TAMIL NADU FOREST DEPARTMENT

Advanced Institute for Wildlife Conservation
(Research, Training & Education)
Vandalur - 600 048.

Report

EVALUATION OF DIFFERENT TRAP MODELS TO MONITOR AND CONTROL HAEMATOPHAGOUS INSECTS WHICH TRANSMIT DISEASES TO CAPTIVE ANIMALS IN ZOOLOGICAL PARKS

Annual Plan of Operatons
2023-24

Tamil Nadu Forest Department

ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(Research, Training & Education)
Vandalur, Chennai – 600 048.

Project Completion Report
on

EVALUATION OF DIFFERENT TRAP MODELS TO MONITOR AND CONTROL HAEMATOPHAGOUS INSECTS WHICH TRANSMIT DISEASES TO CAPTIVE ANIMALS IN ZOOLOGICAL PARKS

Project Sanctioned under

Annual Plan of Operations (APO) 2023-24

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CENTRE FOR ANIMAL CARE SCIENCES
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Abbreviations and symbols

AAZP	Arignar Anna Zoological Park
AHS	African Horse Sickness
ATR	Anamalai Tiger Reserve
AZP	Amirthi Zoological Park
BG	Biogents
BLAST	Basic Local Alignment Search Tool
Bp	base pair
BTV	Bluetongue virus
CCFV	Crimean-Congo fever virus
CDC	Centre for Disease Control
CDC-LT	Centre for Disease Control Light trap
CO_2	Carbon dioxide
COX	Cytochrome c oxidase I
DNA	Deoxyribose Nucleic Acid
EDTA	Ethylenediaminetetraacetic acid
EHD	Epizootic Hemorrhagic Disease
gm	gram
h	hour
ha	hectare
HKY	Hasegawa-Kishino-Yano
ICAR	Indian Council of Agricultural Research
ICMR	Indian Council of Medical Research
IVRI	Indian Veterinary Research Institute
KEC	Kozhikamuthi Elephant Camp
KFD	Kyasanur Forest Disease
KFDV	Kyasanur Forest Disease Virus
KZP	Kurumbapatti Zoological Park
M	Molarity
MEGA	Molecular Evolutionary Genetics Analysis
mg	milligram
min	minute
mA	milliampere
mL	millilitre
mm	millimetre
MSA	Multiple Sequence Alignment
MTR	Mudumalai Tiger Reserve
NCBI	National Center for Biotechnology Information
NICD	National Institute of Communicable Diseases
NIL	Nothing/Zero
OD	Optical Density
PCR	Polymerase Chain Reaction
pM	picomole
R4	Radius vein 4 in insect wing venation
R5	Radius vein 5 in insect wing venation
rRNA	Ribosomal Ribonucleic Acid
sp.	species
spp.	multiple species
Sec	second
TAE	Tris Acetic EDTA
TEC	Theppakadu Elephant Camp
TN92	Tamura Nei-1992
UV	Ultra Violet
V	Volt
virB9	Virulence B9
VSG	Variant Surface Glycoprotein
µl	Microlitre
18s	small subunit of eukaryotic ribosomes
%	Percentage
°C	Degree Celsius

CONTENT

Sl. No	Title	Page No.
1	Abstract	2
2	Introduction	4
3	Objectives	9
4	Review of Literature	10
5	Materials and Methods	16
6	Results	30
7	Discussion	46
8	Conclusion	49
9	Summary	50
	References	52

Project Report

Title of the Project:	**'EVALUATION OF DIFFERENT TRAP MODELS TO MONITOR AND CONTROL HAEMATOPHAGOUS INSECTS WHICH TRANSMIT DISEASES TO CAPTIVE ANIMALS IN ZOOLOGICAL PARKS'**
Project Category:	Annual Plan of Operations (APO) - 2023-24
Project Period	10 months (May 2024 – March 2025)
Implementing Institute and Centre	Centre for Animal Care Sciences, Advanced Institute for Wildlife Conservation, Vandalur – 600 048.
Research Team	Dr M. Gabriel Paulraj, Project Scientist, Ms. K. Yuvasri, Project Associate
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1. ABSTRACT

Haematophagous insects are blood-feeding insects belonging to the Phylum Arthropoda and Class Insecta. Haematophagy or blood-feeding behaviour is found in a wide variety of arthropods and insects. Mosquitoes, fleas, lice, biting midges, tabanid flies (horse flies and deer flies) and ticks have evolved specialized mouthparts to pierce the skin and suck blood from vertebrate hosts for their development and reproduction.

The haematophagous insects and ticks have significant negative effects on humans, domestic animals and wild animals. They transmit many diseases to the host animals while sucking blood and thus act as vectors. Captive animals in zoological parks are particularly vulnerable to vector-borne diseases due to their living conditions within a confined environment. Avian malaria, babesiosis, trypanosomiasis, and leishmaniasis are common vector-borne diseases of captive animals which may lead to chronic stress, anemia, allergic reactions, and, in severe cases, mortality. Moreover, captive animals can act as reservoirs, transmitting these diseases to humans.

Several incidents of captive animal deaths in zoological parks due to vector-borne diseases have been reported both in India and abroad. The prevalence of vector-borne diseases among captive wildlife populations has been studied by several investigators around the world.

Ticks and lice are obligate ectoparasites and generally considered more harmful than facultative ectoparasitic arthropods like mosquitoes, biting midges and tabanids. Ticks are grouped into two main families: the Ixodidae (hard ticks) and Argasidae (soft ticks). There are nearly 1000 tick species in the world and all tick species are haematophagous.

A study was conducted from May 2024 to March 2025 to document haematophagous insect and tick species of captive animals. Insects and ticks

were collected from five different places of Tamil Nadu viz., Arignar Anna Zoological Park (AAZP) in Vandalur, Kurumbapatti Zoological Park (KZP) in Salem, Amirthi Zoological Park (AZP) in Vellore, Theppakadu Elephant Camp (TEC) in Mudumalai Tiger Reserve (MTR) and Kozhikamuthi Elephant Camp (KEC) in Anamalai Tiger Reserve (ATR). Insects and ticks were collected by traps and hand-picking method. Five different traps namely CDC (Centre for Disease Control) light trap, Malaise trap, BG sentinel trap, horse fly trap and NZI trap were used. Ticks were also collected from a Pangolin that died in AAZP. The collected insects and ticks were preserved in 70% ethanol for morphological identification, DNA barcoding and molecular screening of parasites.

The present study showed that captive animals in the study areas serve as hosts for variety of blood-feeding insects, such as *Tabanus* spp., *Haematopota* spp., *Culicoides* spp. and two tick genera (*Haemaphysalis* and *Amblyomma*). Morphological identification of the genera was further confirmed through DNA barcoding method. Results on molecular screening of parasites in the vectors showed that two specimens of *Haematopota* sp., one *Tabanus* sp. and one tick species (*Haemaphysalis* sp.) were positive for *Trypanosoma* and *Hepatozoon.*

Among the trapping methods used, the CDC light trap was found to be the best device for capturing *Culicoides* spp., mosquitoes and other biting midges. Malaise trap, being a generalist trap, successfully collected a wide range of insects including haematophagous insects.

Keywords: captive animals, blood-feeding insects, protozoan parasites, *Trypanosoma* spp., *Babesia* sp., vector-borne disease, ticks

2. INTRODUCTION

Vector-borne diseases are a major cause of death in both humans and animals. Domesticated animals, wild animals and animals kept in zoological parks/ captivity are exposed to a wide range of pathogens, putting their health and lives at risk. Blood-feeding insects (haematophagous insects) and other arthropods, like ticks, play an important role in transmitting pathogens between animals. These disease-spreading organisms (vectors) can cause large-scale damage to livestock, wildlife and humans. Mosquitoes, fleas, sand flies, lice, ticks and mites, horse flies, deer flies and stable flies are major arthropod vectors which transmit pathogens between animals and also between animals and humans during blood feeding. For example, mosquitoes are known to transmit avian malaria, and sand flies are responsible for the transmission of leishmaniasis to animals.

Some blood feeding insects like Triatomine bugs feed by piercing the host's skin by their sharp mouth parts and directly accessing the blood vessels. In contrast, small insects like biting midges feed by forming a pool of blood on the skin's surface and then sucking from it (Fig. 1).

Zoological parks house a diverse range of wild animals and birds for education, research and conservation purposes. These captive animals, however, are increasingly becoming the focus of scientific research on vector-transmitting diseases. Captive animals in zoological parks may also serve as reservoirs of infections for both humans and other animals (Chomel *et al.,* 2007). Leishmaniasis in animals is caused by the protozoan parasite of the genus Leishmania (Fig. 2), which is transmitted through the bites of female phlebotomine sand flies. *Leishmania infantum* infection has been recently reported in tigers in a wildlife safari park in southern Italy, demonstrating the risk of zoonotic parasite transmission (Iatta *et al.*, 2020).

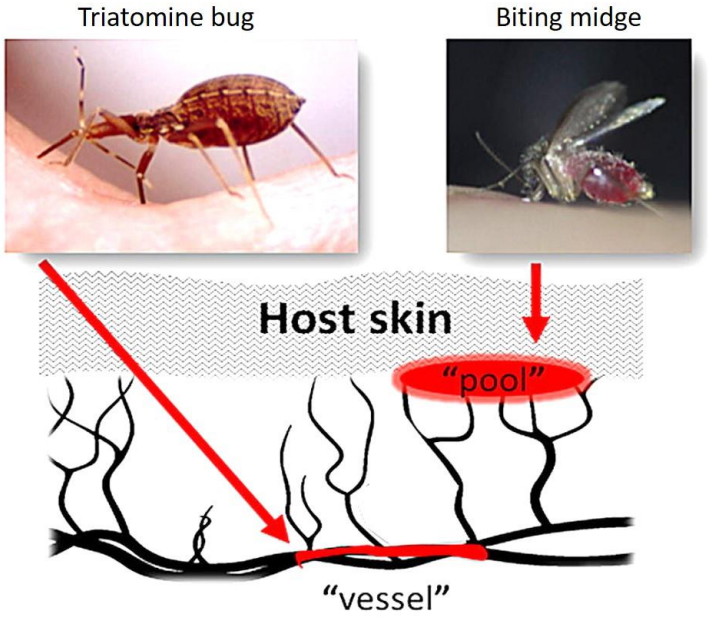


Figure 1. Feeding mechanism in haematophagous insects (Image credit: Sant'Anna *et al.*, 2017)

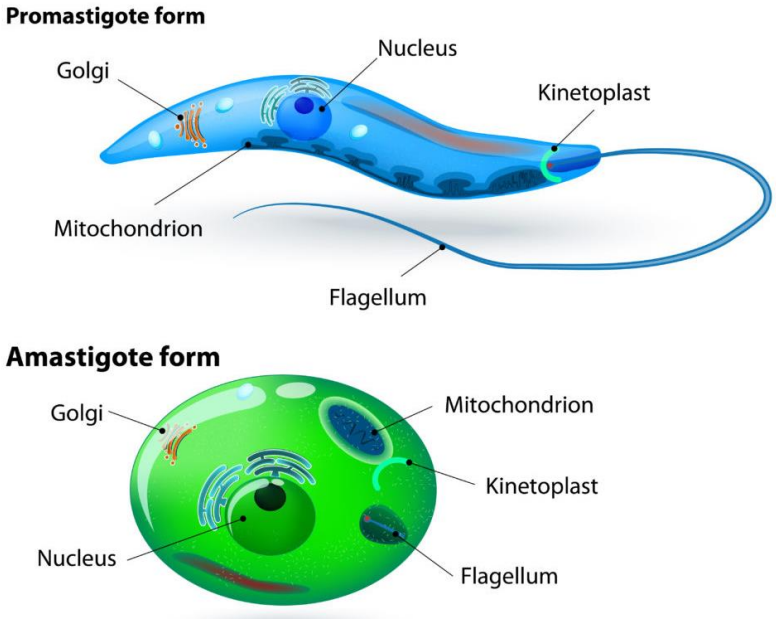


Figure 2. *Leishmania* parasite. Two forms of *Leishmania* cell during the life cycle (Image credit: Leish@York website)

Horse flies (Diptera: Tabanidae) and Stable flies (Diptera: Muscidae) are significant dipteran hematophagous insects with considerable medical and veterinary importance. Female horse flies act as vectors for different pathogens, including *Trypanosoma theileri*, *Trypanosoma evansi*, *Bacillus anthracis*, *Clostridium chauvoei* and *Pasteurella multocida,* which affect both wild and domestic animals (Baldacchino *et al.*, 2014). Their bites can induce considerable stress to the animals and indirectly lead to secondary infections, allergic reactions and anaemia due to blood loss. In India, *Trypanosoma* (Fig. 3) is primarily transmitted by Tabanids (horse flies) and *Stomoxys* (stable flies).

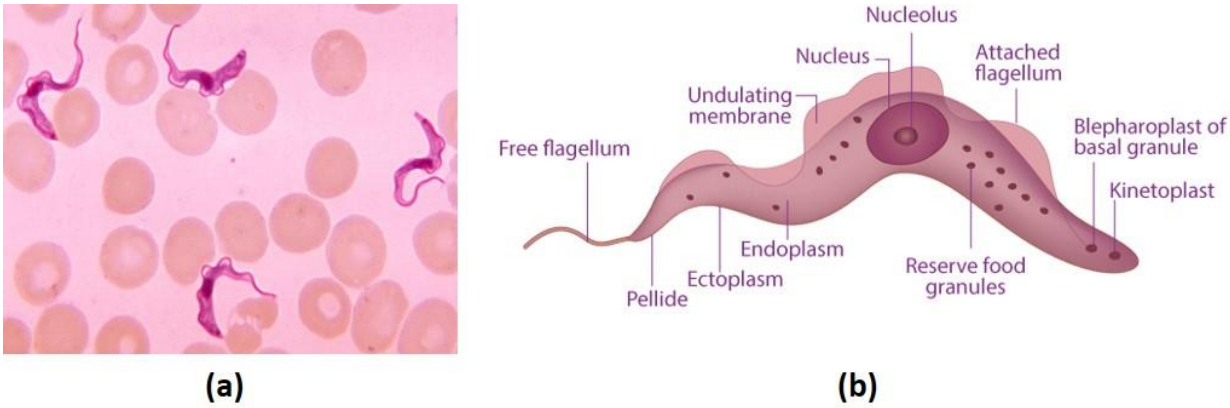


Figure 3. (a) *Trypanosoma* in blood smear and (b) *Trypanosoma*'s cell structure (Image credit: (a): Wikipedia and (b): https://byjus.com/biology/trypanosoma-diagram/)

Stable fly, *Stomoxys calcitrans* is another significant blood sucking fly. Stable flies suck blood from mammals and heavy infestation lead to anaemic conditions and reduced milk production in cows. Stable flies are carriers of trypanosomatid parasites and *Bacillus anthracis*, the causative agent of anthrax (Turell & Knudson, 1987). Studies showed that Stable fly infestation may lead to a 19% reduction in animal body weight gain and a 40–60% reduction in milk production (Campbell *et al.*, 2001). *S. calcitrans* bites also cause skin lesions like exudative dermatitis on the legs of horses, necrotic dermatitis at the tips of dogs' ears and in the "hair whirlpools" on the back of

calves (Yeruham & Braverman, 1995). Some investigators have detected *Hepatozoon felis* (Pawar *et al.,* 2012), *Mycoplasma haemofelis* (Haefner *et al.,* 2003) and *Toxoplasma gondii* (Dorny & Fransen, 1989; Goodrich *et al.*, 2012) in tigers using molecular, parasitological and serological tests, respectively. Protozoan and bacterial vector-borne diseases are very common in captive wild animals worldwide.

In 2000, the Nandankanan Zoological Park in India experienced the loss of 12 tigers due to Trypanosomiasis. A subsequent survey in the Nandankanan Zoo revealed the presence of 11 species of blood sucking flies and one species of hard tick in and around the tiger and deer enclosures, all of which were identified as potential vectors of trypanosome. This highlights the critical importance of surveilling vector insects and arthropods within zoological parks for the early detection and effective management of vector-borne diseases in captive animals.

Trapping systems serve as effective and practical tools for arthropod vector surveillance and control. Numerous studies have evaluated the efficacy of traps as a component of integrated vector management studies. Traps for horse flies are designed to attract the flies using sensory cues such as visual stimuli and/or a natural or synthetic odour.

Traps baited with fermented cow urine were found to be 2.5 times more effective in trapping flies compared to unbaited traps (Metri *et al.*, 2022). Similarly, the chemical compound 1-Octen-3-ol has been identified as a potential attractant, significantly increasing the capture rate of stable flies (*Stomoxys calcitrans*) by up to 3.7-fold at dosages ranging from 0.2-2.0 mg/h (Mihok *et al.*, 1995).

In addition, Mushroom extracts possess attractant properties against hematophagous insects. Chaiphongpachara *et al.* (2018) assessed the efficacy of ethanol extracts of five mushroom species viz., *Pleurotusostreatus*, *P. kumm*, *Thaeogyroporus porentosus*, *Volvariella volvacea*, *Pleurotussajor-caju* and

Lentinus edodes, against mosquitoes. *L. edodes* was found to be the most effective attractant against mosquitoes

A detailed study on the diversity of hematophagous insects within zoological parks across Tamil Nadu, conducted at different seasons, is critically warranted to enhance vector surveillance and disease prevention strategies. In response to this need, the present study was undertaken. Three zoological parks, namely Arignar Anna Zoological Park (AAZP) (Vandalur), Kurumbapatti Zoological Park (Salem) and Amirthi Zoological Park (Vellore), were selected as study places. Besides these Zoological Parks, insect sampling was also done in elephant camps at Theppakadu, Mudumalai Tiger Reserve (MTR) and Kozhikamuthi, Anamalai Tiger Reserve (ATR).

3. OBJECTIVES

1) To evaluate the attractant property of selected semiochemicals, cow urine and carbon dioxide (CO_2) gas individually and their blends against hematophagous insects in Arignar Anna Zoological Park (AAZP), Kurumbapatti Zoological Park (KZP) and in Theppakadu Elephant camp, Mudumalai.
2) To study the species composition, seasonal abundance and dominant species of hematophagous insects in both AAZP and KZP.
3) To study the parasitic load of *Trypanosoma* spp. in the hematophagous insects.
4) To identify the parasite species by PCR-based molecular technique
5) To develop a significant control measure for the eradication/management of hematophagous insects inside zoological parks.

4. REVIEW OF LITERATURE

4.1. International Status

Wild animals, both free-ranging and captive, are susceptible to several infectious diseases caused by viruses, bacteria, protozoa, nematodes and fungi (Ostrowski & Gilbert, 2016; Otranto and Deplazes, 2019; Rondón *et al.*, 2021; Laurie *et al.*, 2024). Many diseases are transmitted by insects, ticks and mites while they are feeding on the blood of the host animal. These blood-feeding insects (haematophagous insects) pose a significant threat to captive animals in zoos, rehabilitation centres, and wildlife centres (Castillo *et al.,* 2024). These infections can cause serious health issues in animals, leading to chronic stress and sometimes death. Moreover, infected animals can serve as reservoirs and transmitters of zoonotic pathogens, posing serious risks to human health. It has been reported that mosquitoes cause malaria in primates and ungulates (Miller *et al.*, 2013).

Tick-borne pathogens, such as *Babesia* and *Borrelia* species, have been documented in wild ungulates in captivity (Moss *et al.,* 2009). Tsetse flies and horseflies are blood-feeding insects that transmit *Trypanosoma theileri, Trypanosoma evansi*, *Bacillus anthracis*, *Clostridium chauvoei*, and *Pasteurella multocida* in both wild and domesticated animals (Baldacchino *et al.*, 2014). These insects not only transmit infectious agents but also cause allergy, anaemia, and stress in both wild and captive animals. Specifically, tsetse flies are responsible for the transmission of *Trypanosoma brucei* in captive African ungulates (Hughes *et al.*, 2011). Furthermore, leishmaniasis has been reported in both mammals and birds, primarily transmitted by infected sandflies (Chauhan *et al.*, 2014)

Hughes *et al.* (2011) discussed the prevalence of *Trypanosoma brucei* in captive African ungulates exposed to tsetse flies within enclosures. Similarly, Chauhan *et al.* (2014) documented cases of sandfly-transmitted leishmaniasis

in captive wildlife species, affecting both mammals and birds. The tick infestations in captive deer cause blood loss, as reported by Gonzalez *et al.* (2017). Stable flies are vectors of trypanosomatid parasites and *Bacillus anthracis*, the causative agent of anthrax (Turell & Knudson, 1987). *Hepatozoon felis* (Pawar *et al.,* 2012), *Mycoplasma haemofelis* (Haefner *et al.,* 2003), and *Toxoplasma gondii* (Dorny & Fransen, 1989; Goodrich *et al.,* 2012) were identified in captive tigers using molecular methods.

Culicoides spp. are small insects that affect captive and wild animals, leading to various health issues. These midges are prominent vectors of several viral diseases, including Bluetongue virus (BTV)**,** Epizootic Hemorrhagic Disease (EHD)**,** and African Horse Sickness (AHS) in wild animals, mostly in deer species. Clinical manifestations of these infections typically include fever, swelling, and severe lesions in the oral cavity.

Some mosquito species have been linked to the transmission of malaria across a range of vertebrate hosts, including primates, ungulates and birds. Members of the Tabanidae family, which comprises various species of blood-feeding flies, are responsible for the transmission of infectious pathogens causing Trypanosomiasis**,** Anaplasmosis**,** and Equine Infectious Anaemia. Furthermore, Tabanid species transmit *Leptospira* spp., the causative organism of leptospirosis, as well as the pathogens responsible for Bovine Viral Diarrhoea in wild populations.

The eradication of arthropod vectors in and around the closed enclosures of captive wild animals requires proper vector-borne disease management measures. Vector sampling is a critical step for early detection and developing effective vector control strategies (Álvaro Eduardo Eiras *et al.,* 2021). Trapping systems play a crucial role in the surveillance and control of arthropod vectors, and researchers have extensively studied their effectiveness as a control strategy.

Horse fly traps are designed to utilize sensory cues such as visual stimuli and natural or synthetic odours to attract flies. Studies have shown that traps baited with fermented cow urine are 2.5 times more effective than unbaited traps (Metri *et al.,* 2022). Similarly, the chemical compound 1-Octen-3-ol has been identified as a strong attractant, increasing stable fly captures by up to 3.7 times at doses of 0.2–2.0 mg/h (Mihok *et al.,* 1995). Additionally, mushroom extracts contain compounds that attract blood-feeding insects. The attractant properties of ethanol extracts from five mushroom species *Pleurotus ostreatus, P. kumm*, *Thaeogyroporus porentosus*, *Volvariella volvacea*, *Pleurotus sajor-caju*, and *Lentinus edodes* were tested against mosquitoes, with *L. edodes* proving to be the most effective (Chaiphongpachara *et al.,* 2018).

Details of some important traps used for vector surveillance and their control are given below:

Light trap: Light traps operate at night and are most efficient with minimal light pollution. The advantages of the light trap are that they are portable and easily powered by a 6-volt battery (Jolyon Medlock *et al.,* 2018). Insects attracted by CDC Light Trap are mainly mosquitoes (Culicidae), biting midges (Ceratopogonidae), sandflies (Psychodidae: Phlebotominae), black flies (Simuliidae) and other dipterans.

Sticky traps: This is the simplest trap which has a colored adhesive board– typically yellow, blue or black – that attracts and captures flies upon contact. Various sticky-trap materials are commercially available, and some traps are additionally equipped with attractants such as lights (Gibb *et al.*, 2020). Blue, black, and yellow are most attractive for hematophagous insects.

Malaise trap: Malaise traps are useful for capturing different types of flying insects and have been employed in ecological studies (DeWalt & Stewart, 1995; Griffith *et al.*, 1998). Malaise traps are tent-like structures made of fine mesh material and are used primarily for the collection of dipteran flies and wasps (Hymenoptera), although they also catch a great many other flying insects.

***NZI trap*:** The NZI trap is specifically designed to capture biting flies, especially haematophagous flies like tsetse flies (*Glossina* spp.) and stable flies (*Stomoxys* spp.). It was developed by Vale (1993). It has a blue and black fabric structure, as these colours are known to be more attractive to biting flies.

***Drag cloth*:** This is a simple yet effective trap used to collect ticks from grasslands and small shrubs. A drag cloth is made from durable, light-coloured cloth attached to a piece of doweling along one edge and can be dragged through long grass or small shrubs to collect ticks (Gibb *et al.*, 2020).

BG-Sentinel trap: The BG-Sentinel trap is a widely used mosquito trap designed to attract and capture mosquito species like *Aedes aegypti* and *Aedes albopictus*, which are vectors of diseases, including dengue, Zika, and chikungunya. This trap uses visual and olfactory cues to attract mosquitoes and is highly effective in urban and peri-urban environments.

4.2. National Status

Our knowledge of scientific studies on blood-sucking arthropods of India dates back to the naturalist, Carolus Linnaeus, who was considered as the first to report on Indian ticks (1758). The next descriptive studies on Indian haematophages were by Rudow (1870) and Neumann (1879).

Further significant contributions were made in the beginning of the 19th century by some researchers like Nuttall (1904), Sharif (1924, 1928), Jeppner (1935), Sen (1938) and Kohls (1947) on the hematophagous arthropods of India. Rao and Kalra (1949) reported that the ticks *Ornithodoros lahoreensis* and *O. tholozani* were vectors of relapsing fever in Kashmir. It was further substantiated and reviewed by Philip (1952). Ansari (1951) contributed a comprehensive paper on *Phthirapteran* lice found on Indian mammals.

In 1957, the Kyasanur Forest Disease (KFD), one of the serious vector-borne diseases of man, was discovered in Shimoga district of Mysore (Work, 1958). These significant investigations driven further research activities in

India on vector ticks at four main Research Institutes, namely Indian Veterinary Research Institute (IVRI) (Izatnagar and Mukteswar), Virus Research Centre (now National Institute of Virology, Pune), Pusa Institute, Delhi, and National Institute of Communicable Diseases (NICD), New Delhi.

The intensive studies at the National Institute of Virology (Pune) since 1958 led to the identification of the etiological agent (KFD-Virus) of the aerosol diseases from a number of tick species viz., *Haemaphysalis spinigera*, *H. wellingtoni*, *H. turturis*, *H. kinneari*, *H. kyasanurensis*, *H. wellingtoni*, *H. bispinosa*, *H. minuta*, *H. cuspidata*; *Dermacentor auratus*; *Rhipicephalus sanguineus*; *Ixodes ceylonicus*; *I. petauristae*, and *Ornithodoros chiropterphilae* (Work, 1958; Trapido *et al.*, 1959; Boshell and Rao, 1963; and Singh *et al.*, 1964).

During this period, Krishnaswami (1965) reported 600 cases of human plague in the Kolar district of Mysore and later, the infection was detected in contiguous areas in Madras, Mysore, and Andhra Pradesh (Krishnaswami, 1965). The reappearance of plague coupled with the discovery of a new disease virus (Chikungunya virus) causing haemorrhagic dengue, along with sandfly fever in Calcutta, and enigmatic Japanese encephalitis, furthered and stimulated research on haematophagous arthropod vectors in India (Wattal, 1971).

These findings demanded collaborative efforts among virologists, medical entomologists, veterinarians, epidemiologists and zoologists to conduct extensive field surveys for the faunistic survey of poorly known haematophagous arthropods and their vector potential for protozoan, bacterial, viral, rickettsial and helminthic diseases in India.

Indian Council of Medical Research (ICMR) and Indian Council of Agricultural Research (ICAR) conducted major studies to explore haematophagous arthropods in India and their disease potential and the outcome of those studies were: 'Ectoparasites of goats' (Garg, 1961); 'A

faunistic survey of ticks in Delhi' (Nagar, 1962); 'Studies on ixodid ticks in Arunachal Pradesh' (Dhanda and Rao, 1964); 'An entomological survey of Doon valley' (Wattal & Tandan, 1965) and 'Research on ectoparasites of mammals in Kanha National Park, Madhya Pradesh' (Mitchell *et al.,* 1966).

Previous and current research activities on vector-borne diseases in India are mainly on humans and domestic animals. Research on vector-borne diseases, disease-causing agents like insects and ticks and their control measures concerning wild animals or captive animals are scanty in India. Given the importance of health monitoring in captive animals, it is essential to identify the vectors responsible for disease transmission in zoological parks. This foundational knowledge is crucial for developing effective control strategies. Hence, the present study was undertaken with this objective.

5. MATERIALS AND METHODS

5.1. Place of the study

Haematophagous insects and ticks were collected using various traps and manual collection method from five locations: Arignar Anna Zoological Park (AAZP) in Vandalur (coordinates: 12.87917°N; 80.08167°E) (Fig. 4a), Kurumbapatti Zoological Park (KZP) in Salem (coordinates: 11.744153°N; 78.170944°E) (Fig. 4b), Amirthi Zoological Park (AZP) in Vellore (12.732432°N; 79.056646°E) (Fig. 4c), Theppakadu Elephant Camp (TEC) in Mudumalai Tiger Reserve (11.579201°N; 76.584012°E) (Fig. 4d) and Kozhikamuthi Elephant Camp (KEC) in Anamalai Tiger Reserve (10.44442° N; 76.84914°E) (Fig. 4e). The map showing the locations of the study areas is given in Figure 5.

AAZP was established in 1855. It spreads over an area of 602 ha, including a 92.45 ha rescue and rehabilitation centre. AAZP houses more than 2,600 animals representing 171 species. KZP was established in 1981 in Salem District in Tamil Nadu. It spans an area of 31.73 ha and houses nearly 157 animals across 17 species. Amirthi Zoological Park in Vellore district was opened in 1967. The zoo covers an area of 25 ha and has waterfalls. It is a home to a variety of species including spotted deer, sambar deer, mongoose, porcupines, bonnet macaques, rose-ringed parakeets, budgerigars, star tortoises, peafowls, crocodiles, snakes including Indian Rock Python.

a) Arignar Anna Zoological Park, Vandalur, Tamil Nadu

b) Kurumbapatti Zoological Park, Salem, Tamil Nadu

c) Amirthi Zoological Park, Vellore, Tamil Nadu

d) Theppakadu Elephant Camp, Mudhumalai Tiger Reserve

e) Kozhikamuthi Elephant camp at Anamalai Tiger Reserve, Tamil Nadu

Figure 4. The study places: a) Arignar Anna Zoological Park in Vandalur; b) Kurumbapatti Zoological Park in Salem; c) Amirthi Zoological Park in Vellore; d) Theppakadu elephant camp in Mudumalai Tiger Reserve and e) Kozhikamuthi elephant camp in Anamalai Tiger Reserve

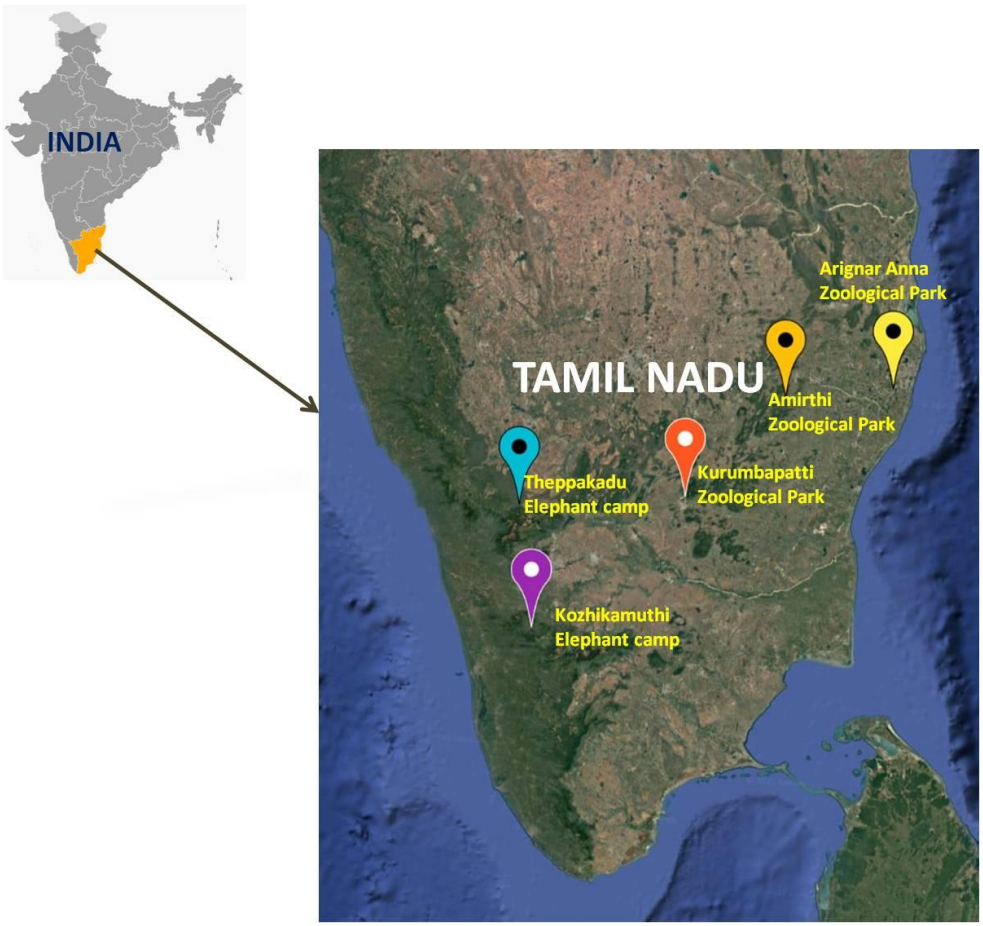


Figure 5. Map showing the places of insect collection in Tamil Nadu

Theppakadu elephant camp in Mudumalai was established in 1927, is one of the oldest elephant camps in India. At present, 26 elephants are maintained at Theppakadu and the nearby Abhayaranyam camp. Kozhikamuthi elephant camp in Anamalai Tiger Reserve is another important elephant camp in India. There are 28 elephants currently maintained at Kozhikamuthi and Varagalayar camps.

5.2. Period of the Study

A total of three field visits were conducted during this study period from May 2024 to March 2025. The first visit took place from October 4 to October 11, 2024, the second visit was conducted from December 11 to December 16, 2024, and the third visit from February 23 to February 28, 2025. During these field visits, different trap models were used to collect the haematophagous

insects. Additionally, flies, ticks, and lice were manually collected from the camp elephants.

5.3. Insect Collection

Traps: Traps are essential devices for collecting haematophagous insects. Different trap models were employed in the present study. Centers for Disease Control Light Trap (CDC-LT) and Biogents BG Pro trap are light-based traps equipped with UV lights (Fig. 6). These traps were installed during night hours to attract blood-feeding insects.

Biogents BG-Pro trap

CDC Light trap

Figure 6. Biogents BG-Pro trap and CDC light trap

Malaise trap and NZI traps were used to collect flying insects, including tabanid flies and deer flies (Fig. 7). Lures such as p-Cresol and 1-Octene -3-ol

were also tried along with malaise and NZI traps to evaluate their attractant properties towards haematophagous flies. Malaise traps, NZI trap and sticky trap were installed in the morning hours and insects were collected in the evening.

Figure 7. Malaise Trap installed in Abhayaranyam elephant camp at MTR

Manual collection of insects and ticks: Ticks, lice, *Haematopota* spp. and *Tabanus* spp. were manually collected from camp elephants (Fig. 8). Some ticks were collected from dead animals during post-mortem examinations at AAZP. High-risk areas such as ears, trunk folds, underbelly, tail base, and legs of the animals were examined, and the ticks and lice were collected.

Figure 8. Manual collection of ticks and lice from camp elephant (Place: Abhayaranyam, Mudumalai Tiger Reserve)

The attractant property of cow urine and chemicals: The attractant properties of two chemicals, p-cresol and 1-Octene -3-ol and cow urine were tested individually. A simple trap made by a plastic container was used for this study (Fig. 9). Each chemical (1 ml) was separately applied on small cotton swab taken in a 2 ml vial and kept inside the plastic container. The trap was monitored daily, and insects trapped inside the container were collected and stored.

Figure 9. Trap used for testing the efficacy of cow urine, p-cresol and 1-Octene -3-ol

5.4. Storage of Insect specimens

Collected insects were refrigerated at 4 °C for 30 min to immobilise them. The haematophagous insects were then separated from other insects, preserved in 70% ethanol and stored in the laboratory for species identification and molecular studies.

5.5. Morphological identification

Morphological identification of haematophagous insects relies on detailed examination of their external structures. The colour and size of the body, microscopic structures of head, antennae, legs, thorax, abdomen, wing

venation, patterns in the wing, structure and position of hairs/cilia on the body and number of segments in the antenna were carefully studied under a stereo zoom microscope (ZEISS Stemi 508, attached with 'Axiocam 208 color' camera) and a digital compound microscope (Magnus MX21iPRO). The morphological characteristics of the specimens were matched with existing identification keys for tabanids (Stone & Philip, 1974; Vasudeva *et al.*, 2007), *Culicoides* (Janke *et al.*, 2023; Wirth & Hubert, 1989; Archana *et al.*, 2016), ticks (Walker *et al.*, 2003; Training Module – ICMR-VCRC, 2022) and elephant lice (Lapage 1956; Bowman *et al.* 2003; Godara *et al.* 2009).

Photographs of the entire specimens, body parts and microscopic structures of body parts were taken.

***Culicoides* spp.**

Culicoides spp. are small dipteran flies belonging to the family Ceratopogonidae. Keys provided by many authors have been used for species identification (Atchley & Wirth, 1979). Species identification of *Culicoides* can be done using some key morphological features. Size ranges from 1 to 4 mm in length; shape of the body is slender and elongated; humped appearance due to the shape of the thorax; colour is light brown to dark brown or black; wings are usually clear or slightly smoky and have a characteristic pattern of veins. The presence of a specific pattern of wing veins is a key identification feature. The wing length is usually 2 to 3 times the length of the body; the mouth parts are long, slender, and adapted for piercing the skin and feeding on blood of the host. The proboscis is typically longer than the head; antennae are segmented and relatively long, with 13 segments in male and 14 in female, presence of distinct sensory structures, antenna of male is bushy or plumose and female antenna is more slender; legs are long and slender, with distinct segments, the tarsi may have species-specific characteristics; shape and size of the male genitalia are species-specific (Janke *et al.,* 2023; Wirth & Hubert, 1989; Archana *et al.,* 2016).

***Tabanids* (*Tabanus* spp. and *Haematopota* spp.)**

***Tabanus* spp.:** *Tabanus* spp are larger flies with stout body. The eyes are often brilliantly colored (green, purple and blue) in live specimens with horizontal bands or iridescent patterns, eyes are separated in females, eyes are holoptic (touching) in males; frontal callus in frons (forehead) is rectangular or triangular in shape; antenna has 3 segments, basal segments are short, the third segment (flagellum) is elongated and often annulated or notched; thorax is usually dark brown or gray with longitudinal stripes; arrangement of bristles (setation) may vary; abdomen is banded, spotted, or uniformly colored and broad/flat or tapered in shape; wings are usually clear, may have infuscation (dark areas) or banding in some species; configuration of wing vein R4 and R5 are also a distinguishing characteristic (Stone & Philip, 1974; Vasudeva *et al.*, 2007).

***Haematopota* spp.:** Key morphological characteristics of *Haematopota* spp. were studied based on the identification keys by Chvála *et al.* (1972). Wings show a distinctive mottled (marbled) appearance, distribution of light and dark spots in the wing (wing pattern), forming a species-specific wing pattern. Antenna has 3 segments – the first two basal segments are short and the third segment is annulated (ringed); eyes are often brilliantly colored with bands of green, purple and red colours in live specimens; thorax usually grayish with darker longitudinal stripes; abdomen has conspicuous patterns, especially in females.

Ticks: Ticks were identified to species level by studying their morphological characteristics. The colour and shape of the body, scutum colour and ornamentation, structure of the mouth parts, presence or absence of eyes, structure of capitulum and basis capitulum, presence or absence of marginal grooves in the body, position of the groove around the anus, structure of spiracular plates, etc., were used for the identification of the genus and species of ticks (Walker *et al.,* 2003; Training Module – ICMR-VCRC, 2022).

5.6. DNA barcoding

Extraction of DNA

Using a commercial DNA extraction kit (Qiagen DNeasy blood and tissue extraction kit), genomic DNA was extracted from the legs of larger insects and from the whole specimen for the smaller insects. The concentration and purity of the extracted DNA were assessed using a Nanodrop 2000 spectrophotometer by measuring the absorbance at 260/280 nm. The extracted DNA was stored in -20 °C for further molecular analysis.

Polymerase Chain Reaction (PCR)

Reconstitution and Dilution of Primer: For DNA barcoding, Folmer's primers (LCO1490 and HCO2198) were used to amplify the Cytochrome oxidase C subunit 1 partial gene (Table 1). The lyophilized primer was reconstituted in molecular biology-grade nuclease-free water to obtain a stock concentration of 200 pM/µL, and the tubes were then briefly vortexed. The stock solution was aliquoted into 10 µL portions and kept at -20 °C until further use. The working solution was prepared just before PCR by mixing one microlitre of stock solution with nine microlitre of molecular biology grade nuclease-free water to achieve a concentration of 10 pM/µL.

Table 1. Primers used for Species identification**.**

Primers	Sequence	Amplicon size	Reference
LCO1490	5'GGTCAACAAATCATAAAGATATTGG 3'	700 bp	Folmer *et al.* (1994)
HCO2198	5'TAAACTTCAGGGTGACCAAAAAATCA 3'		

Optimization of polymerase chain reaction conditions: The optimization of PCR conditions was achieved through gradient PCR (Thermal cycler). Modifications in different time-temperature combinations were used for annealing and extension steps. The temperature gradient that yielded the best amplification results was selected for all downstream use. The reaction was

carried out in 0.2 mL PCR tubes, and the PCR reaction mix details are provided in Table 2. A negative control was prepared using nuclease-free water. PCR was performed in the thermal cycler (ProFlex PCR System, Thermo Fisher Scientific) using the programme described in Table 3.

Table 2. Optimized concentration of PCR reagents for the amplification

Sl. No.	Constituents	Volume (µL)
1	Template DNA	1.5
2	Forward primer (10 pM/µL)	0.5
3	Reverse primer (10pM/µL)	0.5
4	Master mix	12.5
5	Nuclease-free water	10
Total		25

Table 3. PCR protocol for amplification of *COX 1* gene

Step		Temperature (° C)	Time
Initial denaturation		95	3 min
38 cycles	Denaturation	94	45 sec
	Annealing	55.7	40 sec
	Extension	72	40 sec
Final extension		72	10 min

Agarose gel electrophoresis

242 gm of Tris base, 57.1 mL of glacial acetic acid, and 100 mL of 0.5 M EDTA (pH 8.0) were dissolved in one liter of triple-distilled water to create Tris Acetic EDTA (TAE) buffer (50X) with a pH of 8.2. The stock solution was diluted to 1X before use. A 100 bp DNA ladder, 2% agarose, and Novel Juice were used.

Method: The PCR result was identified using a 2% agarose gel in TAE buffer (1X). The agarose was dissolved by heating the mixture. The comb was held in place, and the casting tray was fastened before the agarose was poured. After the gel solidified, the gel tray was placed in the electrophoresis tank. A

significant amount of buffer (1X TAE) has been added to the tank. The PCR products of samples, positive control, negative control, and 100 bp DNA ladder along with novel juice were all loaded into the appropriate wells. Electrophoresis was performed at 100 V and 400 mA until the dye had travelled two-thirds of the length of the gel. Then the gel was visualized using a UV transilluminator, and the outcomes were recorded using a gel documentation system.

Gel Extraction and Purification of PCR Products: All successfully amplified PCR products were chosen for purification. A clean scalpel was used to excise the specific band from the agarose gel. The DNA fragments were then purified using the QIAquick Gel Purification Kit, following the manufacturer's instructions. The eluted product was confirmed via agarose gel electrophoresis to confirm the presence and quality of the desired fragment.

Sanger Sequencing

Purified PCR products were sent for Sanger dideoxy nucleotide sequencing using Applied Biosystems 3500 Series Genetic Analyzer.

Species Identification via BLAST Analysis

After obtaining the raw sequencing data, the reads were assembled to construct a contig, which was then analyzed using the Basic Local Alignment Search Tool (BLAST) for sequence similarity search, leading to species identification.

5.7. Phylogenetic analysis

For phylogenetic analysis, sequences of closely related insect species were selected from GenBank, National Center for Biotechnology Information (NCBI). The obtained sequences were aligned with downloaded sequences using the Clustal W programme of MEGA X software. The maximum likelihood method was used to interpret evolutionary history with 1000 bootstrap replications.

5.8. Parasite Screening

Parasite screening using PCR is a sensitive and specific molecular method to detect and identify parasites. DNA was extracted from the vector insects using commercial kits (Qiagen DNeasy Blood & Tissue Kit). DNA quality and concentration were assessed using a spectrophotometer. Specific primers targeting parasite DNA (species-specific genes) were used for amplification (Tables 4-7).

Table 4. Primers used for amplification of the partial 18s rRNA gene of *Babesia* spp.

Primers	Sequence	Amplicon size	Reference
Ba103F	5' CCAATCCTGACACAGGGAGGTAGTGACA 3'	619 bp	Kledmanee *et al.* (2009)
Ba721R	5'CCCCAGAACCCAAAGACTTTGATTTCTCT CAAG 3'		

Table 5. Primers used for amplification of the full-length 18S rRNA gene of *Trypanosoma*

Primers	Sequence	Amplicon size	Reference
1127F	5′-AGGCATTCTTCAAGGATACCTTCC-3′	836 bp	Kostygov *et al.* (2022)
1958R	5′-TGATGAGCTGCGCCTACGAGA-3′		

Table 6. Primers used for amplification of the partial VSG gene of *Trypanosoma evansi*

Primers	Sequence	Amplicon size	Reference
TevF	5' TGCAGACGACCTGACGCTACT 3'	227 bp	Ravindran *et al.* (2008)
TevR	5' CTCCTAGAAGCTTCGGTGTCCT 3'		

Table 7. Primers used for amplification of the partial virB9 gene of *Ehrlichia canis*

Primers	Sequence	Amplicon size	Reference
Ehr1401F	5'CCATAAGCATAGCTGATAACCCTGT TACAA 3'	377 bp	Kledmanee *et al.* (2009)
Ehr1780R	5"TGGATAATAAAACCGTACTATGTAT GCTAG 3'		

Optimization of polymerase chain reaction conditions: The details of reaction mix for the PCR is given in Table 8. After mixing the ingredients, the tubes were placed in an automatic thermal cycler for amplification. The amplification protocols are given in Tables 9-12.

Table 8. Optimized concentration of polymerase chain reaction reagents for the amplification

Sl. No.	Constituents	Volume (µL)
1	Template DNA	1.5
2	Forward primer (10 pM/µL)	0.5
3	Reverse primer (10 pM/µL)	0.5
4	Master mix	12.5
5	Nuclease free water	10
	Total	25

Table 9. PCR protocol for Partial 18S rRNA gene of *Babesia* spp.

Step		Temperature (° C)	Time
Initial denaturation		95	3 min
30 cycles	Denaturation	94	45 sec
	Annealing	60	40 sec
	Extension	72	40 sec
Final Extension		72	10 min

Table 10. PCR protocol for the full-length 18S rRNA gene of *Trypanosoma*

Step		**Temperature (°C)**	**Time**
Initial denaturation		95	5 min
30 cycles	Denaturation	95	30 sec
	Annealing	55	1 min
	Extension	72	1 min
Final Extension		72	10 min

Table 11. PCR protocol for partial virB9 gene of *Ehrlichia canis*

Step		**Temperature (°C)**	**Time**
Initial denaturation		95	3 min
30 cycles	Denaturation	94	45 sec
	Annealing	60	40 sec
	Extension	72	40 sec
Final Extension		72	10 min

Table 12. PCR protocol for partial VSG gene of *Trypanosoma evansi*

Step		**Temperature (°C)**	**Time**
Initial denaturation		95	3 min
30 cycles	Denaturation	94	45 sec
	Annealing	60	40 sec
	Extension	72	40 sec
Final Extension		72	10 min

6. RESULTS

6.1. Collected Insects

Three field visits were conducted from June 2024 to March 2025 to collect haematophagous insects from Theppakadu, Topslip, Kurumbapatti Zoo and Amirthi Zoo. Haematophagous insects were also collected from Arignar Anna Zoological Park and Rescue Centre, Vandalur during 10 different occasions. Four different traps viz., CDC light trap, BG sentinel trap, Malaise trap and NZI trap were tested for their effectiveness in trapping haematophagous insects.

Insects were also manually collected from camp elephants whenever they were observed actively feeding on their blood. Six different groups of blood-feeding arthropods, including five insect groups were collected during the study period. They were: mosquitoes, *Culicoides* spp., *Tabanus* spp., *Haematopota* spp., elephant lice and ticks. The collected specimens were identified up to genus level.

6.2. Predominant insect/arthropod species

Based on the direct sightings, it was clear that *Tabanus* spp., *Haematopota* spp. and mosquitoes were predominant blood sucking insects in elephant camps and zoological parks. *Tabanus* and *Haematopota*, both belonging to the family Tabanidae, approach the camp elephants in the morning and evening hours, mainly during bathing time. Three different species of *Tabanus* were caught in the present study. Figures 10 and 11 show two different *Tabanus* spp. which were collected from Abhayaranyam elephant camp, Mudumalai. *Haematopota* spp. were also found to be the most common blood-feeding insect of camp elephants (Fig. 12 & 13). They were mainly active during the evening hours.

Figure 10. *Tabanus* sp.1: Dorsal view (a), ventral view (b), side view (c) and wing structure and venation (d)

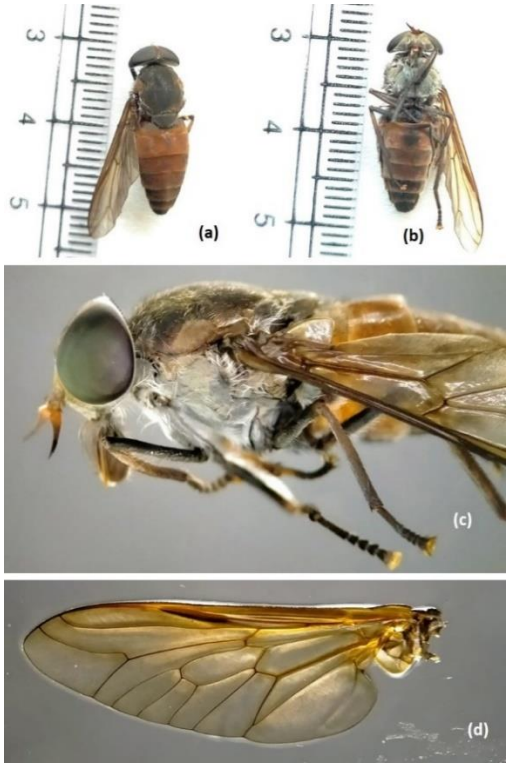


Figure 11. *Tabanus* sp.2: Dorsal view (a), ventral view (b), side view (c) and wing structure and venation (d)

Figure 12. *Haematopota* flies biting a camp elephant (indicated by arrow marks) and the enlarged flies are shown inside the circle

Figure 13. *Haematopota* sp.: Whole insect (a), wing venation and patterns in the wing (b), head, thorax and abdomen (c), head with antennae dorsal view (d) and antenna structure (e)

Culicoides spp., the tiny blood-feeding insects, were not directly sighted on animals and were caught by light traps such as CDC light trap and BG sentinel trap (Fig. 14-15).

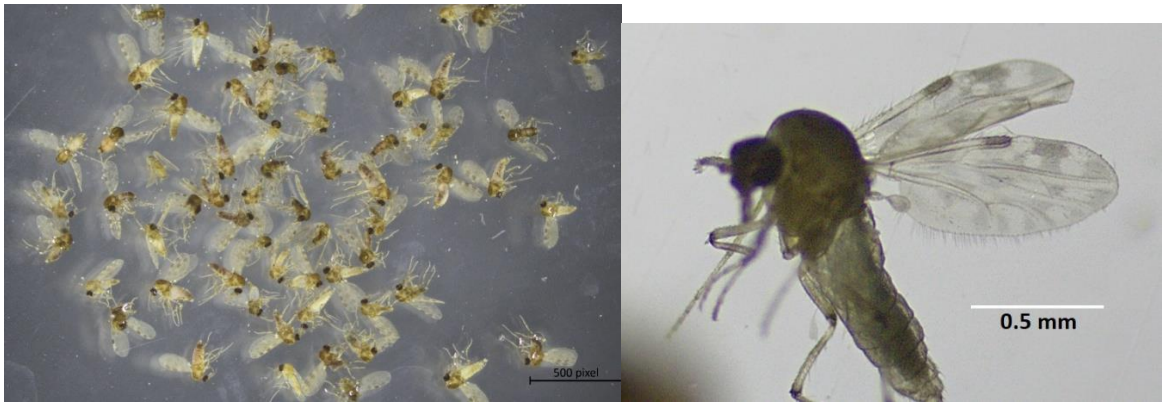


Figure 14. *Culicoides* sp. collected from AAZP by CDC light trap

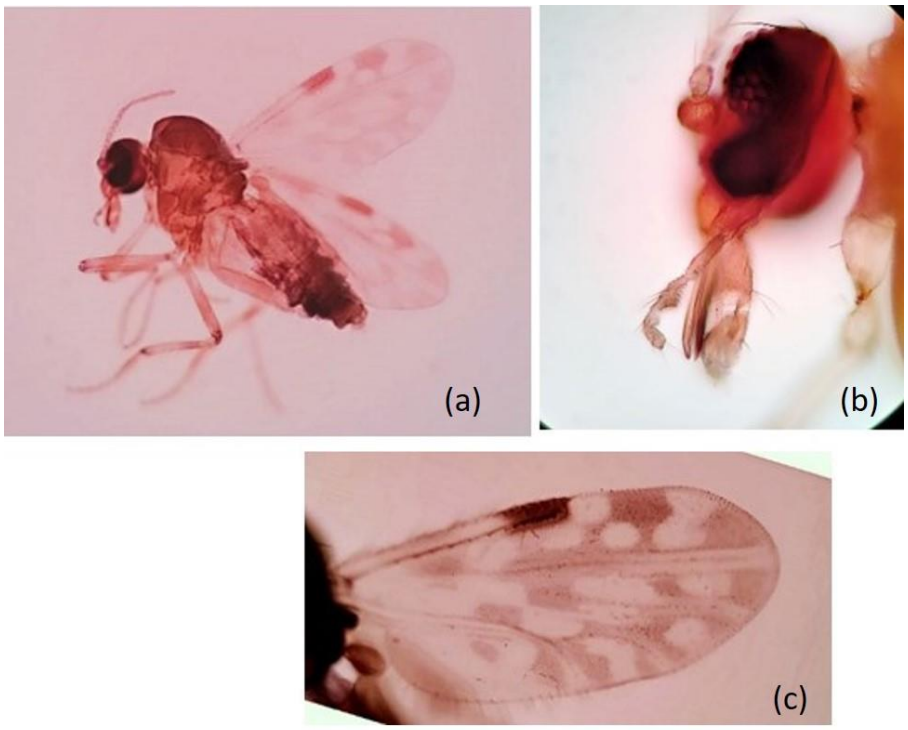


Figure 15. *Culicoides* whole specimen (a), head and mouthparts (b) and wing pattern (c)

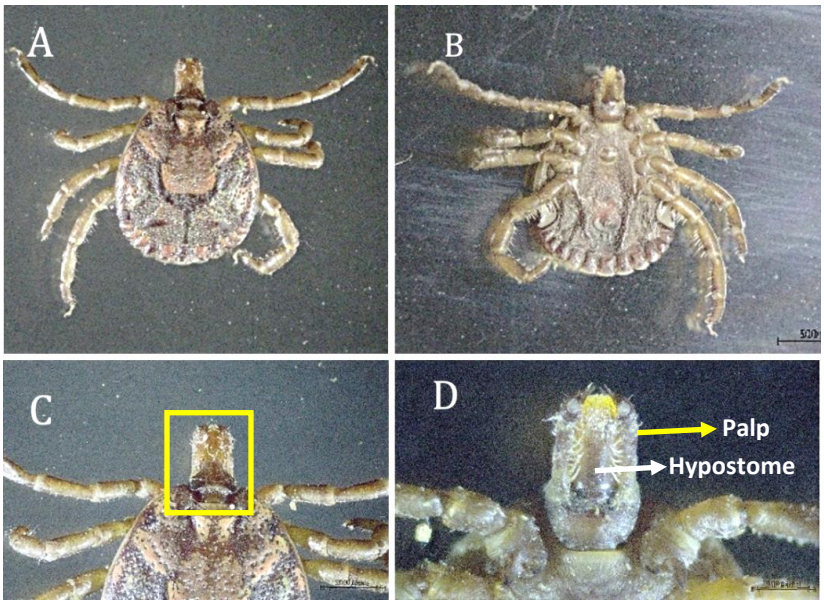
Among the six different arthropods, *Culicoides* were found to be the most active haematophagous insects throughout the study period and their activity was successfully monitored by light traps. When using the UV light in both the CDC and BG sentinel traps, mosquitoes were also attracted, along with *Culicoides* spp. The mosquito species identified in the collection were: *Aedes* spp., *Culex* spp. and *Armigeres* spp. (Table 13).

Table 13. Details of date and Haematophagous insect species collected from Arignar Anna Zoological Park, Kurumbapatti Zoological Park, Theppakadu and Abhayaranyam elephant camps at Mudumalai Tiger Reserve and Kozhikamuthi elephant camp, Anamalai Tiger Reserve.

S. No.	Place and Date of Field visit	Insects/ticks collected	Trap/ Manual method	Number of specimens
1	Arignar Anna Zoological Park & Rescue centre (29.06.2024)	*Culicoides* spp.	CDC light trap	22
2	Arignar Anna Zoological Park (06.09.2024)	Ticks (from dead Pangolin)	Manual collection	4
3	Arignar Anna Zoological Park (September, 2024)	*Culicoides* spp.	CDC light trap	550
4	Arignar Anna Zoological Park (31.7.2024)	*Culicoides* spp.	CDC light trap (near wolf enclosure)	45
5	Arignar Anna Zoological Park (30.01.2025)	Non-biting midges	CDC light trap (Near bison enclousure)	10
6	Theppakadu elephant camp and Kurumbapatti Zoo (4.10.2024 to 11.10.2024)	NIL	CDC light trap	0
7		*Haematopota* sp.	Manual collection	5
8		*Tabanus* spp.	Manual collection	6
9	11.12.2024 to 16.12.2024	NIL	----	0
10	Theppakadu elephant camp, Kozhikamuthi elephant camp and Kurumbapatti Zoo 23.02.2025 to 28.02.2025	*Culicoides* spp.	CDC light trap and BG sentinel trap	70
		Mosquitoes	CDC light trap	25
		Haematopota spp.	Manual collection	5
		Tabanus sp.	Manual collection	7
		Ticks	Manual collection	16
		Elephant lice	Manual collection	8

The malaise trap was an effective tool for collecting flying insects, however, it collected all types of insects, both harmful and beneficial, such as moths, hymenopteran wasps, honeybees, butterflies, ants, bugs, blow flies etc. Four tick specimens were also recovered from the Malaise trap.

Tabanus spp. and *Haemtopota* spp. were active at elephant camps, but they could not be caught by the traps. These insects were manually collected from camp elephants in the morning and evening hours. Ticks were also manually collected from camp elephants. Two different tick species namely *Amblyomma* sp. and *Haemaphysalis* sp. were found to be the major tick species in the elephant camps at Theppakadu in Mudumalai and Kozhikamuthi at Anamalai Tiger Reserve (Fig. 16-20). Elephant lice, *Haematomyzus elephantis* were also collected from camp elephants (Fig. 21).



Amblyomma species 1: A – Dorsal side; B – Ventral side; C – Capitulum and basis captilula; D – Palp and Hypostome

Amblyomma sp. 2 (Dorsal view)

Amblyomma sp.2 (Ventral view)

Figure 16. Two different *Amblyomma* species - collected from camp elephants at Theppakadu camp

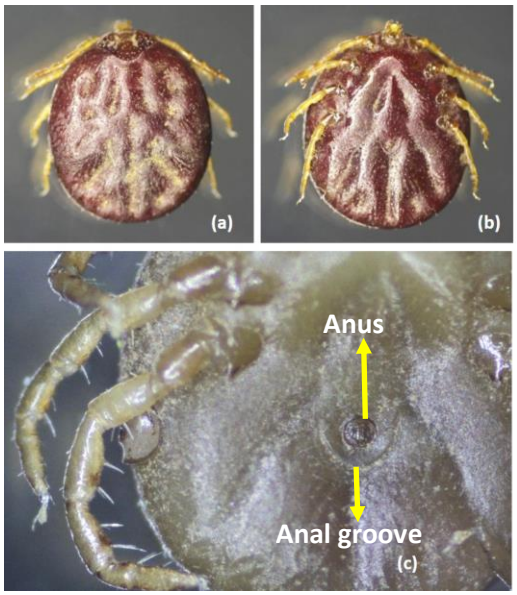


Figure 17. Engorged *Amblyomma* species collected from camp elephants at Kozhikamuthi camp: a) Dorsal side; b) ventral side; c) anus and anal groove

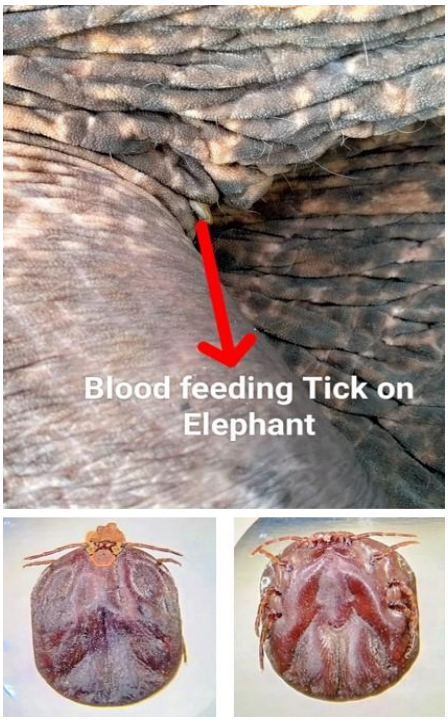


Figure 18. *Haemaphysalis* sp. feeding blood on a camp elephant at Theppakadu. Engorged *Haemaphysalis* sp. female dorsal and ventral sides are shown below

Figure 19. *Haemaphysalis* sp. Male: Dorsal (a) and ventral (b) views. The mouth parts are shown below (c).

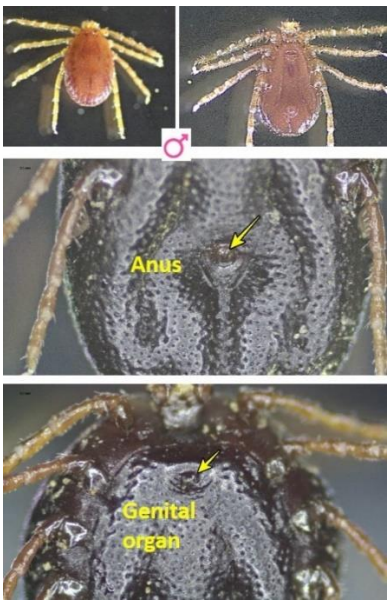


Figure 20. *Haemaphysalis* sp. Male collected from dead Pangolin at AAZP, during post-mortem

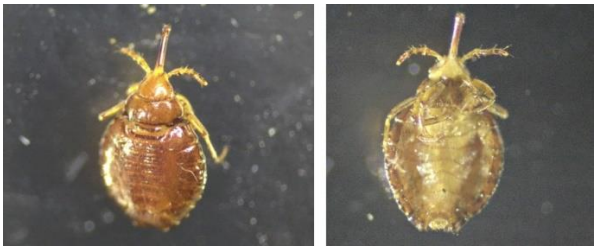


Figure 21. Elephant lice *Haematomyzus elephantis*

6.3. DNA barcoding of insects and ticks

Following morphological identification, DNA was extracted from the insect samples and subjected to DNA barcoding using the Folmer primers targeting the mitochondrial cytochrome c oxidase subunit I (*COX 1*) gene.

Figure 22 shows the amplification of the 700 bp region. Out of the 51 specimens processed, nine failed to yield high-quality sequences suitable for analysis. The remaining 42 samples produced high-quality sequences, which were then analyzed using the NCBI BLAST tool for species identification. *Culicoides* spp., which were collected from AAZP and rescue centre showed similarity with *Culicoides oxystoma* (Table 14).

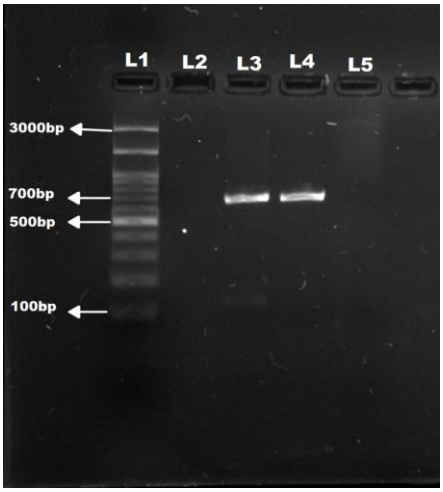


Figure 22. Gel image of amplified *COX 1* gene. L1 – molecular weight marker (NEB 100 bp DNA ladder); L2 – Blank; L3 – sample 1, L4 – sample 2, L5 – Negative control.

Haematopota specimen that was collected from the camp elephant at ATR showed similarity with *Haematopota cilipes* and the *Haematopota* specimens from MTR camp elephants were found to be *Haematopota javana.* Two Pangolin ticks showed similarity with *Haemaphysalis bispinosa* and *Haemaphysalis intermedia* based on DNA barcoding

Two different *Tabanus* spp. viz., *Tabanus crassus* and *Tabanus thurmani* were identified by DNA barcoding of specimens collected from MTR camp

elephants. The morphologically identified *Amblyomma* spp. that were collected from MTR camp elephants showed similarity with *Amblyomma marmoreum* and *A. dissimile* in DNA barcoding analysis (Table 14).

Table 14. Details of insect specimens used for DNA barcoding and sequence similarity against barcode sequences in GenBank

Sl. No	Specimen ID	Date and place of Collection	Host Species	BLAST Result For Barcoding	Trap used
1	PHI 1	AAZP 04.07.24	Near store room	No similarity found	CDC Light trap
2	PHI 2a	AAZP 30.07.24	Near store room	No similarity found	CDC Light trap
3	PHI 2b	AAZP 30.07.24	Near store room	*Culicoides oxystoma*	CDC Light trap
4	PHI 2c	AAZP 30.07.24	Near store room	*Culicoides oxystoma*	CDC Light trap
5	PHI 3a	Rescue Center 31.07.24	Near tiger enclosure	*Culicoides oxystoma*	CDC Light trap
6	PHI 3b	Rescue Center 31.07.24	Near tiger enclosure	*Culicoides oxystoma*	CDC Light trap
7	PHI 3c	Rescue Center 31.07.24	Near tiger enclosure	*Culicoides oxystoma*	CDC Light trap
8	PHI 3d	Rescue Center 31.07.24	Near tiger enclosure	*Culicoides oxystoma*	CDC Light trap
9	PHI 3e	Rescue Center 31.07.24	Near tiger enclosure	*Culicoides oxystoma*	CDC Light trap
10	PHI H1P	ATR	Elephant	*Haematopota cilipes*	Hand pick
11	PHI PNG 1	AAZP 10.09.24	Pangolin	*Haemaphysalis bispinosa*	Hand pick
12	PHI PNG 2	AAZP 10.09.24	Pangolin	*Haemaphysalis bispinosa*	Hand pick
13	PHI PNG 3	AAZP 10.09.24	Pangolin	*Haemaphysalis intermedia*	Hand pick
14	PHI 6	MTR 06.10.24	Elephant	*Amblyomma marmoreum*	Hand pick

15	PHI 14	MTR 05.10.24	Elephant	*Tabanus crassus*	Hand pick
16	PHI 15	MTR 05.10.24	Elephant	*Tabanus crassus*	Hand pick
17	PHI 16	MTR 05.10.24	Elephant	*Tabanus thurmani*	Hand pick
18	PHI 17	MTR 05.10.24	Elephant	*Tabanus crassus*	Hand pick
19	PHI 18	MTR 05.10.24	Elephant	*Haematopota javana*	Hand pick
20	PHI 20a	MTR 06.10.24	Elephant	*Polypedilum japonicum*	CDC light trap
21	PHI 20b	MTR 06.10.24	Elephant	*Tabanus thurmani*	CDC light trap
22	PHI F3 4	MTR 24.02.25	Elephant	No amplification	Hand pick
23	PHI F3 5(i)	MTR 24.02.25	Elephant	*Amblyomma marmoreum*	Hand pick
24	PHI F3 5(ii)	MTR 24.02.25	Elephant	*Amblyomma dissimile*	Hand pick
25	PHI F3 6	MTR 24.02.25	Elephant	*Amblyomma marmoreum*	Hand pick
26	PHI F3 8	MTR 24.02.25	Elephant	*Haematopota javana*	Hand pick
27	PHI F3 12	MTR 24.02.25	Elephant	*Haematopota javana*	Hand pick
28	PHI F3 13	MTR 24.02.25	Elephant	*Haematopota javana*	Hand pick
29	PHI F3 20(i)	MTR 24.02.25	Elephant	*Haematopota javana*	Hand pick
30	PHI F3 20(ii)	MTR 24.02.25	Elephant	*Haematopota javana*	Hand pick
31	PHI F3 20(iii)	MTR 24.02.25	Elephant	No amplification	Hand pick
32	PIHAC 01	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
33	PIHAC 02	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
34	PIHAC 03	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
35	PIHAC 04	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
36	PIHAC 05	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
37	PIHAC 06	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
38	PIHAC 07	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap

39	PIHAC 08	AAZP 03.01.2025	Near Deer Enclosure	No Amplification	CDC Light trap
40	PIHAC 09	AAZP 03.01.2025	Near Deer Enclosure	*Culicoides oxystoma*	CDC Light trap
41	PIHAC 10	AAZP 03.01.2025	Near Deer Enclosure	No Amplification	CDC Light trap
42	PIHRC 01	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides anophelis*	CDC Light trap
43	PIHRC 02	Rescue Center 24.01.2025	Near Tiger Enclosure	No Amplification	CDC Light trap
44	PIHRC 03	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides anophelis*	CDC Light trap
45	PIHRC 04	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides oxystoma*	CDC Light trap
46	PIHRC 05	Rescue Center 24.01.2025	Near Tiger Enclosure	No Amplification	CDC Light trap
47	PIHRC 06	Rescue Center 24.01.2025	Near Tiger Enclosure	No Amplification	CDC Light trap
48	PIHRC 07	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides oxystoma*	CDC Light trap
49	PIHRC 08	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides oxystoma*	CDC Light trap
50	PIHRC 09	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides oxystoma*	CDC Light trap
51	PIHRC 10	Rescue Center 24.01.2025	Near Tiger Enclosure	*Culicoides oxystoma*	CDC Light trap

6.4. Molecular screening of parasites in insects

Morphological identification and molecular barcoding were performed on 51 specimens. These samples were screened for haemoprotozoan diseases, including *Babesia* spp., *Trypanosoma* spp., and *Ehrlichia canis*, using PCR with species-specific primers listed in Table 1 and following the protocols outlined in Table 2. Out of the 51 samples tested, three showed positive results for *Trypanosoma* spp. (Fig. 23), and one sample tested positive for *Hepatozoon felis* (Fig. 24; Table 15)

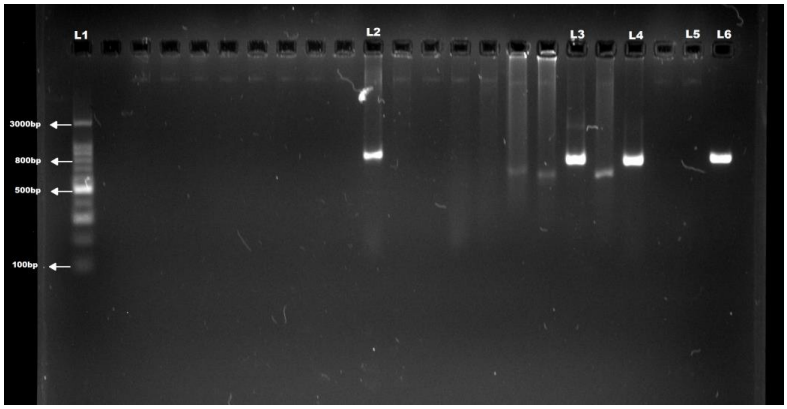


Figure 23. PCR amplification of *Trypanosoma* spp. specific gene. L1 – molecular weight marker (NEB 100 bp DNA ladder); L2 – PHI H1P ; L3 – PHI 16, L4 – PHI 18, L5 – Negative control and L6-Positive control

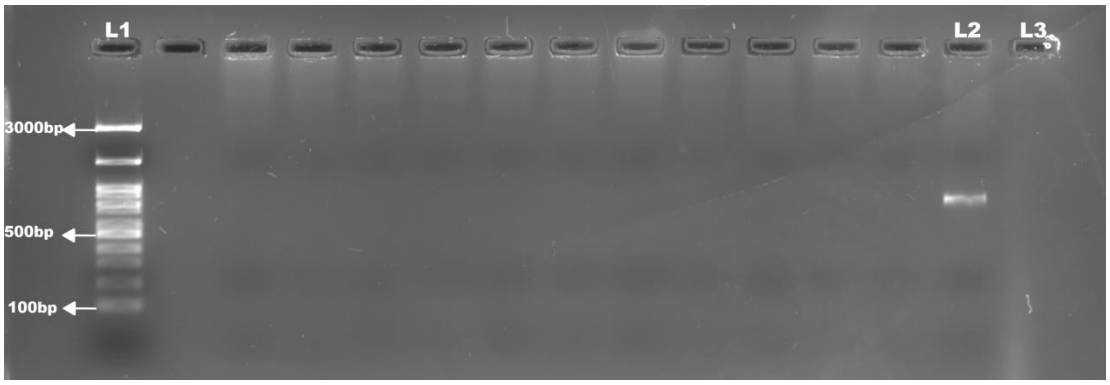


Figure 24. PCR amplification of *Hepatozoon* spp. specific gene. L1 – molecular weight marker (NEB 100 bp DNA ladder); L2 – PHIPNG3; L3- Negative control

Table 15. Amplification of parasite genes in insects using specific primers and sequence similarity against reference sequences in GenBank

Host insect and specimen ID	Sequence of amplified DNA region of parasite	Parasite name
Haematopota cilipes (PHI H1P) (from ATR)	ATCGAAGATGATTAGAGACCATTGTAGTCCACACTGCA AACGATGACACCCATGAATTGGGGAATTTTTGGTCGTA GGCGAGGTCGGGTTCATCTCGCTCCTCGCCTCGCCAA TGGATATCAATTTACGTGCATATTCTTTTCGGTCCTCGC AAGGGGGCCTTTAACGGGAATATCCTCAGCACGTTATC TGACTTCTTCACGCGAAAGCTTTGAGGTTACAGTCTCA GGGGGGAGTACGTTCGCAAGAGTGAAACTTAAAGAAAT TGACGGAATGGCACCACAAGACGTGGAGCGTGCGGTT TAATTTGACTCAACACGGGGAACTTTACCAGATCCGGA CAGGGTGAGGATTGACAGATTGAGTGTTCTTTCTCGAT CCCCTGAATGGTGGTGCATGGCCGCTTTTGGTCGGTG GAGTGATTTGTTTGGTTGATTCCGTCAACGGACGAGAT CCAAGCTGCCCAGTAGGATTCAGAATTGCCCATAGGAT AGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGGGGCG ATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTGTTTT GCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTGTGAT GCTCCTCAATGTTCTGGGCGACACGCGCACTACAATGT CAGTGAGAACAAGAAAAACGACTTTTGTCGGACCTACT TGATCAAAAGAGTGGGAAAACCCCGGAATCACATAGAC CCACTTGGGACCGAGTATTGCAATTATTG	*Trypanosoma* sp.
Haemaphysalis intermedia (PHI PNG3) (from Pangolin)	TTTTTGTAAATTGGRAATGATAGAAATTTAAACACTTTTT AAAGTATCAATTGCSAGGGCAAGTCTGGTGCCAGCAGC CGCGGTAATTCCAGCTCCMWTAGCGTATATTAAAATTG TTGCAGTTAAAAAGCTCGTAGTTGAATTTCTGTTAAAAA TAACCGGTCTGCTTTTAATAAAAGTGGTATCTTGGTGTG TTTTTAGCAATAATGTCCTTTGAAATGTTTTTTACTTTATT GAATAAATAATATTCAGGATTTTTACTTTGAGAAAATTAK AGTGTTTCTARCAGGCTAACGCTTTGAATACTGCAGMA TGGAATAATAAAATAGGATTTTAGTTCTACATTATTGGTT TTAAGAACTAAATTAATGATTGATASGGACAGTTGGGGG CATTTGTATTTAACTGTCAGAGGTGAAATTCTTAKATTTG TTAYAGACAAACTACTGCRAAAGCWTTTGCCAAAGATG TTTTCATTAATCAAGAACGAAAGTTAKGGGATCRAAGAC GATCASATACCGTCGTARTCTTAACTATAAACTATGCCG ACTAGAGATTGGAGGTYGTCTTTATAAA	*Hepatozoon felis*
Tabanus thurmani (PHI 16) (from MTR)	TGGGGGGAWCGGAAGATGATTAGAGACCATTGTAGTC CACACTGCAAACGATGACACCCATGAATTGGGGAATTT TTGGTCGCAGGCGGGGTCGAGTTCATCTCGCTCCTCG CCTCGCCAATGGATATCAATTTACGTGCATATTCTTTTC GGTCCTCGCAAGGGGGCCTTTAACGGGAATATCCTCA GCACGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTT ACAGTCTCAGGGGGGGAGTACGTTCGCAAGAGTGAAAC TTAAAGAAATTGACGGAATGGCACCACAAGACGTGGAG CGTGCGGTTTAATTTGACTCAACACGGGGAACTTTACC	*Trypanosoma theileri*

	AGATCCGGACAGGGTGAGGATTGACAGATTGAGTGTTC TTTCTCGATCCCCTGAATGGTGGTGCATGGCCGCTTTT GGTCGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACG GACGAGATCCAAGCTGCCCAGTAGGATTCAGAATTGCC CATAGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGG AGGGGCGATATTCGTTTGTATCCTTCTCTGCGGGATTC CTTGTTTTGCGCAAGGTGAGATTTTGGGCAACAGCAGG TCTGTGATGCTCCTCAATGTTCTGGGCGACACGCGCAC TACAATGTCAGTGAGAACAAGAAAAACGACTTTTGTCG GACCTACTTGATCAAAAGAGTGGGAAAACCCCGGAATC ACATAGACCCACTTGGGACCGAGTATTGCAA	
Haematopota javana (PHI 18) (from MTR)	GTGGGGGAWCGAAGATGATTAGAGACCATTGTAGTCC ACACTGCAAACGATGACACCCATGAATTGGGGAATTTTT GGTCGCAGGCGGGGTCGAGTTCATCTCGCTCCTCGCC TCGCCAATGGATATCAATTTACGTGCATATTCTTTTCGG TCCTCGCAAGGGGGCCTTTAACGGGAATATCCTCAGCA CGTTATCTGACTTCTTCACGCGAAAGCTTTGAGGTTACA GTCTCAGGGGGGGAGTACGTTCGCAAGAGTGAAACTTAA AGAAATTGACGGAATGGCACCACAAGACGTGGAGCGT GCGGTTTAATTTGACTCAACACGGGGAACTTTACCAGA TCCGGACAGGGTGAGGATTGACAGATTGAGTGTTCTTT CTCGATCCCCTGAATGGTGGTGCATGGCCGCTTTTGGT CGGTGGAGTGATTTGTTTGGTTGATTCCGTCAACGGAC GAGATCCAAGCTGCCCAGTAGGATTCAGAATTGCCCAT AGGATAGCAATCCCCTCCGCGGGTTTTTCCCAAGGAGG GGCGATATTCGTTTGTATCCTTCTCTGCGGGATTCCTTG TTTTGCGCAAGGTGAGATTTTGGGCAACAGCAGGTCTG TGATGCTCCTCAATGTTCTGGGCGACACGCGCACTACA ATGTCAGTGAGAACAAGAAAAACGACTTTTGTCGGACC TACTTGATCAAAAGAGTGGGAAAACCCCGGAATCACAT AGACCCACTTGGGACCGAGTATTGCAATTA	*Trypanosoma* spp

6.5. Phylogenetic analysis

MEGA XI software was used to construct the Maximum Likelihood phylogenetic trees to evolutionarily differentiate the identified species from the rest of the related species in NCBI. A dendrogram was generated by MSA (Multiple Sequence Alignment) of contig sequence with similar species sequence and outgroup species (distant). Maximum Likelihood method of the phylogenetic tree construction with 1000 bootstrap replicates was done using HKY+I model computed for *Hepatozoon* species 18s gene sequence (Fig. 25) and TN92+I model computed for *Trypanosoma* species 18s gene sequence (Fig.

26)

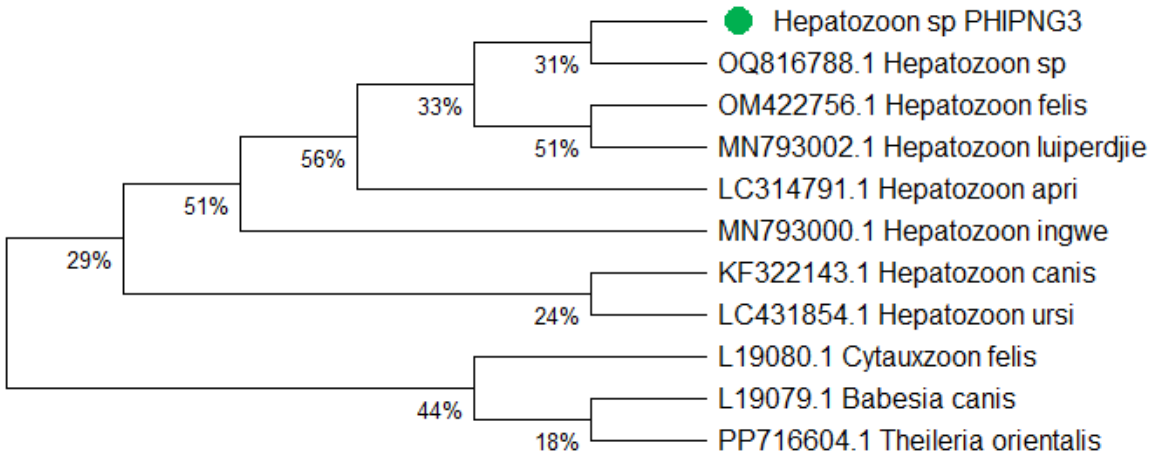


Figure 25. Phylogenetic tree for *Hepatozoon* spp.

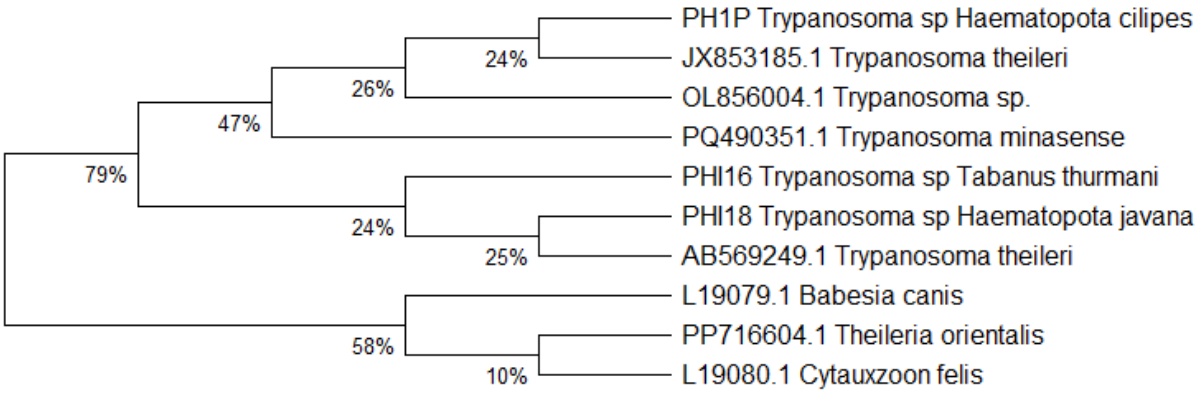


Figure 26. Phylogenetic tree for *Trypanosoma* spp.

7. DISCUSSION

Vector insects are the most serious organisms in disease transmission between animals. Mosquitoes are responsible for the spread of West Nile Virus, while ticks transmit diseases such as Lyme disease and ehrlichiosis. Flea infestations may lead to anaemia, dermatitis and secondary infections. *Leishmania* spp., a group of protozoan parasites, are transmitted by sandflies.

Captive animals are often housed in higher densities, providing a conducive environment for vectors to thrive and spread diseases more rapidly. Captivity can induce stress in animals, which may compromise their immune systems, making them more susceptible to infection. Different species may exhibit varied immunological responses to infections, affecting disease dynamics within a captive population.

In the present study, five different types of haematophagous insects/ arthrodpods, namely *Culicoides* spp., *Tabanus* spp., *Haematopota* spp., ticks and elephant lice, were recorded from the captive animals and their environment. *Culicoides* spp. were the most dominant organisms in terms of numbers. Though the *Culicoides* population was slightly decreased during monsoon season, its activity was recorded during the entire study period. In contrast, ticks, *Tabanus* spp. and *Haematopota* species were not active during the winter period.

Ticks are ectoparasites that infest humans, domestic and wild animals, birds, reptiles and amphibians. They are vectors of several serious diseases like Kyasanur Forest Disease Virus (KFDV), *Babesia conorii, Anaplasma marginale*, Crimean-Congo fever virus (CCFV) etc., (Grisi *et al.,* 2014; Dantas-Torres *et al.,* 2012). About 982 species of ticks have been reported worldwide and 125 species from India (Nijhof *et al.,* 2019; Dantas-Torres *et al.,* 2019; Guglielmone *et al.*, 2023).

Flies belonging to the family Tabanidae are found to be the major blood sucking ectoparasites of the camp elephants. Tabanid flies are potential vectors of bovine viral diarrhoea virus, *Bacillus anthracis, Brucella* spp. and *Trypanosoma evansi*. They give a painful bite to the host animal. In the present study, two genera of tabanid flies were collected: *Haematopota* spp. and *Tabanus* spp. The genus *Haematopota* belongs to the subfamily Tabaninae within the family Tabanidae. It is commonly called 'Cleg flies'.

CDC light trap was found to be the best device in collecting *Culicoides* spp. The use of CDC light traps for collecting haematophagous insects in environments with captive animals has several implications, benefits, and considerations. The effectiveness of CDC light traps can vary based on multiple factors, including the specific type of haematophagous insect targeted, environmental conditions, and the behaviour of the insects. CDC light traps are well-established for capturing mosquitoes. However, it is also effective in trapping *Culicoides*. But the effectiveness in trapping *Culicoides* may vary, because some *Culicoides* species are more attracted to carbon dioxide than light, making additional bait options necessary. The UV lights used in CDC traps can effectively lure many species of mosquitoes.

Incorporating carbon dioxide or octenol (a mosquito attractant) alongside light can enhance capture rates for certain species, particularly those that rely on olfactory cues. In the present study, we evaluated the efficacy of Octenol and p-cresol in attracting blood-feeding insects. However, these attractants did not demonstrate any significant enhancement on insect capture, suggesting limited responsiveness of the local insect fauna to these specific chemical cues.

Placement of traps in relation to captive animal enclosures can greatly affect capture rates. Traps positioned in proximity to areas frequently used by captive animals may capture more insects seeking blood meals. Variables such as temperature, humidity, and wind speed can influence insect activity and trap performance. Warm, humid conditions may enhance attraction and capture rates.

In our study, traps were strategically placed near enclosures in AAZP, KZP and rescue centres. Notably, a higher number of biting midges (*Culicoides* spp.) were captured using CDC light traps when positioned near the wolf enclosure at AAZP. Conversely, traps placed near the tiger and gaur~~bison~~ enclosures yielded no captures. AAZP produced the highest number of trapped insects overall. In contrast, no haematophagous insects were collected using any of the trap types at Amirthi Zoo, indicating either low insect abundance or trap inefficacy in that location.

8. CONCLUSION

The present study clearly showed that biting midges were the predominant blood-feeding insects in AAZP and elephant camps. *Tabanus* spp. and *Haematopota* spp. were identified as notable vectors of blood parasites in camp elephants; however, these species could not be effectively captured using traps. Additionally, ticks were found to pose a significant threat to the health of the camp elephants. The molecular screening of insect samples revealed the presence of *Trypanosoma* spp. and *Hepatozoan felis,* suggesting that these parasites may be predominantly transmitted by ticks, *Haematopota* spp. and *Tabanus* spp.

The study was limited by a time constraint, with only three sample collections being conducted. To gain a comprehensive understanding of insect population dynamics and the seasonal variation in their prevalence, sampling should be done in multiple seasons. The molecular detection of Trypanosoma parasites in ticks and Haematopota spp. in this study serves as a cautionary indication that preventive measures, including compulsory precautionary treatments, should be implemented for the health of the captive animals.

We recommend the use of CDC light traps for monitoring and controlling the population of *Culicoides* spp. These traps are effective for surveillance of insect populations and disease vectors, providing a valuable tool, for vector management and disease prevention in captive animal environments.

9. SUMMARY

- Haematophagous insects and ticks were collected from five different places such as Arignar Anna Zoological Park (AAZP) in Vandalur, Kurumbapatti Zoological Park in Salem (KZP), Amirthi Zoological Park in Vellore (AZP), Theppakadu elephant camp in Mudumalai Tiger Reserve (MTR) and Kozhikamuthi elephant camp in Anamalai Tiger Reserve (ATR). Insects were also collected from Animal Rescue Centre of AAZP in Vandalur.
- Five different traps were used to collect the insects and ticks: CDC light trap, BG sentinel light trap, Malaise trap, horse fly trap and NZI trap
- The study period was from May 2024 to March 2025
- Morphological identification showed the presence of two genera from Tabanidae *(Haematopota* and *Tabanus)* one genera of biting midge *(Culicoides),* one lice species *(Haematomyzus elephantis)* and two tick genera *(Amblyomma* and *Haemaphysalis)* in the study areas
- DNA barcoding results revealed that there were two *Haematopota* species (*H. javana* and *H. cilipes*), two *Culicoides* species (*C. oxystoma* and *C. anophelis*), two *Haemaphysalis* species (*H. bispinosa* and *H. intermedia*), two *Amblyomma* species (*A. marmoreum* and *A. dissimile*) and two *Tabanus* species (*T. crassus* and *T. thurmani*) in the study areas.
- Molecular screening of parasites in the insects and ticks revealed the presence of *Trypanosoma* sp. in *Haematopota* flies, *Trypanosoma theileri* in *Tabanus* sp. and *Hepatozoon felis* in the Pangolin tick, *Haemaphysalis intermedia*.
- Among the five different traps, CDC light trap was the most efficient to collect the Culicodes and mosquito species.

- The chemical attractants p-cresol and 1-Octene -3-ol and cow urine were tested two times for their insect-attracting properties. But they were found to be ineffective. Due to the limited period, this study was not replicated more than twice.
- The study clearly showed that disease transmitting insects and ticks were present in the study areas. This underlines the need for active strategies to safeguard the health of captive animals and also to prevent the possible transmission of pathogens to humans or other wildlife.
- Routine health screenings, ecofriendly vector control measures (like UV light traps, sticky traps) and continuous ecological monitoring can help.

REFERENCES

Archana, M., Placid, E., Souza, D., Renuka Prasad, C. & Byregowda, S.M. (2016). Prevalence of different species of Culicoides in Bangalore rural and urban districts of South India. *J Parasit Dis.*, 40(3): 591-604.

Atchley, W. R., & Wirth, W. W. (1979). A Review of the Culicoides haematopotus Group in North America (Diptera: Ceratopogonidae). *Journal of the Kansas Entomological Society*, 52(3), 524–545, http://www.jstor.org/stable/25083943

Baldacchino, F., Desquesnes, M., Mihok, S., Foil, L. D., Duvallet, G., & Jittapalapong, S. (2014). *Tabanids: Neglected subjects of research, but important vectors of disease agents! Infection, Genetics and Evolution, 28:* 596–615, doi:10.1016/j.meegid.2014.03.029.

Bowman, D.D., Lynn, R.C., Eberhard, M.L., & Alcaraz, A. (2003). Georgi's parasitology for veterinarians. 8. St. Louis: Saunders-an imprint of Elsevier, pp. 1–82.

Campbell, J.B., Skoda, S.R., Berkebile, D.R., Boxler, D.J., Thomas, G.D., Adams, D.C., & Davis, R. (2001). Effects of stable flies (Diptera: Muscidae) on weight gains of grazing yearling cattle. *Journal of Economic Entomology*, 94: 780–783.

Castillo, A.P., Colácio, N., Rodrigues, P.H.C., Miranda, J.V.O., Lima, P.C.S., Motta, R.O.C., Tinoco, H.P., Coelho, C.M., & da Silveira, J.A.G. (2024). Parasitic protozoa and other vector-borne pathogens in captive mammals from Brazil. *Journal of Zoological and Botanical Gardens*, *5*(4): 754-773, https://doi.org/10.3390/jzbg5040050

Chaiphongpachara, T., Padidpoo, O., Chansukh, K.K., & Sumruayphol, S. (2018). Efficacies of five edible mushroom extracts as odor baits for resting boxes to attract mosquito vectors: A field study in SamutSongkhram Province, Thailand. Tropical Biomedicine 35(3): 653–663.

Chomel, B.B., Belotto, A., & Meslin, F.X. (2007). Wildlife, exotic pets, and emerging zoonoses. *Emerging infectious diseases*, *13*(1): 6–11, https://doi.org/10.3201/eid1301.060480.

Dantas-Torres, F., Chomel, B.B., & Otranto, D. (2012). Ticks and tick-borne diseases: A one health perspective. *Trends in Parasitology*, 28: 437–446.

Dantas-Torres, F., Martins, T.F., Munoz-Leal, S., Onofrio, V.C., & Barros-Battesti, D.M. (2019). Ticks (Ixodida: Argasidae, Ixodidae) of Brazil: Updated species checklist and taxonomic keys. *Ticks and Tick-borne Disease,* 10(6): 101252.

Dorny, P., & Fransen, J. (1989). Toxoplasmosis in a Siberian tiger (*Panthera tigris altaica*). *Vet. Rec.,* 125: 647.

Eiras, Á.E., de Almeida Batista, E.P., & de Resende, M.C. (2021). Sampling methods for blood-feeding insects diversity. In: Santos, J.C., Fernandes, G.W. (eds) Measuring arthropod biodiversity. Springer, Cham., https://doi.org/10.1007/978-3-030-53226-0_21.

Godara, R., Singh, S., Dev, R., & Sharma, R.L. (2009). Occurrence of *Haematomyzus elephantis* in semi-arid Rajasthan. *J Vet Parasitol.*, 23(1): 69–71.

Goodrich, J.M., Quigley, K.S., Lewis, J.C.M., Astafiev, A.A., Slabi, E.V., Miquelle, D.G., Smirnov, E.N., Kerley, L.L., Armstrong, D.L., Quigley, H.B., & Hornocker, M.G. (2012). Serosurvey of free-ranging amur tigers in the Russian far East, *J. Wildl. Dis.*, 48: 186-189.

Grisi, L., Leite, R.C., Martins, J.R., Barros, A.T., Andreotti, R., Cançado, P.H., Léon, A.P., Pereira, J.B., & Villela, H.S. (2014). Reassessment of the potential economic impact of cattle parasites in Brazil. *Revista brasileira de parasitologia veterinaria = Brazilian Journal of Veterinary Parasitology*, 23(2): 150-156.

Guglielmone, A.A., Nava, S., & Robbins, R.G. (2023). Geographic distribution of the hard ticks (Acari: Ixodida: Ixodidae) of the world by countries and territories. *Zootaxa*, 5251(1): 1–274.

Haefner, M., Burke, T.J., Kitchell, B.E., Lamont, L.A., Schaeffer, D.J., Behr, M., & Messick, J.B. (2003). Identification of *Haemobartonella felis* (*Mycoplasma haemofelis*) in captive nondomestic cats. *J Zoo Wildl Med.*, 34(2):139-43.

ICMR-VCRC (2022). Hands-on training module on medically important hard ticks. ICMR Vector Control Research Centre (VCRC), Puducherry, 44 pages.

Iatta, R., Trerotoli, P., Lucchese, L. *et al.* (2020). Validation of a new immunofluorescence antibody test for the detection of *Leishmania infantum* infection in cats. *Parasitol. Res.,* 119: 1381–1386, https://doi.org/10.1007/s00436-020-06627-1.

Janke, L.A.A., Vigil, S., Lindsay, K.G., Stoffer, T.F., Colucci, N., Ambagala, A., & Hanner, R. (2023). *Culicoides* (Diptera: Ceratopogonidae) of Ontario: A Dichotomous Key and Wing Atlas. *Canadian Journal of Arthropod Identification*, No. 50, doi:10.3752/cjai.2023.50.

Lapage, G. (1956). Veterinary parasitology. 1. London: Oliver and Boyd LTD; pp. 364–588.

Laurie, P., Alain, H., Sena, A.S., Mutien, G., & Fany, B. (2024). Zoonotic pathogens in wild Asian primates: a systematic review highlighting research gaps. Frontiers in Veterinary Science, 11; https://www.frontiersin.org/journals/veterinary-science/articles/10.3389/fvets.2024.1386180

Medlock, J., Balenghien, T., Alten, B., Versteirt, V., & Schaffner, F. (2018). *Field sampling methods for mosquitoes, sandflies, biting midges and ticks. EFSA Supporting Publications,* 15(6). doi:10.2903/sp.efsa.2018.en-1435.

Metri, R., Puttalakshmamma, G.C., Dhanalakshmi, H., & Jaya, N.L. (2022). Control of biting flies with KVAFSU Tabanid trap (Indigenous Mechanical Device). *International Journal of Science and Research*, 11(3): 970-976.

Mihok, S., Kang'ethe, E.K., & Kamau, G.K. (1995). Trials of traps and attractants for *Stomoxys* spp. (Diptera: Muscidae). *Journal of Medical Entomology*, 32(3): 283–289.

Nijhof, A.M., Guglielmone, A.A., & Horak, I.G. (2019). Ticks Base (version 5.6, Jun 2005). In: Species 2000 & ITIS Catalogue of Life, Annual Checklist (Roskov Y., Ower G., Orrell T., Nicolson D., Bailly N., Kirk P.M., Bourgoin T., DeWalt

R.E., Decock W., Nieukerken E. van, Zarucchi J., Penev L., eds.). Digital resource at www.catalogueoflife.org/annual-checklist/2019. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X//Last accession 01.10.2020.

Ostrowski, S., & Gilbert, M. (2016). Diseases of free-ranging snow leopards and primary prey species. *Snow Leopards*, 6: 97–112, doi: 10.1016/B978-0-12-802213-9.00009-2. Epub 2016 Jul 15. PMCID: PMC7149802.

Otranto, D., & Deplazes, P. (2019). Zoonotic nematodes of wild carnivores, International Journal for Parasitology: *Parasites and Wildlife*, 9: 370-383.

Pawar, R.M., Poornachandar, A., Srinivas, P., Rao, K.R., Lakshmikantan, U., & Shivaji, S. (2012). Molecular characterization of *Hepatozoon* spp. infection in endangered Indian wild felids and canids, Veterinary Parasitology, 186(3–4): 475-479

Rondón, S., Cavallero, S., Renzi, E., Link, A., González, C., & D'Amelio, S. (2021). Parasites of Free-Ranging and Captive American Primates: A Systematic Review. *Microorganisms*, *9*(12), 2546,https://doi.org/10.3390/microorganisms9122546

Sant'Anna, M.R.V., Soares, A.C., Araujo, R.N., Gontijo, N.F., & Pereira, M.H. (2017). Triatomines (Hemiptera, Reduviidae) blood intake: Physical constraints and biological adaptations, *Journal of Insect Physiology*, 97: 20-26.

Stone, A. & Philip C.B., (1974). The Oriental Species of the Tribe Haematopotini (Diptera, Tabanidae). Agricultural Research Service,United States, Department of Agriculture, Washington, D.C. Technical Bulletin No. 1974;1489:149-150.

Turell, M.J., & Knudson, G.B. (1987). Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti and Aedes taeniorhynchus*). *Infection and Immunity*, 55, https://doi.org/10.1128/iai.55.8.1859-1861.1987

Vasudeva, P., Hegde, S., & Veer, V. (2007). Tabanid Flies (Diptera: Tabanidae) of Rajiv Gandhi National Park, Karnataka, India. *J. Ent. Res. Soc.* 9(1): 53-62.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I. G., Latif, A.A., Pegram, R.G., & Preston, P.M. (2003). Ticks of domestic animals in Africa: a guide to identification of species. Biosciences report, Edinburgh, 221.

Wirth, W.W., Hubert, A.A. (1989). The Culicoides of Southeast Asia (Diptera: ceratopogonidae). *Mem. Amer. Ent. Inst.* no 44.

Yeruham, I., & Braverman, Y. (1995). Skin lesions in dogs, horses and calves caused by the stable fly *Stomoxys calcitrans* (L.) (Diptera: Muscidae). *Revue d'élevageet de médecinevétérinaire des pays tropicaux*, 48, 347–349

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