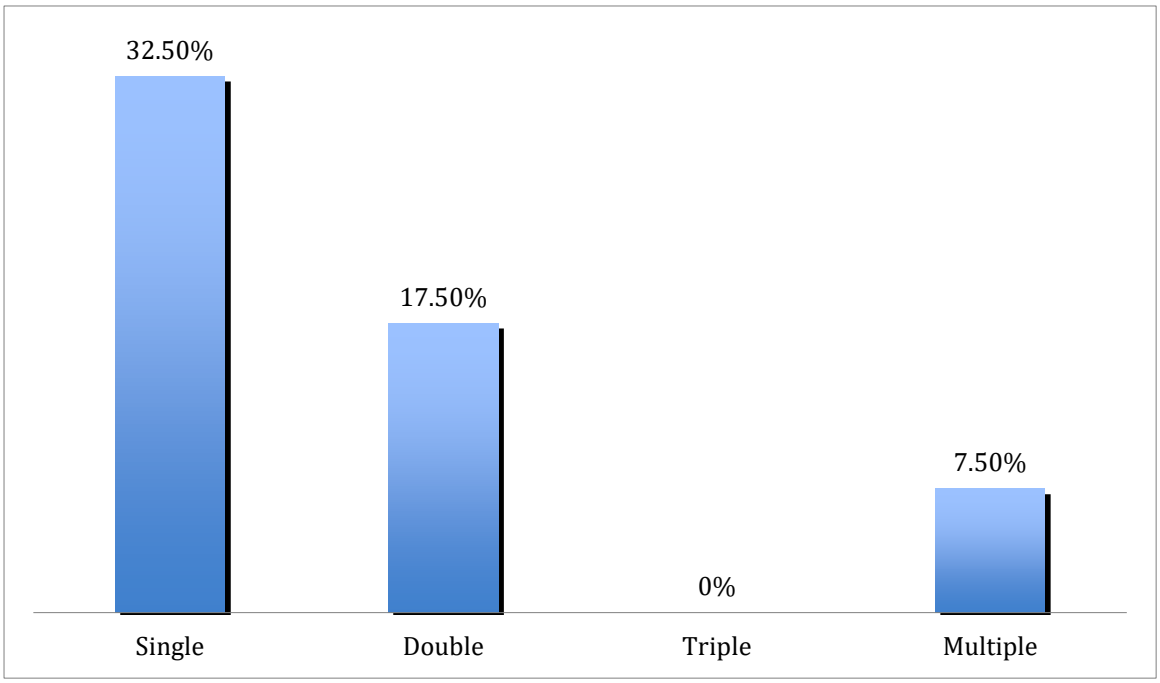


Figure 5: Mixed infection in captive/domestic elephants

4.2.5. Intensity of parasitic eggs/cysts in captive/domestic elephants

During the study, heavy infection was not found but 5 samples showed moderate intensity of *Strongyloide* sp. (23%). Maximum 5 samples showed the mild intensity of *Ascaris* sp. (45%), followed by 3 samples with *Strongyloide* sp. (15%), 2 samples with *Haemonchus* sp. (67%) and 1 sample with *Eimeria* sp. (33%). Maximum 13 samples showed the light intensity of *Strongyloide* sp. (62%), followed by 6 samples with *Ascaris* sp. (55%), 2 samples with *Eimeria* sp. (67%) and each of one sample with *Haemonchus* sp. (33%) and *Bunostomum* sp. (100%) was recorded with light intensity.

Table 6: Intensity of parasitic eggs/cysts in captive/domestic elephants

S.N	Class	Parasites	Light (+)	Mild (++)	Moderate (+++)	Heavy (++++)
1.	Protozoa	*Eimeira* sp.	2 (67%)	1 (33%)	–	–
2.	Nematode	*Ascairs* sp.	6 (55%)	5 (45%)	–	–
		Strongyloide sp.	13 (62%)	3 (15%)	5 (23%)	–
		Haemonchus sp.	1 (33%)	2 (67%)	–	–
		Bunostomum sp.	1 (100%)	–	–	–

4.3 Comparative analysis of gastro-intestinal parasite in between wild and captive/domestic elephant

From the present observation, statistically the prevalence of gastro-intestinal parasites in between wild (90%) and captive/domestic elephant (57%) was found significantly difference ($\chi 2$= 5.0931, P-value= 0.02). In case of protozoan parasites, prevalence rate showed by *Eimeria* sp. (15%) and (7.5%) in wild and captive/domestic elephants respectively. Statistically, there was not significant difference in prevalence of intestinal protozoan parasites in between wild and captive/domestic elephants. (Table: 7)

Among the eight genera of nematode, 85% and 52.5% prevalence rate showed by the *Strongyloide* sp. in wild and captive/domestic elephants respectively. . Statistically, there was significant difference in prevalence of *Strongyloide* sp.in between wild and captive/domestic elephants. 45% and 27.5% prevalence rate showed by the *Ascaris* sp. in wild and captive/domestic elephants respectively where as 25% and 7.5% prevalence rate showed by the *Haemonchus* sp. in wild and captive/domestic elephants respectively while 10% ad 2.5% prevalence rate showed by the *Bunostomum* sp. in wild and captive/domestic elephants respectively. Statistically there was no significant difference in the prevalence of *Ascaris* sp. *Haemonchus* sp., *Bunostomum* sp.in between wild and

captive/domestic elephants. But *Dromeostrongylus* sp. (30%) *Nematodirus* sp(10%). *Trichostrongylus* sp(10%). *Chabertia* sp. (15%) were the nematode parasites reported only from wild elephants and there was significant difference in the prevalence of *Dromeostrongylus* sp. and *Chabertia* sp. in between wild and captive/domestic elephants whereas there was no significant difference in the prevalence of *Nematodirus* sp (10%). *Trichostrongylus* sp (10%). in between wild and captive/domestic elephants.(Table:7)

The cestode parasite 10% prevalence rate of *Anoplocephala* sp. was only reported from wild elephant and was absent in captive/domestic one. Statistically, there was no significant difference in prevalence of *Anoplocephala* sp. in between wild and captive/domestic elephants. (Table: 7)

Table 7: Comparative analysis of prevalence of gastro-intestinal parasites in between wild and captive/domestic elephants

S. N	Class	Parasites	Prevalence in Wild elephants (N=20)	Prevalence in captive/Domestic elephant (N=40)	$\chi 2$	P- value
1.	Protozoa	*Eimeria* sp.	3 (15%)	3 (7.5%)	0.20833	0.6481
2.	Cestode	*Anoplocephala* sp.	2 (10%)	0	1.6164	0.2036
3.	Nematode	*Ascaris* sp.	8 (45%)	11(27.5%)	0.47176	0.4922
		Dromeostrongylus sp.	6 (30%)	0	10.208	0.001398
		Nematodirus sp.	2 (10%)	0	1.6164	0.2036
		Haemonchus sp.	5 (25%)	3 (7.5%)	2.1815	0.1397
		Strongyloide sp.	17(85%)	21(52.5%)	4.7458	0.02937
		Trichostrongylus sp.	2(10%)	0	1.6164	0.2036
		Chabertia sp.	3(15%)	0	3.5526	0.05945
		Bunostomum sp.	2(10%)	1(2.5%)	0.39474	0.5298

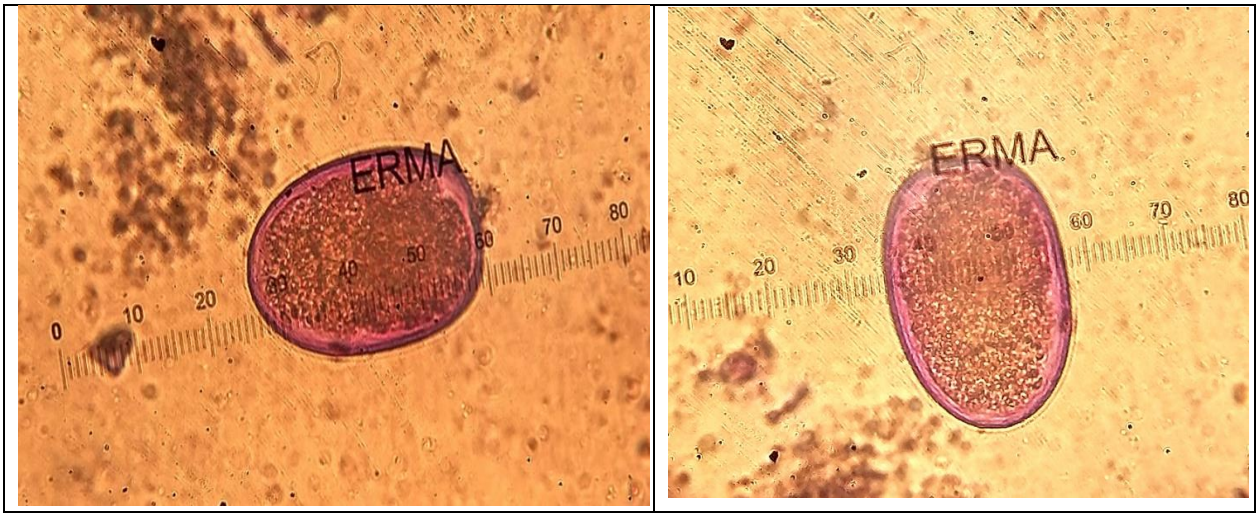
4.4 Diameter of eggs/cysts of different gastro-intestinal parasites of Asian elephants

In the present study, the diameter of the eggs/cysts of different gastro intestinal parasites was measured/ calculated. Reference values were given based on published book as

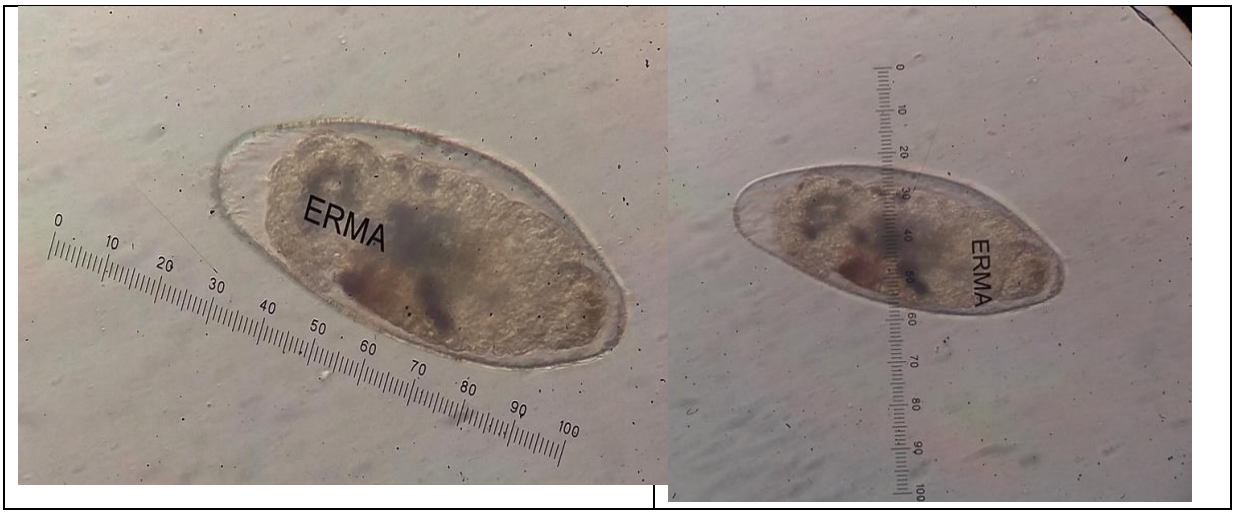
Table 8. Description of the eggs of identified gastro-intestinal parasites in 10X×40X.

S.N.	Name of parasites	Range of diameter of eggs and cysts (in µm)		Morphological characters	Reference values (Soulsby, 1982)
		Length	Width		
1.	*Eimeria* sp.	32.5	-	Cysts are small, rounded or spherical with four nucleus.	15-55µm
2.	*Anoplocephala* sp.	75	-	-	-
3.	*Strongyloide* sp.	75	42.5	Eggs are small in size, oval with rounded edges or allipsodial, thin shelled and contain fully developed larvae that can be seen under low power.	70-80µm × 41-48µm
4.	*Ascaris* sp.	47.5	-	Oval, thick shells brownish yellow in color, albuminous layer bear prominent projections.	50-75µm × 40-50µm
5.	*Dromeostrongylus* sp.	137.5	75		
6.	*Bunostomum* sp.	95	46	Bluntly rounded, embryonic cells darkly granulated.	79- 92µm × 47-50µm
7.	*Haemonchus* sp.	87.5	50	Embryo divided into 16-32 cells wen passed in faeces	70-85µm × 41-48µm
8.	*Chabertia* sp.	125	70	-	
9.	*Trichostrongylus* sp.	70	42.5	Eggs are irregular, ellipse dissimilar, kidney shaped not very wide poles, one of which more rounded than the other, dissimilar side –walls.	70-108µm × 30-40µm
10.	*Nematodirus* sp.	212.5	75	Brown shell, thin, parallel sides.	152-182µm × 66-77µm

PHOTOGRAPHS OF THE EGGS OF IDENTIFIED GASTRO-INTESTINAL PARASITES IN 10X × 40X

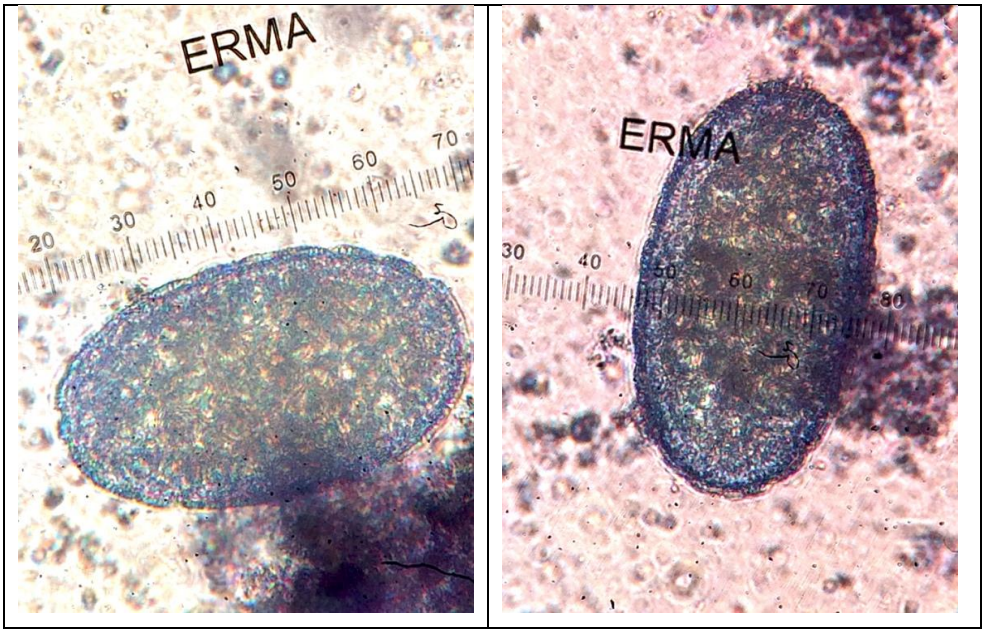


Photograph 11. Eggs of *Haemonchus* sp. (87.5 µm × 50 µm)

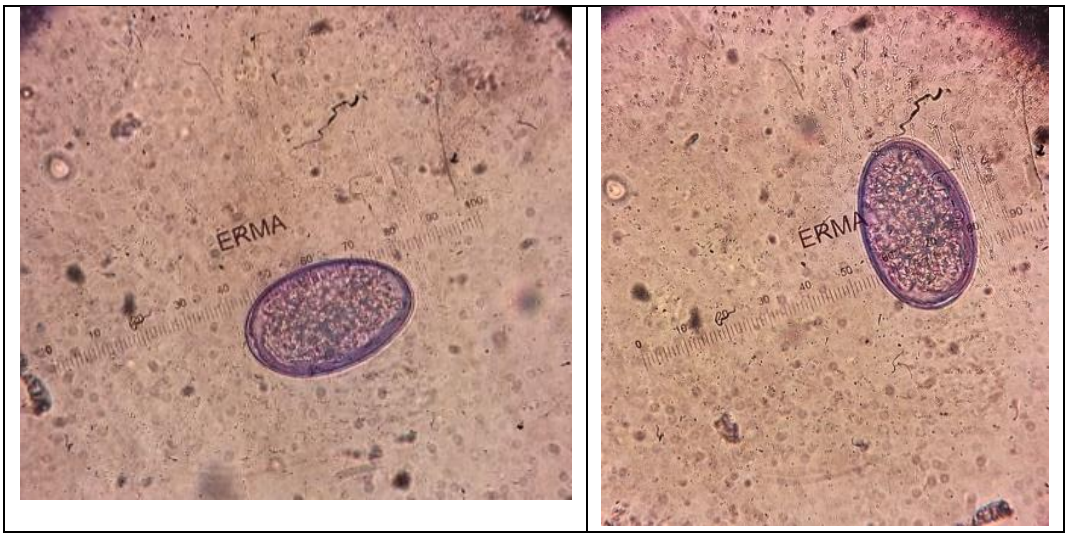


Photograph 12. Eggs of *Nematodirus* sp. (212.5 µm ×75 µm)

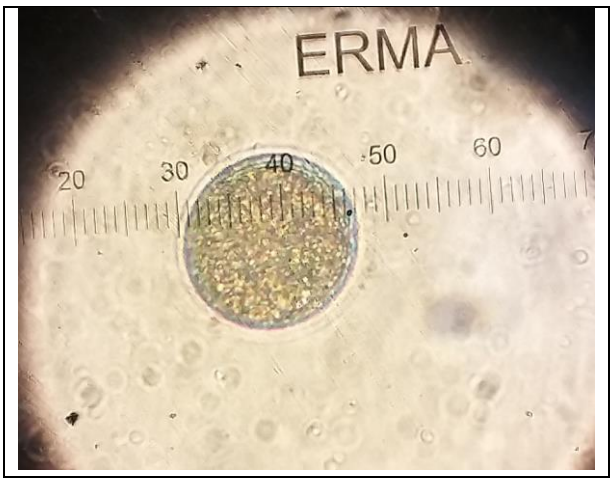
Photograph 13. Eggs of *Trichostrongylus* sp. (70 µm ×42.5 µm)



Photograph 14. Eggs of *Dromeostrongylus* sp. (137.5 µm ×75 µm)

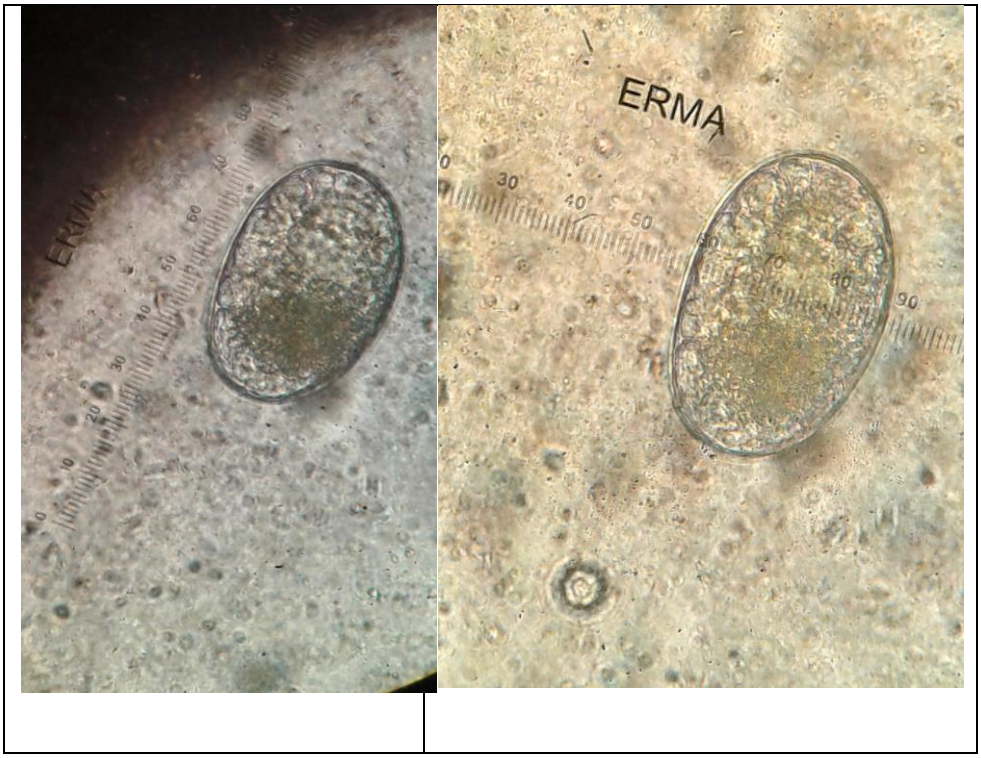


Photograph 15. Eggs of *Bunostomum* sp. (95 µm ×46 µm)

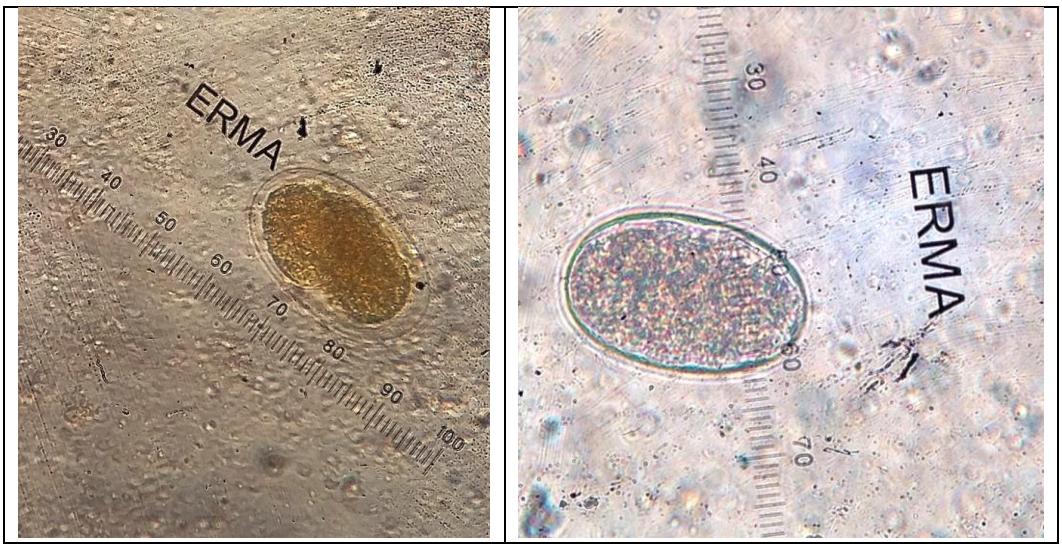


Photograph 16. Egg of *Ascaris* sp. (47.5 µm)

.

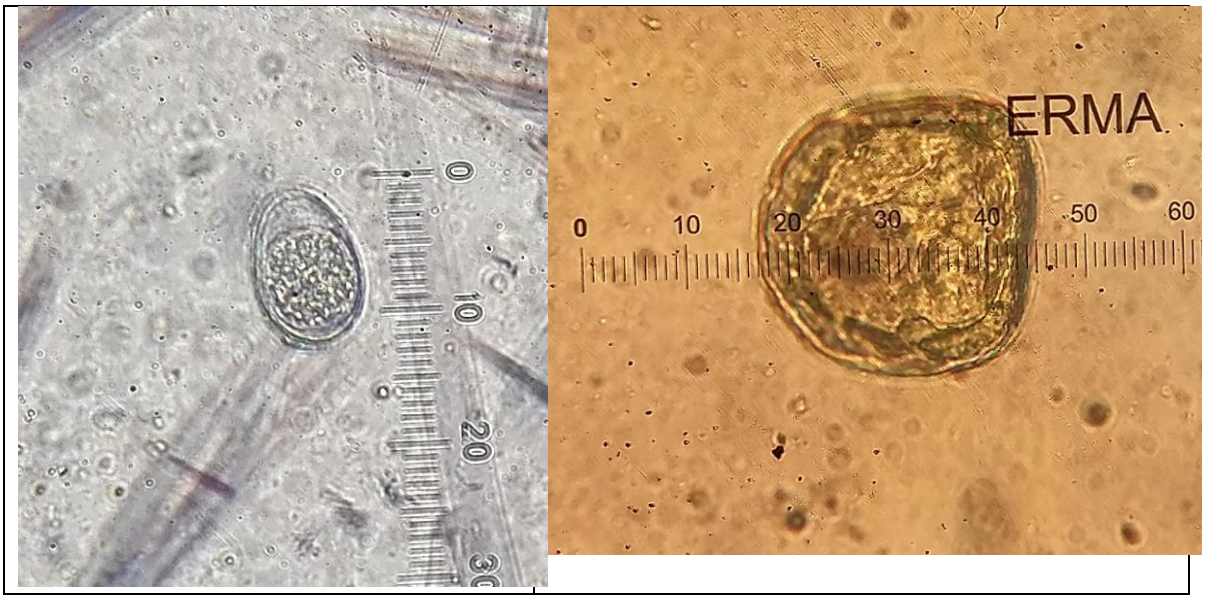


Photograph 17. Eggs of Chabertia sp. (125 µm ×70 µm)



Photograph 18. Eggs of *Strongyloide* sp. (75 µm ×42.5 µm)

Photograph 19. Larva of *Strongyloide* sp.



Photograph 20. Eggs of *Eimeria* sp. (left) 32.5µm and *Anoplocephala* sp. (right) 75 µm

5. DISCUSSION

Although, the gastro-intestinal parasites are the parasites that can infect the gastrointestinal tract of the animals, these endoparasites are not generally considered as the life threatening in a healthy adult animals but can lead to change in disease dynamics due to altered host, environment or pathogen factors and result in some clinically significant endoparasitic infection (Lioyd, 1995). Asian elephant (*Elephas maximus),* which is well adapted to live in diverse habitats by exploiting a wide spectrum of plant species, are infected by various types of gastrointestinal parasites. In context of our country, there have not been adequate research works in the elephants as compared to work done in our neighboring country, India and other countries as well as continents. The present study explores the parasitic prevalence, mixed infection, intensity of the parasites in both wild and domestic Asian elephants and their comparison in the parasitic prevalence. Hence, this present researches study the gastrointestinal parasites in Asian elephant in Chitwan National Park comparing the wild and domestic elephants in Nepal.

The present study revealed that there is higher prevalence of gastrointestinal parasites (90%) among the wild Asian elephants of CNP. The overall prevalence is almost similar to the findings of Hing *et. al.* (2013) and Nishanth *et. al.* (2012) who had reported the prevalence of parasites in all 104 samples of Asian Bornean elephants in Sabah, Malaysia and 82.66% in India respectively. However, the result is comparatively higher than the previous study done by the Mbaya *et. al.* (2013) in Chad Basin National Park, Nigeria (36.86%). The variation in the prevalence of gastrointestinal parasites might be due to intrinsic as well as extrinsic factors.

Coccidian parasites are the most prevalent among protozoa that infects large number of wild animals including wild elephants, which has significant effect on wild animals. *Eimeria* sp. is the most common coccidian parasites among wild animals. *Eimeria* sp. cannot be differentiated into the species level in the present study because it needs a culture to identify the species of this parasite. The present study shows that the wild elephants in CNP were infected by Protozoan coccidian parasite belonging to the genus *Eimeria* sp. (15%). However, the result is comparatively less than the previous study done by Baines *et. al.* (2015) which was 51% in Botswana whereas coccidian infection was reported to be common in African elephants (Fowler and Mikton, 2006). Although, *Eimeria* sp. are common coccidian parasites of herbivores, the parasite has not been reported from wild Asian elephants earlier.

The result of this study showed that among the helminthes parasites, wild elephants were found highly infected with nematode parasites belonging to eight different genera which is found similar to findings of Baines *et. al.* (2015) in Botswana in which highest prevalence of nematodes were reported (77%).The reason for this finding might be due to the direct life cycles of nematodes and as they are mostly present in soil and grasses grown in soil also may contain eggs and L3 larva of nematodes which can easily enter to the host body through penetration as well as ingestion of contaminated food. Among eight different genera of nematodes the highest infection was with *Strongyloide* sp., which is 85%. This result is similar to the findings of Vidhya and Sukumar (2002) who reported 84.4% Strongyle egg from southern India. The result was slightly higher than the finding

of Hing *et.al.* (2013) which was 66.3% but comparatively lower than the finding of Phuphisut *et. al.* (2016) who have reported *Strongylids* (93%) from Thailand. Similarly, the prevalence of other nematodes in wild elephants among eight genera in present study was *Ascaris* sp. (45%), which shows high prevalence in comparison to the previous study done in Thailand by Phuphisut *et. al.* (2016) i.e 2.3%. Whereas the other nematodes such as *Dromeostrongylus* sp. (30%), *Haemonchus* sp. (25%), *Chabertia* sp. (15%) and with each of 10% of *Nematodirus* sp., *Bunostomum* sp., *Trichostrongylus* sp. were reported from wild elephants for the first time in Nepal.

One genus of the cestode parasite was found infecting the wild elephant in CNP, Nepal i.e. *Anoplocephala* sp. (10%), which was similar to the finding of Nishanth *et. al.* (2012) i.e. 13.33%. However, the result is comparatively less than previous finding done by Hing *et. al.* (2013) which was 50%. The presence of the cestode *Anoplocephala* sp. might be due to the presence of their intermediate host i.e. grass mites which get ingested along with grass by the elephants while grazing. However, the trematodes were not reported from the wild elephants of CNP which is similar to Nishanth *et. al.* (2012) in India and Vidhya and Sukumar (2002) in Kerala. While the trematode egg (27%) were reported in the wild African elephants by Baines *et. al.*, (2015) from Botswana.

Location wise analysis in six area of CNP revealed prevalence rate higher in the Chure area (30%) with maximum eight different types of parasitic genera such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Dromeostrongylus* sp., *Haemonchus* sp., *Trichostrongylus* sp., *Chabertia* sp., *Bunostomum* sp. The reason for this finding might be due to the collection of maximum amount of samples (40%) from this chure area as it is also considered as the core area of wild elephants according to the governmental Mahouts of CNP, the samples collected might be of Ronaldo (which is known wild elephant named by the local people) or of Govinda (also another baby wild elephant named by the local people) or of the other wild elephants roaming in that area. Whereas the prevalence rate was 20% in the Tiger tops area with 6 different genera of parasites such as *Strongyloide* sp., *Haemonchus* sp., *Dromeostrongylus* sp., *Chabertia* sp., *Trichostrongylus* sp. and *Anoplocephala* sp. which was only reported from this area and the samples from this area might be of Dhurbe (one of a another known wild elephant named by the local people) or of Ronaldo or other wild elephant. Similarly, a less prevalence rate in the Temple Tiger area (10%) with four different genera of parasites such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp. and *Haemonchus* sp. that might be of Dhurbe or other wild elephant followed by the prevalence rate in khosar area (10%) with three different genera of parasites such as *Ascaris* sp., *Strongyloide* sp. and *Haemonchus* sp., that might be of Ronaldo or Govinda and similarly the prevalence rate in Magartole (10%) with three different genera of the parasites such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., that can be of Ronaldo (according to the local people) and the prevalence rate in Nandan tal area (10%) with two different genera of parasites such as *Strongyloide* sp. and *Nematodirus* sp were found. The location wise variation in the prevalence rate might be due to the variation in total number of samples collected from six different area of CNP such as Chure area (40%), Tiger tops (20%), Temple tiger area (10%), Khosar area (10%), Nandantal area (10%) and Magartol area (10%).

Present study revealed that Ronaldo, Dhurbe and Govinda might be infected by the four different genera of parasites such as *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp. and *Haemonchus* sp while other wild elephants might be infected by *Eimeria* sp., *Ascaris* sp., *Strongyloide* sp., *Dromeostrongylus* sp., *Haemonchus* sp., *Trichostrongylus* sp., *Chabertia* sp., *Bunostomum* sp., *Nematodirus* sp. and *Anoplocephala* sp.

Among 20 samples, the mixed parasitic infection was found more common in wild elephants in CNP. In the present study, triple infection was observed maximum i.e. 40% followed by double infection (35%) and multiple infection (15%) whereas no single infection was observed i.e. all the positive sample have mixed infection. The result is comparatively higher than the finding of Hing *et. al.* (2013) which was 65.4% and Vidhya and Sukumar, (2002) that is 11.9% of sample as mixed infection. This might be due to the high contamination of their habitat sharing with other wild animals of CNP.

The intensity of different parasites in wild elephants of CNP was observed in this study. According to the result maximum of wild elephants were found infected with light infection, which are considered to cause less infection. However, mild as well as moderate infection with some parasites that might cause some general dysfunction in wild elephants. While some of the dung samples of the wild elephant found to be positive for heavy infection by *Strongyloides* sp. This heavy infection might indicate some symptomatic condition anemia in wild elephants as they suck the blood.

Among 40 captive/domestic Asian elephants, 22 governmental captive elephants and 18 domesticated elephants from three different private lodges were examined in and around CNP. Out of which, 57% of the elephants were found to be positive with the parasitic prevalence, which is found comparatively less than the several other previous finding reported by Karki (2008) from Nepal which was 100% and from India by Ahmed and Doley (2017) and Jani (2008) which was 68% and 62.5% respectively. However, the result is comparatively higher than the previous study done by Saseendran *et. al.* (2003) which was 17.7% prevalence rate.

Infection regarding the coccidian oocysts in captive/circus elephants has been reported by Bhoyar *et. al.* (2014) in Bidar, Karnataka state of India. However, the present study revealed the presence of protozoan parasite belonging to genus *Eimeria* sp. without micropyle in 15% samples of domestic Asian elephants so far for the first time in Nepal. The reason for this might be due to the opportunistic infection in the captive/ domestic elephants.

From the result of this present study it shows that the captive elephant in and around the chitwan national park were infected with the nematode parasites with four different genera such as *Strongyloide* sp., *Ascaris* sp., *Haemonchus* sp. and *Bunostomum* sp. Among these nematodes, *Strongyloide* sp. was found significantly infective (52.5%) to the captive elephants, which is similar to the findings of Dutta and Bordoloi (1989), Roa *et. al.* (1992) and Cheeran (1999) in India. However, the result was found significantly higher from the previous studies done by Saseendran *et. al.* (2003), Arunachalam *et. al.* (2007), Ahmed and Doley (2017) and Abeysinghe *et. al.* (2017) who reported Strongyle sp. 10.10%, 36%, 5%, 14.71% and 38% respectively from the captive elephants in India and Pandit *et. al.* (2015) who reported *Strongyloide westeri* (23%) from Nepal. The present finding seems somehow similar to the result reported by Suresh *et. al.* (2001),

63.64% Strongylosis which is caused by *Strongyloide* sp. in S.V dairy farm, Tirupati. Likewise, the present finding seems comparatively less than the finding reported by the previous studies done by Chandrasekharan (1995) in India (91.27%), Suresh *et. al.,* (2001) in Nehru (87.5%), and Abeysinghe *et. al.* (2017) in privately owned elephants in Srilanka.

The other genera of nematodes in captive/domestic elephants in the present study were reported as *Ascaris* sp. (27.5%), which is comparatively higher than the finding of Ahmed and Doley (2017), which is 14.71%. Similarly, in the result of present study the other nematode genera i.e. *Haemonchus* sp. (7.5%) and *Bunostomum* sp. (2.5%) were also reported.

No trematodes and Cestode parasites were reported from the captive elephants in and around the CNP, which disagree with finding of Karki (2008) who have recorded the differerent genera of trematodes as well as Pandit *et. al.* (2015) who have reported *Fascioloide magma* (9%) in the captive elephants of Nepal. This might be due to proper adoption of the deworming treatment in the majority of stables of captive elephant as well proper maintenance of hygiene in captive condition.

Location wise analysis in this present study revealed that among all five location, the elephants from the Machang country villa were infected by the maximum five different genera of parasites with 5% of positive sample was reported, followed by the elephants from Temple Tiger lodge found infected with four different genera of parasites with 10% positive sample Likewise, the elephants from Tiger Tops Tharu lodge, Khorsar breeding center and Sauraha sector, Hattisar found infected with two different genera of parasites with 15% , 15% and 12.5% positive samples respectively. However, the variation in the positive sample in different location might be due to the different in sample size wit location where as the variation in the number of the parasitic genera with different location might be due to lack of proper maintenance of sanitation and hygiene in the captive condition.

Among 40 samples from the captive/ domestic elephant in or around CNP, both single and mixed infection was recorded. Single infection was found highest i.e. 32.50% in comparison to the mixed infection (25%) which includes double infection (17.50%) and multiple infection (7.50%) in the captive/domestic elephants. Present study encountered the highest single infection of *Strongyloide* sp. (92.3%) in captive/domestic elephant, which was found to be comparatively higher than the previous study done by Jani (2008) who reported 5% single infection of *Strongyloide* sp. in India. Likewise, the present result shows the single infection of *Ascaris* sp. (7.7%), which higher than the finding of Jani (2008) which is 2.5%. However, double and multiple infections were only reported as mixed infection. Double infection (17.50%) was found with highest prevalence than multiple one (7.50%) with the highest prevalence of *Ascaris* sp. with *Strongyloide* sp. followed by *Eimeria* sp. with *Ascaris* sp. (14.3%). The movement of elephants from their captive stables to inside CNP for feeding, tourism operation as well as other several purposes could have favor the mixed parasitic infection in them.

The intensity of different parasites in captive/ domestic elephant in and around the Chitwan national park was observed in this study. According to this present finding, maximum number of the elephants in captive or domestic conditions in and around CNP

is found to be infected by the light infection which is asymptomatic condition and cannot causes serious damage or disease to the animals while less number of the elephants were infected with mild infection was revealed with *Eimeria* sp., Ascaris sp., *Strongyloide* sp. and *Haemonchus* sp. and some with moderate infection was also revealed with Strongyloide sp. which may be symptomatic or asymptomatic in animals.

The comparative analysis of gastro-intestinal parasite in between wild and captive/ domestic elephants in and around Chitwan national park is so far for the first time in Nepal. Wild elephants were found more infected than the captive/ domestic elephants as the prevalence of parasites in wild elephants and captive elephant was 90% and 57% respectively. The reason for this variation in result in between them might be due to the high vulnerability of wild animal to the parasites because of their wild habitat as well the adoption of medication/ deworming treatment. However, the prevalence of protozoan parasites was compared in between wild and captive elephants and no significant difference was obtained as both of them were reported with same genus of protozoa i.e. *Eimeria* sp.

Among the helminthes parasites, wild elephants were infected by 8 different genera of nematode and one cestode whereas captive/ domestic elephants were infected by four different genera of nematode only. No trematodes were observed in both wild as well as captive/ domestic elephants in and around the CNP. The reason behind this might be due to the lack of advance technique/ process for collection, storage and examination of the dung sample for identification of parasite's egg.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Asian elephants in Chitwan National Park (CNP) were found infected with different gastrointestinal parasites. Statistically, the overall prevalence of gastrointestinal parasites in wild and captive/domestic elephants was found significantly difference ($\chi 2$= 5.0931, P-value= 0.02). The identified parasites were one protozoan (*Eimeria* sp.), one cestode (*Anoplocephala* sp.), and eight genera of nematodes (*Ascaris* sp., *Strongyloide* sp., *Haemonchus* sp., *Trichostrongylus* sp., *Dromeostrongylus* sp., *Chabertia* sp., *Bunostomum* sp., and *Nematodirus* sp.) based on the morphology of the eggs.

The parasites recorded in this study were higher in wild elephants than captive/ domestic elephants in and around CNP. One protozoan parasite, one cestode and eight genera of nematodes were reported from wild whereas one protozoan parasite and 4 genera of nematode were reported from captive/domesticated elephants. Between both the wild and domestic elephants, infection of *Strongyloide* sp. was found as dominate i.e. 85% and 52.5% respectively. From this finding it can be assumed that the parasite in wild habitats are more infective than in captive/domesticated habitat.

Among the wild elephants the dung sample collected from the Chure area were found highly infected by the gastrointestinal parasites whereas least from the Nandan tal area. Likewise in case of captive or domestic elephants, the dung sample collected from the Tiger Tops Tharu lodge and Khorsar breeding center were found highly infected with gastrointestinal parasites whereas least was reported from, Machang country villa.

In case of wild elephants the mixed infection was observed with maximum of triple infection (40%) followed by double (35%) and multiple infection (15%) but there was no single infection. However, in case of Captive/ domestic elephants single infection was found highest (32.50%) with the *Strongyloide* sp. (92.3%) than the mixed infection with double infection (17.50%) and multiple (7.50%) were reported.

Furthermore, the intensity of gastrointestinal parasite in the dung samples of wild elephants had maximum of light infection with mild, moderate and heavy infections whereas in case of captive/ domestic elephants maximum of light infection was found in the dung samples with some mild and moderate infection but heavy infection was not observed. This finding shows that the Asian elephants in CNP are susceptible to different gastrointestinal parasites, which in its heavy infection can cause disease.

In addition, the prevalence of gastrointestinal parasites in wild elephants has significant difference. Similarly there was significant difference in the prevalence of gastrointestinal parasites in case of captive/domestic elephants too.

6.2 Recommendations

On the basis of conclusion following recommendation have been made to reduce the risk of gastrointestinal parasites in both wild and domestic elephants.

- Morphologically similar parasites were found in both wild and captive/domestic elephants but the verification or identification in molecular basis should be done.
- Considering the detection of mostly the nematode parasites in the captive/domestic elephants in both the governmental sector and private lodge, the routine checkup and medication programs should be more emphasize.

7. REFERENCES

Abeysinghe, S.K., Perera, A.N.F., Pastorini, J., Isler, K., Mammides, C. and Fernando, P. 2017. Gastrointestinal Strongyle infections in captive and wild elephants in Srilanka. Gajah, **46**: 21-27.

Ackermann, M., Hatt, J.M., Schette, N. and Steinmetz, H. 2017. Identification of shedders of elephant endotheliotropic herpesviruses among Asian elephants (*Elephas maximus*) in Switzrland. Plos One, **12**(5).

Ahmed, N and Doley, S. 2017. Prevalence of gastro-intestinal parasitic load of Asian elephants (Elephas maximus) in Unakoti, Tripura. Journal of entomology and zoology studies, **5**(4): 1514-1515.

Angkawanish, T., Waijwalku, W., Sirimalaisuwan, A., Mahasawangkul, S., Kaewsakhorn, T. and Boonsri, K. *et. al*. 2010. *Mycobacterium tuberculosis* infection of domesticated Asian elephants, Thailand. Emerging Infectious Disease, **16**(12): 1949-1951.

Arunachalam, K., M. Raman and T.J. Harikrishnan. 2007. Incidence of helminth ova in Indian elephants *Elephas maximus* at Theppakadu Nilgiris, Tamil Nadu. Zoos' Print Journal, **22**(11): 2898–2899.

Baines, L., Morgan, E.R., Ofthile, M. and Evans, K. 2015. Occurrence and seasonality of internal parasites infection in elephants, *Loxodonta africana*, in the Okavango Delta, Bostwana. International Journal for Parasitology: Parasites and Wildlife, **4**(1): 43-48.

Barman, N.N., Choudhury, B., Kumar,V., Koul, M., Gogoi, S.M. and Chakroborty, A. *et. al.* 2017. Incidence of elephant endotheliotropic herpesvirus in Asian elepants in India. Veterinary Microbiology, **208**: 159-163.

Baskaran, N., Balasubramanian, M., Swaminatha, S. and Desai, A.A. 2010. Feeding ecology of the Asian elephant *Elephas maximus*, Linnaeus in the Nilgiri biosphere reserve, southern India. Journal of the Bombay Natural History Society, **107**(1): 3-13.

Bhoyar, R., B.S. Pradeep., Kulkarni, S., Kasaralikar, V.R. and Patil, N.A. 2014. Schistosomiasis in Asian elephants. Gajah, **40**: 35-38.

Bhusal, N.P. 2007. Chitwan National Park: A prime destination of eco-tourism in central tarai region, Nepal. Department of Geography Education. The Third Pole, **5**(7): 70-75.

Brant, S.V., Pomajbíkova, K., Modry, D., Petrzelkova, K.J., Todd, A. and Loker, E.S. 2013. Molecular phylogenetics of the elephant schistosome *Bivitellobilharzia loxodontae* (Trematoda: Schistosomatidae) from the Central African Republic. Journal of

Helminthology, **87:** 102-107.

Chandrasekharan, K., K. Radhakrishnan, J.V. Cheeran, K.N. Muraleedharan& T. Prabhakaran. 1995. Review of the incidence, ecology and control of common diseases of Asian Elephants with special reference to Kerala, pp. 439–449. *In* Daniel, J.C. & H.S. Datye (eds.). 1993. A Week with Elephants: Proceedings of the International Seminar on the Conservation of Asian Elephant. Oxford University Press, Oxford.

Chandrasekharan, K., Radhakrishnan, K., Cheeran, J.V., K. N., Muraleedharan and T. Prabhakaran. 1995. Review of the incidence, ecology and control of common diseases of Asian Elephants with special reference to Kerala. *In* A Week with Elephants: Proceedings of the International Seminar on the Conservation of Asian Elephant. Daniel, J.C. and Datye, H.S. (eds.). Oxford University Press, Oxford, pp. 439–449.

Chandrasekharan. K., Radhakrishnan. K., Cheeran, J.V., Muraleedharan, K.N. and Prabhakaran. T. 2009. Review of the incidence, etiology and control of common diseases of Asian elephants with special reference to Kerala. *In* Healthcare Management of Captive Asian Elephants. G, Ajitkumar., K.S., Anil. and P.C., Alex. (eds.). Kerala Agricultural University Press, Mannuthy, Thrissur, India, pp. 92-100.

Cheeran, J.V. 1999. Habitat utilization of animals and their parasitic burden with the special reference to elephants in Vazhachal forest division. Kerala. Zoo's Print, **14**(2): 44.

Cheeran, J.V. and Poole, T.B. 2009. The exploitation of Asian elephants. *In* Healthcare management of captive Asian elephants. G, Ajitkumar., K.S., Anil. and P.C., Alex. (eds.). Kerala Agricultural University Press, Mannuthy, Thrissur, India, pp. 28-32.

Choudhury AU. 1999. Status and conservation of the Asian elephant Elephas maximus in North-eastern India. Mammal Review, **29**: 141-173.

Choudhury, A., Choudhary, L., Desai, D.K., Duckwort, J.W., Easa, P.S. and Johnsingh, A.J.T. *et. al.* 2008. *Elephas maximus*. The IUCN Red List of Threatened Species 2008: e.T7140A12828813. doi.org/10.2305/IUCN.UK.2008.RLTS.T7140A12828813.en. Acces sed on 26 February 2018.

Chungath, J.J. 2009. Anatomy of elephant. *In* Healthcare Management of Captive Asian Elephants. G, Ajitkumar., K.S., Anil. and P.C., Alex. (eds.). Kerala Agriculture University, Mannuthy, Thrissur, India. pp. 12-15.

Cohn, J.P. 2006. Do elephants belong in zoos? BioScience, **56**(9): 714-717.

Coop, R.L. and Holmes, P.H. 1996. Nutrition and parasite interaction. International Journal for Parasitology, **26**(8): 951-962.

Coop, R.L. and Kyriazakis, I. 1999. Nutrition–parasite interaction. Veterinary parasitology, **84**(3): 187-204.

Delgado, E., Fonseca, I.P., Fazendeiro, I, Matos, O., Antunes, F. and Cunha, M.B. 2003. Cryptosporidium spp. in ruminants at the Lisbon Zoo. Journal of Zoo and Wildlife Medicine, **34**(4): 352-356.

Devkota, R., Brant, S.V., Thapa, A. and Loker, E.S. 2014. Sharing Schistosomes: The elephant Schistosome *Bivitellobilharzia nairi* also infects the greater one-horned rhinoceros (*Rhinoceros unicornis)* in Chitwan National Park. Journal of Helminthology, **88**(1): 32-40.

Dharmarajan, G. 2000. Epidemiology in wild and domestic herbivores at the Mudumalai Wildlife Sanctuary, Tamil Nadu. M.V.Sc Thesis. Tamil Nadu University of Veterinary and Animal Sciences, Chennai, Tamil Nadu, 75 pp.

Dhungel, S., C. Browner and S. Yoder. 1990. Elephant training and management in Nepal. Tiger paper, **17**: 1-6.

DNPWC. 2008. The Elephant Conservation Action Plan for Nepal Ministry of Forest and Soil Conservation, Department of National Park and Wildlife Conservation, Kathmandu, Nepal.

DNPWC. 2011. Nepal Elephant Tuberculosis Control and Management Action Plan (2011- 2015). Government of Nepal, Ministry of Forest and Soil Conservation, Department of National Parks and Wildlife Conservation, Kathmandu, Nepal.

Dutta, G.C. and Bodoloi. 1989. Prevalence of helminthic infection in elephants of Tiger project of Manas, Assam. Zoo's Print, **4**(3): 14.

Fagiolini, M., Lia, R.P., Laricchiuta, P., Cavicchio, P., Mannella. R. and Cafarchia, C. *et.al*. 2010 Dec. Gastrointestinal parasites in mammals of two Italian zoological gardens. Journal of Zoo and Wildlife Medicine, **41**(4): 662-70.

Fowler, M.E. and Mikota, S.K. 2006. Biology, Medicine and Surgery of Elephants. Blackwell Publishing, Ames, Iowa, 565 pp.

Friyal, S. and Naureen, A. 2007. Elephant as a veterinary patient. Pakistan Veterinary Journal, **27:** 48-54.

Gairhe, K.P. 2002. A Case Study of Tuberculosis in Captive Elephants in Nepal. A Report submitted to Department of National Parks and Wildlife Conservation, Kathmandu, Nepal.

Gairhe, K.P. 2012. Veterinary care and breeding of elephants in Nepal. Gajah, **37**: 27-30.

Ghielmetti, G., Coscolla, M., Ruetten, M., Fridel, U., Loiseau, C. and Feldmann, J. *et. al*. 2017. Tuberculosis in Swiss captive Asian elephants: microevolution of *Mycobacterium tuberculosis* characterized by multilocus variable- number tandem-repeat analysis and whole-genome sequencing. Scientific Reports, **7**: 14647.

Goodwin, G. 1951. The Crowinshield Elephant. The Natural History Magazine. http://www.naturalhistorymag.com/editors_ accessed on 12 April, 2010.

Gürelli, G.and Ito, A. 2014. Intestinal ciliated protozoa of the Asian elephant *Elephas maximus* Linnaeus, 1758 with the description of *Triplumariaizmirae* n. sp. European Journal of Protistology, 5**0**(1): 25-32.

Hing, S., Othman, N., Nathan, S. S. K. S., Fox, M., Fisher, M. and Goossens, B. 2013. First parasitological survey of Endangered Bornean elephants *Elephas maximus borneensis*. Endangered species research, **21**: 223–230.

Huang, D.S. 1981. Three trematodes parasitizing an elephants (*Elephas maximus).* Chinese journal of Veterinary Medicine, **7**(4): 17-18.

Jani, R.G. 2008. Prevalence and Haemato-Biochemical Studies of Gastro-intestinal Parasites of Indian Elephants (Elephas maximus). Veterinary World, **1**(10): 296-298.
Jayewardene, J. 2014. Illegal Captures - A Serious Threat to Asian Elephants. Gajah, **40***:* 1-2.

Karki, K. 2008. Incidence of gastrointestinal helminths in captive Elephants in Wildlife Reserves of Nepal. Article base. http://www.articlesbase.com/health-articles/incidence-of-gastrointestinal-helminthes-in-captive-elephants-in-wildlife-reserves-of-nepal.
accessed on: 23rd December 2013.

Kemf, E. and Santiapillai, C. 2000. Asian Elephants in the wild. A WWF species status report, WWF, Gland, Switzerland.

Kendalla, R., Howard, L., Masters, N. and Grant, R. 2016. The impact of elephant endotheliotropic herpesvirus on the captive Asian elephant (Elephas *maximus*) population of the United Kingdom and Ireland (1995-2013). Journal of Zoo and Wildlife Medicine, **47**(2): 405- 418.

Kharel, F.R. 2001. The challenge of managing domesticated Asian elephants in Nepal. *In* Giants on Our Hands. Baker, I. and Kashio, M. (eds.). Proceedings of the International Workshop on the Domesticated Asian Elephant, Bangkok, Thailand, pp. 103-110.

Kinsella, J.M., Deem, S.D., Blake, S. and Freeman, A.S. 2004. Endoparasites of African forest elephants (*Loxodonta africana cyclotis*) from the Republic of Congo and Central African Republic. Comparative Parasitology, **71**: 104-110.

Latimer, E., Zong, J., Heaggans, S., Richman, L., Hayward, G. 2011. Detection and evaluation of novel herpes viruses in routine and pathological samples from Asian and African elephants: Identification of two new probosciviruses (EEHV5 and EEHV6) and two new gammaherpesviruses (EGHV3B and EGHV5). Veterinary Microbiology, **147**(1-2): 28-41.

Lei, R., Brennerman, R.A., Schmitt, D.L. and Louis, E.E. 2012. Genetic diversity in North American captive Asian elephants. Journal of Zoology, **286**: 37-47.

Lloyd, S. 1995. Environmental influences on host immunity. *In* Ecology of Infectious Diseases in Natural Populations, Grenfell, B.T. and Dobson, A.P. (eds) Cambridge University Press, U.K., pp 327- 362.

Lotfy, W.M., Brant, S.V., DeJong, R.J., Le, T.H., Demiaszkiewicz, A., Raiapakse, R.P.V.J., *et al.* 2008. Evolutionary origins, diversification, and biogeography of liver flukes (Digenea, Fasciolidae). American Journal of Tropical, Medicine and Hygiene, **79**: 248-255.

Lynsdale C.L., Franco dos Santos D.J., Hayward, A.D., Mar, K.U., Htut. W. and Aung H.H. *et al.* 2015. A standardized faecal collection protocol for intestinal helminth egg counts in Asian elephants, *Elephas maximus*. International Journal for Parasitology: Parasites and Wildlife **4**: 307-315.

Maisels, F., Strindberg, S., Blake, S., Wittemyer, G., Hart, J. and Williamson, E.A. 2013. Devastating decline of forest elephants in Central Africa. Plos One, **8**(3): e59469.

Mandal, R.K. and Khadka, K.K. 2013. Health Status of Captive Asian Elephants in Chitwan National Park, Nepal. Gajah, **39**: 37-39.

Mawdsley, J.L., Bardgett, R.D., Merry, R.J., Pain, B.F. and Theodorou, M.K. 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Applied Soil Ecology, **2**(1): 1-15.

Mbaya, A.W., Ogwiji, M. and Kumshe, H.A. 2013. Effects of host demography, season and rainfall on the prevalence and parasitic load of gastrointestinal parasites of free-living elephants (*Loxodonta africana*) of the Chad Basin National Park, Nigeria. Pakistan Journal of Biological Science. **16**(20): 1152-1158.

Mikota, S.K. 2008. Review of tuberculosis in captive elephants and implications for wild populations. Gajah, **28**: 8-18.

Mikton, S.K., Larsen, R.S. and Montali, R.J. 2000. Tuberculosis in elephants in North American. Zoo Biology, **19**: 393-403.

Morgan, E.R., Shaw, S.E., Brennan, S.F., De Waal, T.D., Jones, B.R. and Mulc-ahy, G. 2006. *Angiostrongylus vasorum*: A real heartbreakers. Trends Parasitology, **21**: 49–51.

Moss, C. 2001. The demography of an African elephant (Loxodonta *africana*) population in Amboseli, Kenya. Journal of Zoology, **255**(2): 145-156.

Nishanth, B., Srinivasan, S.R., Jayathangaraj, M.G. and Sridhar R. 2012. Incidence of endoparasitism in free-ranging elephants of TamilNadu State, TamilNadu. Journal of Veterinary and Animal Sciences, **8** (6): 332–335.

Nunn, C.L., Thrall, P.H., Leendertz, F.H. and Boesch, C. 2011. The spread of fecally transmitted parasites in socially structured populations. Plos One, **6**(6).

Obanda, V., Iwaki, T., Mutinda, N.M.and Gakuya, F. 2011. Gastrointestinal parasites and associated pathological lesions in starving free-ranging African elephants. South African Journal Wildlife Research, **41**: 167-172.

Pandit, A., Dhakal, I.P. and Gairhe, K.P. 2015. Prevalence of endoparasitic disease in elephants of Chitwan National Park, Nepal. Internatioal Journal of Recent Scientific Research, **6**(8): 5768-5771.

Pandit, A., Dhakal, I.P., Gairhe, K.P., Rana, H.B. and Karmacharya, D. 2014. Surveillance of the elephant endoteliotropic herpesvirus (EEHV) in Chitwan district, Nepal. International Joural of Recent Scientific Research, **5**(10): 1749-1755.

Phuphisut, O., Maipanich, W., Pubampn, S., Yindee, M., Kosoltanapiwat, N. and Nuamtanong, S. *et. al.* 2016. Molecular identification of the strongyloid nematode *Oesophagostomum aculeatum* in the Asian wild elephant *Elephas maximus*. Journal of Helminthology, **90**(4): 434-440.

Pradhan, N.M.B., Wegge, P., Moe, S.R and Shrestha, A.K. 2008. Feeding ecology of two endangered sympatric megaherbivores: Asian elephant *Elephas maximus* and greater one-horned rhinoceros *Rhinoceros unicornis* in lowland Nepal. Wildlife Biology, **14:** 147-154.

Pradhan, N.M.B., Williams, A.C. and Dhakal, M. 2011. Current status of Asian elephants in Nepal. Gajah, **35**: 87-92.

Rao, D..S.T., S, Yathiraj., Choudhuri, P.C. and Reddy, K.P. 1992. Treatment of helminthiosis in elephants. Indian Journal of Animal Science, **62**(12): 1155-1156.

Reid, C.E., Hildebrandt, T.B., Marx, N., Hunt, M., Thy, N. and Revnes, J.M. *et. al.* 2006. Endotheliotropic elephant herpes virus (EEHV) infection: The first PCR-confirmed fatal case in Asia. Veterinary Quarterly, **28**(2): 61-64.

Richman, L.K., Montali, R.J., Schmitt, C.D., Hardy, D., Hildbrandt, T. and Bengis, R.G. *et. al.,* 2000. Clinical and pathological findings of a newly recognized disease of elephants caused by endotheliotropic herpesviruses. Journal of wildlife diseases, **36**(1): 1-12.

Rosen, L.E., Hanyire, T.G., Dawson, J., Foggin, C.M., Michel, A.L. and Huyvaert, K.P. *et. al.* 2017. Tuberculosis serosurveillance and management practices of captive African elephants (*Loxodonta africana*) in the Kavango-Zambezi Transfrontier Conservation Area. Transboundary and Emerging Diseases, **3** (1): 1-11.

Samansiri, K.A.P. and Weerakoon, D.K. 2007. Feeding behavior of Asian elephants in the Northwestern region of Srilanka. Gajah, **27**: 27-30.

Saseedran, P.C., S. Rajendran, H. Subramanian, M., Sasikumar, G. Vivek and K.S. Anil. 2004. Incidence of helminthic infection among annually de-wormed captive elephants. Zoos' Print Journal, **19**(3): 14-22.

Schmitt, D.L., Hardy, D.A., Montali, R.J., Richman, L.K., Lindsay, W.A. and Isaza, R. 2000. Use of famciclovir for the treatment of endotheliotrophic herpesvirus infections in Asian elephants (*Elephas maximus*). Journal of Zoo and Wildlife Medicine, **31**(4): 518-522.

Seaman, J.T. and Finnle, E.P. 1987. Acute mycocarditis in a Captive African elephant (*Loxodonta africana*). Journal of Wildlife Disease, **23**(1): 170-171.

Shrestha, S. and Gairhe, K.P. 2006. Veterinary problems of geographical concern- section VII, Nepal. *In* Biology, Medicine and surgery of elephants. Fowler M.E. and Mikton S.K. (eds.). Blackwell Publishing, U.K., pp 78-80.

Shrestha, T.K. 2003. Wildlife of Nepal: A study of Renewable Resources of Nepal Himalayas. 2nd ed. Curriculum Development Centre, Kathmandu, 720 pp.

Soulsby, E.J.L. 1982. Textbook of veterinary clinical parasitology Vol I, Helminth Oxford Blackwell Scientific, London.

Sukumar, R. 2004. The living elephants: Evolutionary ecology, behavior, and conservation. Journal of Mammalogy, **85**(3): 581-582.

Suresh, K.P., Choudhuri, C., Nalini, K.K., Hafeez, M.and Hamza, P.A. 2001. Epidemiological and clinico-therapeutic studies of strongylosis in elephants. Zoos' Print

Journal, **16**(7): 539-540.

Thurber, M.I., O'Connell-Rodwell, C.E., Turner, W.C., Nambandi, K., Kinzley, C. and Rodwell T.C., *et al.* 2011. Effects of rainfall, host demography, and musth, on *Strongyl*e fecal egg count in African elephants (*Loxodonta africana*) in Namibia. Journal of Wildlife Disease, **47**: 172-181.

Vanitha, V. 2007. Studies on the status and management of captive Asian Elephants (*Elephas maximus*) at Tamil Nadu in southern India. PhD Thesis. Bharathidasan University, Tiruchirapalli, India.

Vanitha, V., Thiyagesan, K. and Baskaran, N. 2011. Prevalence of intestinal parasites among captive Asian elephants *Elephas maximus*: effect of season, host demography, and management systems in Tamil Nadu, India. Journal of Threatened Taxa **3**(2): 1527-1534.

Verma, K.S., Abraham, D., Dendukuri, N., Cheeran, J.V., Sukumar, R. and Balaji, K.N. 2012. Serodiagnosis of Tuberculosis in Asian Elephants (*Elephas maximus*) in Southern India: A Latent Class Analysis. Plos one, **7**(11): e49548.

Vidya, T.N.C. and Sukumar, R. 2002. The effect of some ecological factors on the intestinal parasite loads of the Asian Elephant *(Elephas maximus*) in southern India. Journal of Bioscience, **27**: 521–528.

Vitovec, J., Kotrla, B., Hussein, H. and Hayles, L.B. 1984. Fatal infection of an elephant calf caused by the trematode *Protofasciola robusta* (Lorenz, 1881) in Somaliland. Zentralblatt fur Veterinarmedizin. Reihe B., **31**:597-602.

Vogelnest, L., Hulst, F., Thompson, P., Lyashchenko, K.P. and Herrin, K.A. 2015. Diagnosis and management of tuberculosis (*Microbacterium tuberculosis*) in an Asian elephant (*Elephas maximus)* with a newborn calf. Journal of Zoo and Wildlife Medicine, **46**(1): 77-85.

Walker, J.W., 1995. Viewpoint: grazing management and research now and in the next millennium. Journal of Range Management, **48**(4): 350-357.

Watve, M.G. 1995. Helminthes parasites of elephant's ecological aspects. *In* A week with elephants: Proceedings of the International Seminar on the Conservation of Asian Elephants. Daniel, J.C. & H.S. Daty (eds). 1993. Oxford University Press, New Delhi. pp. 289-295.

Wiese, R. 1997. Demographic analysis of the Asian elephant population in North America. Proceeding of the Elephant Manager Association workshop. 18-57

Wolfe, N.D., Switzer, W.M., Carr, J.K., Bhullar, V.B., Shanmugam, V., Tamoufe, U. *et al.* 2004. Naturally acquired simian retrovirus infections in Central African hunters. Lancet, **363** (9413): 932–937.

8. ANNEXES

Annex 1 Name of elephants of Khorsar breeding centre

S.N	Name of elephants	*Eimeria* sp.	*Ascaris* sp.	*Strongyloide* sp.	*Haemonchus* sp.	*Bunostomum* sp.
1.	Ambe Kali	-	+	+	-	-
2.	Rimjim Kali	-	+	+	-	-
3.	Simsim Kali	-	-	-	-	-
4.	Pooja Kali	-	-	-	-	-
5.	Lucky Gaj	-	-	+	-	-
6.	Chitwan Kali	-	-	+	-	-
7.	Khorsar Gaj	-	-	+	-	-
8.	Tej Gaj	-	-	-	-	-
9.	Prena Kali	-	-	-	-	-
10.	Ganesh Kali	-	-	+	-	-
11.	Chanchan Kali	-	-	-	-	-
12.	Narayani Kali-	-	-	-	-	-

Annex 2. Name of elephants of Saurah sector, Hattisar

S. N	Name of elephants	*Eimeria* sp.	*Ascaris* sp.	*Strongyloide* sp.	*Haemonchus* sp.	*Bunostomum* sp.
1.	Ram Gaj	-	-	+	-	-
2.	Somsar Prasad	-	-	-	-	-
3.	Sundarmala	-	-	+	-	-
4.	Bahadur Gaj	-	-	-	-	-
5.	Kasara Gaj	-	-	-	-	-
6.	Bhola Prasad	-	-	+	-	-
7.	Dipendra Gaj	-	-	-	-	-
8.	Binayak Prasad	-	-	-	-	-
9.	Moti Prasad "Kha"	-	+	+	-	-
10.	Rampiyari	-	+	+	-	-

Annex 3. Name of elephants of Machang country villa

S. N	Name of elephants	*Eimeria* sp.	*Ascaris* sp.	*Strongyloide* sp.	*Haemonchus* sp.	*Bunostomum* sp.
1.	Pawan Kali	-	+	+	+	+
2.	Phool Kali	+	+	-	-	-

Annex 4. Name of elephants of Temple Tiger Lodge

S.N	Name of elephants	*Eimeria* sp.	*Ascaris* sp.	*Strongyloide* sp.	*Haemonchus* sp.	*Bunostomum* sp.
1.	Champa Kali	-	-	-	-	-
2.	Sundar Kali	-	+	+	-	-
3.	Rangamala Kali	+	+	+	+	-
4.	Kanti Kali	-	-	+	-	-
5.	Durga Kali	+	+	_	_	-

Annex 5. Name of elephants of Tiger Tops Tharu Lodge

S. N	Name of elephants	*Eimeria* sp.	*Ascaris* sp.	*Strongyloide* sp.	*Haemonchus* sp.	*Bunostomum* sp.
1.	Chandra Kali	-	+	-	-	-
2.	Gulab Kali	-	-	+	-	-
3.	Raj Kali	-	-	-	-	-
4.	Hira Kali	-	-	+	-	-
5.	Dibya Kali	-	-	-	-	-
6.	Pawan Kali	-	+	+	-	-
7.	Dipendra Kali	-	-	+	-	-
8.	Sita Kali	-	-	-	-	-
9.	Champa Kali	-	-	-	-	-
10.	Sona Kali	-	-	-	-	-
11.	Saraswoti Kali	-	-	+	-	-