

Are bugs bugging monkeys?

Endoparasitic prevalence and associated physiological changes in Rhesus Macaques (*Macaca mulatta*) across areas of different intensities of human use.



Dissertation submitted by (in partial fulfilment of Masters course)

Sakshi Nulkar

Endoparasitic prevalence and associated physiological changes in Rhesus Macaques (*Macaca mulatta*) across areas of different intensities of human use.

Dissertation submitted to

Saurashtra University, Rajkot
In partial fulfilment of Master's Degree in Wildlife Science.

Dissertation submitted by
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Under the supervision of
Dr. Gautam Talukdar
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भारतीय वन्यजीव संस्थान
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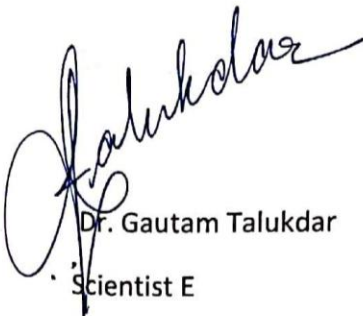
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CERTIFICATE

This is to certify that Ms. **Sakshi Nulkar** has carried out an original piece of research in partial fulfilment of Master's Degree in Wildlife Science of the Saurashtra University, Rajkot. The topic of her dissertation is "**Endoparasitic prevalence and associated physiological changes in Rhesus Macaques (*Macaca mulatta*) across areas of different intensities of human use**". The study was carried out under my supervision from December 2018 to June 2019. I hereby certify that this work has not been submitted for any degree to any university.


Date: 30th June 2019

Place: Dehradun

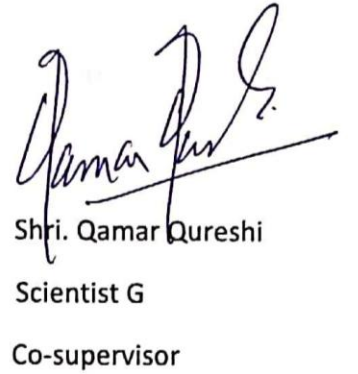


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DECLARATION

I, Sakshi Nulkar, hereby declare that the research work entitled “**Endoparasitic prevalence and associated physiological changes in Rhesus Macaques (*Macaca mulatta*) across areas of different intensities of human use**”, carried out in partial fulfilment of M.Sc (Wildlife Science) degree of Saurashtra University, Rajkot is an original piece of research work. This research work was carried out under the supervision of Dr. Gautam Talukdar, Dr. Lallianpuiii Kawlni and Prof. Qamar Qureshi, at the Wildlife Institute of India from November 2018 to June 2019. I hereby declare that this work has not been submitted for any other degree of any university.

Date: 30th June 2019
Place: Dehradun

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Executive Summary

This study was conducted on the endoparasites of Rhesus Macaques in two areas namely Chandrabani and Rajaji-Chidiyapur during the months of January to April 2019. The aim of this study is to understand whether, between two study areas, there is a difference in the parasite prevalence levels, and incidence of mixed infections of parasites in Rhesus macaques. Also, the aim is to see if a relationship exists between parasite prevalence and the blood parameters. Parasitism is considered to be a natural phenomenon present within any population. However, it is worth questioning whether the parasite populations share many host species in an area of high human-wildlife interface and whether human parasites find refuge in macaques also. Rhesus macaques were studied in Chandrabani area (higher intensity of human use) and Rajaji-Chidiyapur (low intensity of human use). Within the study areas of Chandrabani and Rajaji-Chidiyapur, the Chandrabani area was speculated to have higher parasite prevalence.

A total of 143 faecal samples were collected from both the areas. These samples were analysed in the laboratory for the morphological identification of the parasite genera. For the blood samples, a total of 49 animals were captured and their blood samples were taken for blood testing. Levels of bilirubin, haemoglobin, total proteins, albumin, differential leukocyte counts, RBC counts, platelets etc. are known to show relationships with the parasite infections and hence these parameters were analysed amongst others. Parasite prevalence was calculated for the two areas separately, which indicated the number of individuals that were infected by a particular parasite in the host populations. Blood parameters were correlated with these parasite prevalences to visualize their relationships.

Parasite prevalence was found to be significantly higher in Chandrabani area than Rajaji-Chidiyapur. However, parasite species richness and the incidence of multi-species (mixed) infections in Rhesus macaques were not different between the two areas. Amongst the blood parameters, the mean values of total bilirubin, total protein, RBC count and haemoglobin values showed strong correlations with parasite prevalences of the troops in Chandrabani area.

This data for the haematological and biochemical parameters is the first such report for free-ranging Rhesus Macaques in northern India.

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1. Introduction

Parasite ecology focuses on the interactions between hosts and parasites as a system. Parasitism is a natural phenomenon, where host-parasite ecosystems have evolved simultaneously, which can be better understood if looked at with the perspective of the evolutionary arms race. It is viewed as one of the limiting factors for wild populations, along with others like predation and competition (Anderson and May, 1982; Caughley and Krebs, 1983). The demographics affected by parasites are host survival and fecundity (Hudson and Dobson, 1998). The host-parasite system has been previously studied to understand parasite species richness and abundance which have given insights about disease risk, morbidity and mortality and the immunological status of individuals in a population (Nunn, 2002). Parasites are one of the factors which can induce local declines and extinctions in wild animal populations. This is an especially alarming threat in human-modified landscapes, where the high presence of directly transmitted parasites among wild animals gives an indication of an increasing risk of emerging infectious diseases due to human-induced modification to the environment (Chakraborty et al., 2015; De Castro and Bolker, 2005; Hussain et al., 2013). Many parasites can harm their hosts which lead to physiological changes, nutritional deficiencies, fatal secondary infections, and even immunosuppression (Toft, 1982). These processes can be further exacerbated by different stress-related factors including human-mediated environmental changes and land-use transformation, affecting the dynamics of disease transmission among wild populations of primates.

1.1 Understanding the link between host behaviour and disease spread

It is widely discussed that disease risk increases when species have large social groups, promiscuous mating systems or high densities (close proximity or direct contact)(Altizer et al., 2003). Sociality in the hosts is an important aspect of study because it has epidemiological consequences and the risk of disease spread can be better understood if the social organization of hosts is known (Mbora and Munene, 2019). Social behaviour influences two ends of the spectrum with regard to parasite/pathogen spread; the cross-transmission of generalist parasites increases if the host behaviour is gregarious and generalist. However, isolated but social hosts may also govern the evolution of unique species-specific parasites and parasite complexes (Altizer et al., 2007; Klaus et al., 2017;

Vitone et al., 2004). Host specificity is a measure of the parasite's evolutionary and infective characteristics. The degree of host specificity can be measured by the number of host taxa infected by a particular parasite and can also be related to host phylogeny (Toft, 1982). Host specificity gives insights about the capability of a parasite/pathogen to infect another host individual of the same species or cross over to another species depending on the route of transmission of the parasite/pathogen. These modes of transmission vary from direct transmission through close contact, transmission through environment-vector dependent transmission or the reliance of intermediate hosts for the completion of the life cycle.

Parasites having intermediate/free-living stages may be more likely to cross over to infect another species than the parasite species which only spread through direct/ close contact of the host individuals. Amongst these strategies, vector transmission is generally more likely to infect multiple hosts and is more common among protozoans while intermediate hosts occur more frequently in helminths (Woolhouse et al. 2001).

1.2 Helminthic parasites

Helminths are parasitic or non-parasitic worms. Helminths are divided into two categories: Platyhelminthes (further divided into classes like Trematoda and Cestoda) and Nematelminthes (containing the classes Nematoda, Nematomorpha and Acanthocephala) (Soulsby, 1968).

Class Trematoda contains at least 14 known families with 53 genera overall of worms; class Cestoda contains 7 known families with 24 genera of worms and class Nematoda, which may well be the largest amongst these, contains 35 families and 107 genera. Most of these species are parasitic, only some being free-living. They are known to infect several mammals, mostly ungulates and domesticated animals. There are over 400 parasite species reported from 119 primate species in the wild (Nunn and Altizer, 2005).

The nematodes which are of importance to this particular study belong to the families of Strongyloididae, Strongylidae and Trichuridae belonging to the class Nematoda.

1.3 Parasitic diseases of primates

Several genera of parasites are known to infect all families of living non-human primates. Due to their close phylogenetic relationship, humans and anthropoid primates show susceptibility to a number of common parasites enabling these parasites to potentially cross interspecific boundaries of transmission. (Nunn et al., 2003; Mücke, 2011). Moreover, some primates have high adaptability to human-dominated areas and have established themselves as commensals in the urban environment created by humans. This has increased the frequency of contact with humans, thus increasing direct and indirect interactions (Ekanayake et al., 2006; Fuentes, 2006; Jha et al., 2011).

Parasite presence in hosts also plays a role in the physiology of the host apart from showing clinical signs of disease, and amongst the haematological parameters that are influenced by parasite presence, eosinophil counts typically showed an increase after helminthic infections and a high variation in leukocyte counts (Nunn, 2002). High parasite load has been correlated to extreme variation in body mass, which could affect their overall health (Bauer et al., 2011; Irwin et al., 2010; Junge et al., 2011; Rayadin and Spehar, 2015; Springer et al., 2015). Studies have suggested that parasite prevalence, monitoring of blood cell levels, serum proteins and bilirubin can give insights about the immunocompetence of populations. Parasite prevalence and multiple infections in primates could be affected by the accessibility to human-provisioned and processed food versus traditional/ natural foods (leaves, fruits, flowers, buds etc.), which in turn will reflect upon the nutritional profile of the hosts (Irwin et al., 2010; Nunn, 2002; Tarlow and Blumstein, 2007).

Habitat fragmentation and deforestation are transforming habitats and forcing the wildlife to move into smaller patches with a matrix of human settlements, agricultural crops, roads, mines, etc., and in the presence of livestock and dogs (Fahrig, 2003). Such a landscape increases the chances of interactions of wildlife with livestock, dogs, and other wild animals which might help the parasites in acquiring newer hosts and increasing their host range (Forson et al., 2018; Morand and Poulin, 1998; Nunn et al., 2003). Most primate populations nowadays live in more or less disturbed landscapes. It is expected that host ranges, transmission dynamics, parasite virulence and transmission rates may change and lead to

changes in disease risks to the host populations (Chapman et al., 2006; Pedersen and Fenton, 2007).

Primate populations in forests and fragments have been previously studied for parasite prevalence and abundances. These studies have assumed a positive correlation between parasite prevalence and richness, host density and anthropogenic disturbances. Other studies, however, recorded the opposite or no effect (Gillespie et al., 2006; Gillespie and Chapman, 2008). In a study by Chapman et al. (2006), there is no clear difference in parasitic prevalence in two different areas (centres and edges of fragments); however, there is a significant difference in the abundances of the same parasites in these two areas. Also, hosts in highly disturbed areas are known to have a higher percentage of multi-parasite infections in the same host individual (Chapman et al., 2006).

Primates, known to be the natural reservoirs of many gastrointestinal and haemoparasites, have also acted as a medium for newly emergent diseases in some countries (Fandeur, 1999; Jeneby, 2011; Lee et al., 2011; Springer et al., 2015). Springer et al. in 2015 showed that the prevalence of haemoparasites significantly changes across different age classes. In hosts infected by parasites, especially in primates, the animals may not show clinical signs of diseases or mortality in large numbers, however, the parasites do multiply and propagate using the hosts as reservoirs even when not causing disease. This knowledge is of special significance in areas where the wild host population and humans are in close enough contact through their environments that infections can spread between host species.

A study on lion-tailed macaques in Anamalais suggests that high prevalence and species richness of gastrointestinal parasites in lion-tailed macaque groups are directly related to habitat fragmentation, high anthropogenic activities and high host density (Hussain et al., 2013). A study by Chakraborty et al. (2015) on mammalian parasites in fragmented areas of India, using variables such as location, habitat and forest type, group size, exposure to livestock have been done where hosts were found to have a very high percentage of multiple infections and a significant number of parasite species known to infect humans. For bonnet macaques in Southern India, locations with a high density of macaques and highly exposed to livestock and macaques in towns showed highest endoparasite richness and abundance, which increased with an increase in group size. Young individuals had higher

endoparasite richness and abundance than adults (Kumar, 2016). However, in a similar study of Nilgiri langurs as the host species, no significant difference in the percentage prevalence of parasite infection was found in between disturbed and undisturbed forest fragments (Tiwari and Reddy, 2017). This may be suggestive of how the langurs' exploratory foraging behaviour may be affecting their parasitic loads, which indicates that the foraging behaviour of the hosts also influences the parasite load in the species, which agrees with earlier findings in colobus monkeys of Kibale National Park, Uganda (Chapman et al., 2006). Ekanayake et al. (2006) studied the differences in the faecal parasite loads of primates and found that the individuals who were frequenting water-sources soiled by human faeces had a greater incidence of *Cryptosporidium* sp. than others. A study carried out on Rhesus macaques in Himachal Pradesh found a significant difference of *Giardia* prevalence amongst three different troops studied. This study also revealed the presence of *Entamoeba* spp. In the study population (Debenham et al., 2017). Such studies only pave the path to show that the risk of human-primate disease transmission increase near human-dominated areas.

1.4 Disease-spread in the perspective of human-wildlife conflict

Human-wildlife conflict is most often reported in the form of crop raiding, livestock predation, attacks on humans etc (Madden, 2004; Saraswat et al., 2015). Rhesus macaques are the major contributors to these types of conflict in North India, along with several other animals (Rao et al., 2002). I chose to study the parasitic prevalence in a commensal species such as the Rhesus macaque in a semi-urban area in India with a rapidly changing human-wildlife interface. Rhesus macaques have managed to adapt well to the changing land use scenario in India and have colonized both rural and urban environments alike in large numbers. Foraging for food, be it on garbage dumps or raiding agricultural crops, in human-dominated landscapes has brought them into the limelight as a conflict species, and the frequency of direct and indirect contact is increasing. However, information available on their parasite diversity and in turn their potential roles as reservoirs for zoonoses are minimal, and their indirect interactions with humans at the human-wildlife interface, through the way of parasites and other microorganisms is little studied. These animals congregate at garbage dumps; which is a potential node for transmission of pathogens (Pirta and Singh, 1982). Animals foraging on garbage dumps in large numbers may have a higher chance of acquiring infectious parasites and also be able to transmit it to their fellow

species-members (Plaza and Lambertucci, 2017). Heavily human subsidized animals also have smaller ranging areas since all their food is available in a smaller area. Thus, patterns of parasitism are known to be influenced by the ranging patterns, density, group size, diet, intraspecific and interspecific contacts of the hosts (Altizer et al., 2007; Gillespie et al., 2006; Nunn et al., 2003).

The broad aim of the present study is to develop baseline data of endoparasites of free-ranging Rhesus macaques. The objective is to see if parasitic differences can be correlated meaningfully with the ecological, behavioural, and physiological data of Rhesus macaques. There are very few studies for establishing data on parasite species richness and abundance of the free-ranging Rhesus macaques in their native distribution ranges in North India.

Previously, novel studies have revealed newer haemoparasites to be capable of infecting humans, which were earlier thought to be restricted to only macaque hosts (Lee et al., 2011; Maamun et al., 2011). Since some generalist parasites can be transmitted among conspecifics, domestic animals, and humans, they pose potential anthrozoonotic risks and also have significant economic impacts. The baseline data from this study can be used to build new studies as above.

Thus, studying patterns of parasite species richness and diversity in a wild animal population, especially ones at the human-wildlife interface may aid in better understanding of their role as a means for the persistence and transmission of zoonotic parasites in the environment. Understanding how different intensities of human use influence the gastrointestinal parasites and subsequent diseases of wild primates has a strong relevance for taking preventive and corrective measures at the human-wildlife interface.

1.5 Hypotheses

1.5. Parasite prevalence and abundance is higher in areas of high human use.

Rationale: Areas which are highly used by humans tend to have more garbage dumps, which are frequently accessed by macaques for food. The presence of more numbers of livestock and domestic animals which act as carriers of eggs and cysts may increase the parasite load

in the environment, leading to an increased chance of transmissions. Due to a higher presence of open sewage, animals will come into contact with it more often, thus having higher exposure to parasites. The availability of high-calorie human-subsidized forage is also high and nutritious forage is low.

1.5. b Parasite species richness and the incidence of mixed infections is higher in areas of high human use.

Rationale: The species richness and incidence of mixed infections may be more in areas used highly by humans because the macaques will come in contact with a diverse group of animals including dogs and domestic livestock which may harbour several species of multi-host parasites.

1.6 Objectives:

1. To understand the differences in parasite prevalence and abundance of gastrointestinal parasites of Rhesus Macaques in areas of high and low human use.
2. To understand the possible differences in exposure to parasites and physiological parameters, and their relation to the parasite abundances in different areas.

1.7 Research Questions:

1. Is there a difference in the parasite prevalence and richness (gastro-intestinal and haemoparasites) in areas of different levels of human density and use?
2. Can body condition assessment and haemato-biochemical evaluation reveal a relationship in the health of the individual and its parasite prevalence?

2. Study Area

The aim of this study is to compare the parasite trends between two different areas having high and low intensities of human use.

For the purpose of this study, high intensity of human use is defined as an area where humans are residing in high densities, in turn having a high output of sewage per day (the exit route of gastro-intestinal parasites). This area has a high number of garbage dumps. These dumps are continually refilled every day, thus providing a constant source of food for animals in this area. Additionally, there is a high density of street dogs, pigs, and cattle, all of which can act as host populations for the parasites in question, along with Rhesus Macaques. Thus, hypothetically, since the host population in this area is high, the parasite populations will be proportionally high in this area (at population levels).

Similarly, the low-intensity human use is defined as an area where human density is low, and proportionally lower sewage outputs. Although these areas have areas used by humans, such as agricultural fields, there are no garbage dumps. In addition, as the human population is less, consequently, the associated feral animals such as pigs and dogs are only found to congregate around human settlements and not throughout the area where macaques are found. Rajaji National Park has a complete absence of garbage dumping and human presence (only tourists and forest guards at short durations during the day). However, Chidiyapur is not totally devoid of humans. Care was taken to choose a macaque troop in an area where the roosting and foraging grounds of macaques far away from any human settlement. This troop usually foraged on leaves and bananas provisioned by travellers on the road. Thus, they did not come in contact with any domesticated animals and were not exposed to garbage dumps.

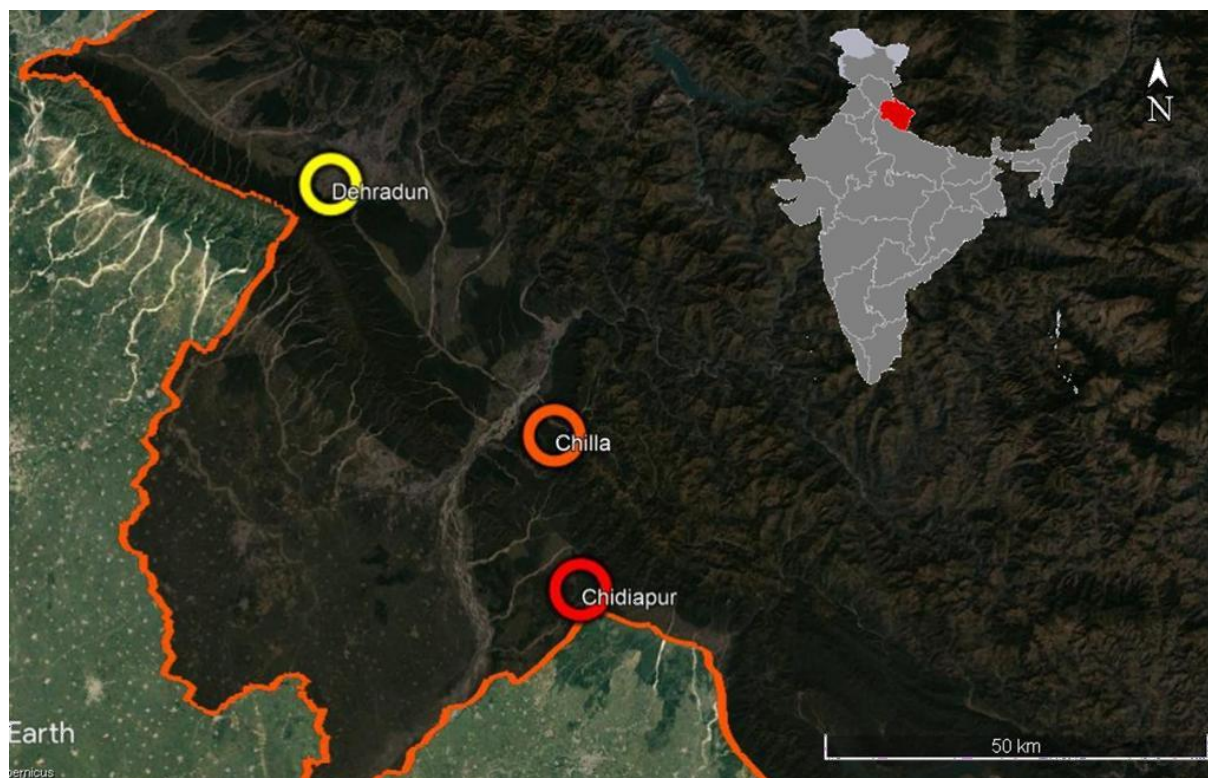


Figure 1: Study areas (circled). The Yellow circle depicts an area of high human use (Chandrabani) and the red (Chidiapur) and orange (Rajaji National Park) circles depict the two study sites of an area of low human use.

2.1 Chandrabani

This semi-urban area lies on the outskirts, towards the south-west, of Dehradun. The maximum rainfall ranges between 60 – 80 mm during the months of July-August. The maximum temperature reaches up to 40° C during the months of May and June and a minimum of 2° - 30° C during December - January.

Being an area of high human use, this area is extensively farmed, the main crop grown being mustard. A huge human influx leading to rapid urbanization and residential area development in this area has led to the degradation of the forest to a great extent. The area of 16sq.km. around Wildlife Institute of India is an area of high intensity of human use. It has open sewage and ample garbage dumps which are frequented by the study species as well as several others like pigs, cattle and dogs.

However, there are distinct patches of forest cover, agricultural lands and residential areas throughout the area. The original forest vegetation has dwindled to a large extent, with the

forests dominantly comprising of *Shorea robusta*. Other vegetation includes *Dalbergia sisso*, *Litsea monopetala*, *Psidium guajava*, *Syzigium cumini*, *Broussonetia papyrifera*, *Cassia fistula* etc. around the residential areas.

A total of 7 troops were sampled in this area. Behavioural observations were carried out on 4 troops, namely A, B, C and D. However, faecal samples were collected from 6 different troops, namely A, B, C, D, E, F. Blood sampling was carried out on the troops A, B, C, D, E, and G. The macaques inhabited the fringes of forest patches all over Chandrabani. The roosting sites of the macaques were in these forest patches, whereas the foraging areas would be towards the urban areas, be it in agricultural fields, home gardens or on garbage dumps.



Figure 2: Study area of high intensity of human use for intensive faecal and blood sampling (Chandrabani)

2.2 Rajaji and Chidiyapur

The Rajaji National Park (NP) is spread across almost 500 sq.km. in Uttarakhand, along the foothills of the Shivalik mountains. The terrain in the Rajaji NP consists of deeply dissected, steep southern slopes forming a series of sharp ridges, which are interspersed with valleys. The southern portion of the sanctuary is flat, constituting parts of the Gangetic Plain (Williams et al., 2001). The fringes are surrounded by agricultural fields in most places.

There has been a strong human presence inside the forest in the form of forest-dwelling Gujjar communities for a long duration; however, they have currently been relocated to the fringes. The forests of this region can be categorized as northern Indian moist deciduous forest and northern tropical dry deciduous forest (Champion & Seth 1968). Chidiyapur forest range is on the fringe of Rajaji National Park, interspersed with forest land and some agricultural fields but mostly devoid of high intensity of human use.

Rhesus macaques are found along the fringes of the National Park, and also in its interiors, where some macaque troops are known to be very shy of humans. The macaques in the interior of the forest which regularly visit some of the forest camps have been released inside by people. These macaques regularly roost in the trees near the forest camps and spend the days inside the forest. A single troop was chosen for faecal sampling in the Mundhal beat of Chilla range, Rajaji National park.

The Chidiyapur range lies in the Haridwar Forest Division. A single troop of macaques was chosen along the Eastern Ganga Canal intersecting the NH 34, near Chidiyapur Forest Rest House. This troop had no garbage dump in their ranging area and was away from any human settlements. This troop was occasionally seen to be provisioned foods like bananas and sugarcane by travellers on the highway.

3. Study species:

3.1 Geographical distribution

The worldwide distribution of Rhesus macaques is from Afghanistan and Pakistan to India, Bangladesh, Bhutan, Nepal all the way to Thailand and Southern China (Smith and McDonough, 2005). The rhesus macaque is the only primate with a very broad geographical distribution (Fooden, 1976; Southwick et al., 1996)). It is distributed throughout northern India up to some extent of central India, in a wide variety of habitat including tropical moist deciduous forest to subtropical pine belt of sub-Himalayas, semi-desert of Rajasthan, mangrove swamps of Sundarbans, West Bengal (Imam and Ahmad, 2013; Seth et al., 2001). Rhesus macaques can adapt to a wide range of habitats including villages, road-side forest, canal banks, towns, temples and agricultural fields (Pirta and Singh, 1982).

3.2 Habit, Habitat and Behaviour

Rhesus macaques are a multi male-multi female society, and they live in groups with the mean group size of 35, though the group size could be up to 250 animals in some of the temples and tourists sites (Altmann, 1962; Jha et al., 2011). They are characterized by furry coats, dusty brown in colour. The thickness of the coats changes according to season. They have little to no fur on their reddish faces, although females do show tufted fur below the cheeks more prominently than males. Male and female are sexually dimorphic (Fooden, 2011). Rhesus macaque is an omnivore and feeds on a wide variety of plant and animal (invertebrate) origin foods. Home range sizes vary a lot depending on the occupied habitat and the resource availability, however, the average home range size is about 5 km² (Kumara et al., 2010; Pirta and Singh, 1982). Rhesus macaques show great flexibility in diet, owing to the fact that it is distributed in such a wide range of habitats. Free-ranging macaques not only feed on several fruits, leaves, flowers, bark and seeds but also act as major seed dispersers for several tree species in the wild (Sengupta et al., 2015).

3.3 People's relationship with rhesus macaques

While the rhesus macaques were once seriously threatened by the rate of capture and export for use in biomedical research, their populations have sprung back rapidly in the absence of a natural predator and very high birth rate (Imam, 1995; Pirta, 1997). Macaques have adapted to human environments and become commensals in the wake of food subsidies that are available to them in the form of food provisioning by humans and foraging on garbage dumps, leading to conflicts. Direct conflict with macaques is seen with crop-raiding, grimacing and grunting at people, snatching food boxes, and very frequently groups of adult macaques target pedestrians. Macaque bites are also frequently reported. (Imam and Malik, 2006).

Even within the study area, several people have been bitten in Chandrabani as well. Along with all the above direct types of conflict, macaques also regularly raid home-gardens and even kitchens to steal food. In several instances, they were seen harassing the chained pet dogs of several households and having aggressive interactions with the free-ranging dogs. Macaques are regularly observed to open the water storage tanks above the house-terraces. They frequently bathe in the water meant for household use without the owners even noticing it. Many of these indirect "contacts" with humans are ideal for the exchange

and spread of gastrointestinal parasites amongst both populations. People incessantly request the researchers working on rhesus macaques to capture them and take them away in the Chandrabani area. Although it is not routinely practised, the macaques have been mass captured and moved to forested areas, and away from human settlements in the recent past.

Study period

The study was carried out in the duration of four months from January to April 2019.

4. Methodology

4.1 Behavioural sampling

In the Chandrabani area, macaques of the same troop tend to spread out on residential buildings and in the surrounding forest. Scan sampling was decided to be suitable for observing the foraging behaviour of macaques in this particular study area. In order to be able to account for all the individuals in a troop in one scan period, the duration of a scan was set to be 10 minutes with a gap of 10 minutes in each scan. A few scans had a gap duration greater than 10 minutes if the troop had moved away and needed some time to be located again. During the scan period of 10 minutes, an effort was made to search for all individual macaques of the troop and note their activity. A total of four macaque troops were observed in the Chandrabani area. Every troop was identified as separate based on their foraging area and the areas where they spent maximum time.

For every scan, date, location, Group ID, presence or absence of sewage or garbage dump in the area of observation, and anecdotal instances of defecation by domestic animals were noted. The macaques were divided into four age-sex classes namely Adult Female (AF), Adult Male (AM), Juvenile (JUV) and Infant (INF). The observations started early in the day and an effort was made to get roost to roost observations from morning to evening. The start time and end time of each scan were noted. The behaviours which were accounted for were divided into categories such as Resting, Feeding, Movement, Grooming, Playing, Sexual, Aggression/ Interactions. Food type for each feeding instance was also noted down as was the place of forage (garbage dump, trees, and ground). The place of forage, and food type were given importance for the following reasons: the place of forage is important because it assumed that the risk of transmission of parasites decreases in the following order garbage > ground > trees. This is so because in many instances, garbage dumps serve as the defecation site for not just macaques, but other domestic animals like dogs and cattle and have the highest risk of faeco-oral transmission of gastrointestinal parasites. The soil can contain several parasite eggs and thus be a point of transmission, and lastly foraging on trees has the least risk of parasite transmission. The food type was recorded to understand how much the macaques feed on human-subsidized processed food as opposed to their natural diet of fruits, leaves, flowers and seeds. Any special observations or anecdotal information was recorded in the 'Remarks' section of the datasheet.

4.2 Capture of Rhesus Macaques

2

For blood sampling, macaques were required to be captured. An area of 16 km was available for capturing the macaques. Thus, macaques were only captured from troops A, B, C, D, E and G which fell into this permitted area. In this area, a cage was set up periodically (until macaques were captured) from 23rd of January to 12th of April 2019. A total of 53 macaques were captured in this duration, however, enough quantities of blood for analyses could only be procured from 49 animals.

Capture procedure: For the capture, a large cage (5 x 1.5 m approximately) with a rope-held trap door was set up in places suitable for capture. Cages were usually set up in shaded spots and away from major human interference, usually near the edges of forest patches. A rope was tied to the trap door and it was lifted up to expose the entrance to the cage. The other end of this rope was then tied to a tree or held by hand, to be released to make the trap door fall when a sufficient number of macaques entered the cage. Up to 8 kgs of fruits, peanuts and split gram was kept inside the cage to attract macaques. The cages were monitored at all times while it was set up for capture. Once macaques entered the cage, the rope was released to close the trap door and macaques were captured.

The captured macaques were then restrained in a squeeze cage one by one before administering anaesthetic drugs. If adult females had infants with them, they were rarely separated from each other, and care was taken to ensure that the animals were not harmed during this process. While restraining the macaques in the squeeze cage, care was taken to push the macaques in a comfortable position and the anaesthesia was administered; the squeeze cage was loosened in a matter of seconds. After administration of anaesthesia, the squeeze cage was covered with a dark cloth to help with the induction of the drugs. Out of the 53 macaques captured, 39 were immobilized with a combination of Ketamine (5 mg/ kg body weight) and Xylazine (0.5mg/ kg body weight), while the remaining 14 were immobilized using a combination of Medetomidine (0.15 mg/kg body weight) and Ketamine (3 mg/ kg body weight) (Naccarato and Hunter, 1975). Both the combinations gave a working period of around 45 mins with every animal on an average. The body measurements of the immobilized animal were taken as follows: nose to head base length, head-base to tail base length, tail base to tail tip length, neck girth, chest girth, stomach girth, hind leg length, shoulder height. BD Vacutainer™ Blood collection tubes (EDTA and

serum tubes) were used to collect blood samples. All immobilized animals were weighed and 5-10 ml blood was collected from each individual depending upon its body weight. These blood samples were immediately sent for analysis of haematological and biochemical parameters on the same day. Respiration rate, heart rate and temperature were, monitored every 10 minutes while the animal was immobilized. Necessary care was taken and reversal drugs were administered if any problems were encountered for the immobilized animal.

4.3 Collection of faecal samples:

Faecal samples were collected from the troops A, B, C, D, E, and F (G was omitted because the samples obtained from these animals were not enough to perform laboratory analysis). A total of 67 faecal samples were collected from Chandrabani and 77 were collected from Rajaji and Chidiyapur combined. Thus, total number of faecal samples collected and analysed were 144.

Once animals were captured in the cage, it was a natural reaction for many animals to defecate out of stress/ fear of capture. Thus, fresh faecal samples of individuals were collected from the cage in containers with 10% formalin and stored at 4^o C until lab analysis was done. These samples were marked with the approximate age and sex of the animal, but were not given individual identification numbers according to the blood sample IDs since it was not possible to ascertain the identity of faecal matter collected from the capture cage with the individual macaque ID given for blood data. However, faecal samples were independent since they were collected from one location at a time, over a short duration of time in which macaques generally do not excrete more than once.

Apart from faecal samples collection during capture, fresh faecal samples were also collected from the field whenever they were found. The samples were usually collected during the behavioural observation period, in the mornings, when macaques were observed to spend most of their time around the same area. Once the troop moved away to another location, the area was scanned for faecal samples and they were collected and preserved in 10% formalin.

4.3.1 Sample size

Sample size was calculated according to Gillespie (2006) as follows: The minimum sample size n required to detect at least 1 infection is calculated via the following formula, where α is the significance level and p is the prevalence in the population:

$$n = \ln(\alpha) / \ln(1 - p)$$

A significance level (α) of 0.05 is generally accepted, recognizing the broad range of values for gastrointestinal parasite prevalence in free-ranging primates. It is recommended that for general surveys for the presence or absence of parasites, a prevalence of 5% should be presumed (Leech and Sellers, 1979; Putt et al., 1988). Thus, the total number of individual faecal samples required is calculated to be 60 independent samples, per area sampled.

4.4 Laboratory analysis of faecal samples:

4.4.1 Qualitative analysis

For concentrating the parasite eggs in the faecal sample, flotation and sedimentation method was carried out. The flotation test is sensitive for the detection of helminths and protozoans in faeces. It works on the principle of concentrating eggs based on their specific gravity. Using a flotation salt/sugar solution with specific gravity higher than the parasite eggs or oocysts helps them to float on the surface of the solution. The requirements for this method were as follows: saturated salt solution (400 gm of salt per 1000 ml of distilled water), faecal sample (1 gm), mortar and pestle, tea-strainer.

Using gloved hands, the weighed out portion of the faecal sample was put in approximately 5 ml of NaCl solution and mixed well with a mortar and pestle. This well-mixed slurry was then sieved through a tea-strainer and the fibrous contents of the faecal sample were discarded. The filtrate was transferred to a 15 ml centrifuge tube and filled to the brim to create a positive meniscus. A coverslip was then placed on this and the set-up was left to stand for 15-20 mins. The coverslip was gently removed from the top of the centrifuge in such a way that the drop of the faecal solution was hanging on the coverslip. This was then placed on a glass slide and observed under a microscope under 10x and 40x magnification. Every slide was scanned until all the fields of the coverslip were covered which took upto 20-30 minutes per sample. Parasite eggs were photographed for identification.

The sedimentation technique is useful to concentrate trematode eggs which are heavier than nematode eggs. As individual macaque faecal samples are comparatively lesser in quantity than that of ungulate/ domesticated livestock, the samples had to be used judiciously. Thus, sedimentation was carried out with the same sample as the one processed for flotation. This technique was developed and routinely recommended for less quantity of faecal samples by the Veterinary Parasitology Department, Indian Veterinary Research Institute (IVRI), Izatnagar (personal communication). After flotation is completed, the heavier contents of the faecal sample settle down at the bottom of the test tube. The flotation solution is decanted carefully and refilled with salt solution and allowed to settle again. This is done until a clear supernatant is obtained and this clear supernatant is carefully discarded. The remaining sediment at the bottom of the test tube is then smeared on a glass slide and covered with a coverslip to examine for any trematode eggs.

Presence of parasite larvae and eggs were recorded and photographs were taken for every unique egg or individual found within a slide. Size, shape, colour of ova and helminth eggs were recorded in detail and used for further identification of the parasitic taxon. Identification keys were used from published literature for the identification (Soulsby, 1968). Some images were sent to Department of Veterinary Parasitology, College of Veterinary Sciences and Animal Husbandary, Selesih, for confirmation.

4.4.2 Identification of parasites

The identification of parasites is done by observing the morphological differences in the parasitic eggs. Morphological identification is helpful for discerning the parasite up to family level especially within the families of Strongyles and Strongylids since the egg morphology of several species within them is very similar. The eggs for these families are thin shelled. All three families are similar with respect to egg size (Soulsby, 1968).



Figure 3: Strongyle spp.: Developing eggs with a 'morula' is seen within.

Strongyles: This family can be identified by the presence of a morula within the egg.



Figure 4: Strongyloides Spp.: Coiled, larval stage is seen within the egg

Strongylids: The identification pointer to this family is the presence of a L2 stage of larvae within the egg.

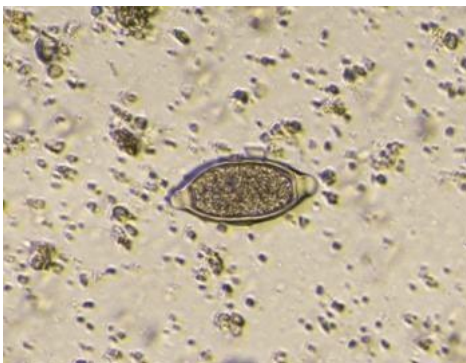


Figure 5: Trichuris spp.: Yellowish, barrel shaped, with mucosal plugs at both ends

Trichurids: Trichuris eggs are characterized by the presence of two polar plugs at the ends of the egg.

4.4.3 Quantitative analysis

In order to determine the number of eggs present per gram of faeces (eggs per gram/ EPG), the McMaster counting technique (Gordon and Whitlock, 1939) is used most commonly. A weighed out portion of 1 gm of the faecal sample was thoroughly mixed with 14 ml of the flotation solution, thoroughly mixed and strained. This strained solution was then taken with a dropper and loaded on to the 2 chambers of a McMaster slide. This slide was allowed to rest for 5 minutes until the eggs floated near the surface of the slide for easier detection, and was then observed under 10x magnification lens. The eggs within the counting chamber were counted and the total of both the chambers was added. This sum of the eggs of the 2 chambers was then multiplied by 50 to obtain the egg per gram count of the faecal sample.

4.5 Analytical methods

4.5.1 Behavioural analysis

The behavioural data was analysed troop-wise (Altmann, 1973; Lehner, 1991) to note the differences in feeding activity patterns of Rhesus macaques in Chandrabani area.

The number of macaques feeding out of the total number of macaques observed per scan was calculated to get the feeding proportion. The average proportion of individuals feeding per hour was calculated separate for each troop.

This average feeding proportion was then plotted against an hourly time interval to get the feeding activity bouts in every troop. This was calculated using Microsoft Excel (2007).

4.5.2 Parasite data analysis

Parasite prevalence calculation

The percentage prevalence of parasites in a population is a measure of how many individuals in a population are infected by a particular parasite. This measure just gives an idea about parasite prevalence at the population level.

$$\text{Prevalence} = (p/n) * 100$$

where, p = number of positive samples

n = total number of samples

To compare the number of positives between Chandrabani and Rajaji-Chidiyapur, a test of proportion was done with the help of the statistical software R (version 3.4). This result yielded the proportion of infection in a population, and tested whether these proportions were significantly different in the two areas.

Eggs per gram (EPG) calculation

EPG counts are indicative of the intensity of the infection in an individual. EPG counts were only calculated for the samples tested positive in the qualitative analysis.

$$\text{EPG} = (x + y) \times 50$$

Where, x = number of eggs counted in chamber 1

y = number of eggs counted in chamber 2

The mean EPG of two areas was reported.

Relationship between troop size and parasite prevalence:

The mean troop size was plotted against the parasite prevalence for each troop to see whether a relationship between the two existed and regressed using lm function in R (version 3.4). The data was normally distributed.

4.5.3 Hematological and biochemical data analysis

A selection of 11 blood parameters (hemoglobin, RBC count, neutrophils, lymphocytes, monocytes, eosinophils, platelet count, total bilirubin, total protein, albumin and A:G ratio) were analysed for correlation with parasite prevalence (J McPherson, 2013). The blood samples were sent for analysis to the veterinary unit of Lal Path Labs, Clement town, Dehradun. While we can make inferences regarding the impact of parasites on these parameters, it should be kept in mind that several other factors may also be acting simultaneously adding to the changes in these parameters.

Mean values with standard errors of all the blood parameters across troops were reported. These were supplemented with reference values from published literature. Troop wise boxplots were made to visualize the blood parameter data from 49 individuals.

The mean values for all blood parameters were calculated troop wise. These 11 values were independently correlated with the percentage prevalence of each troop. Pearson's correlation test was used for each correlation to obtain the correlation coefficients and the relationship was plotted in R.

5. Results:

The study was done in Chandrabani and Rajaji-Chidiyapur areas to evaluate exposure of populations of Rhesus macaques to gastrointestinal parasites. Faecal samples were collected from the above areas and analysed for presence of parasites. Macaques were captured in an area of 2 km² around the WII campus to obtain 49 blood samples.

Table 1: Total effort for sample collection

Area	Troops	Average group size	Total number of days observed	Number of faecal samples collected	Number of blood samples collected
Chandrabani	A	46	5	13	14
Chandrabani	B	39	5	13	3
Chandrabani	C	41	4	11	13
Chandrabani	D	32	4	13	6
Chandrabani	E	40	NA	8	4
Chandrabani	F	80	NA	8	NA
Chandrabani	G	NA	NA	NA	3
Rajaji	RAJ	30	NA	29	NA
Chidiyapur	CP	50	NA	48	NA

5.1. Feeding activity pattern amongst four troops of Chandrabani area.

Activity pattern was visualized for troops A (semi-forested), B (forest-residential area interface), C (Crop field-forest-residential area interface) and D (Forest-residential area interface). The total number of garbage dumps present in the ranging areas of the troops was counted to be 58. The troops show variation in the peak hours of activity during the day. Activity differs across the troops.

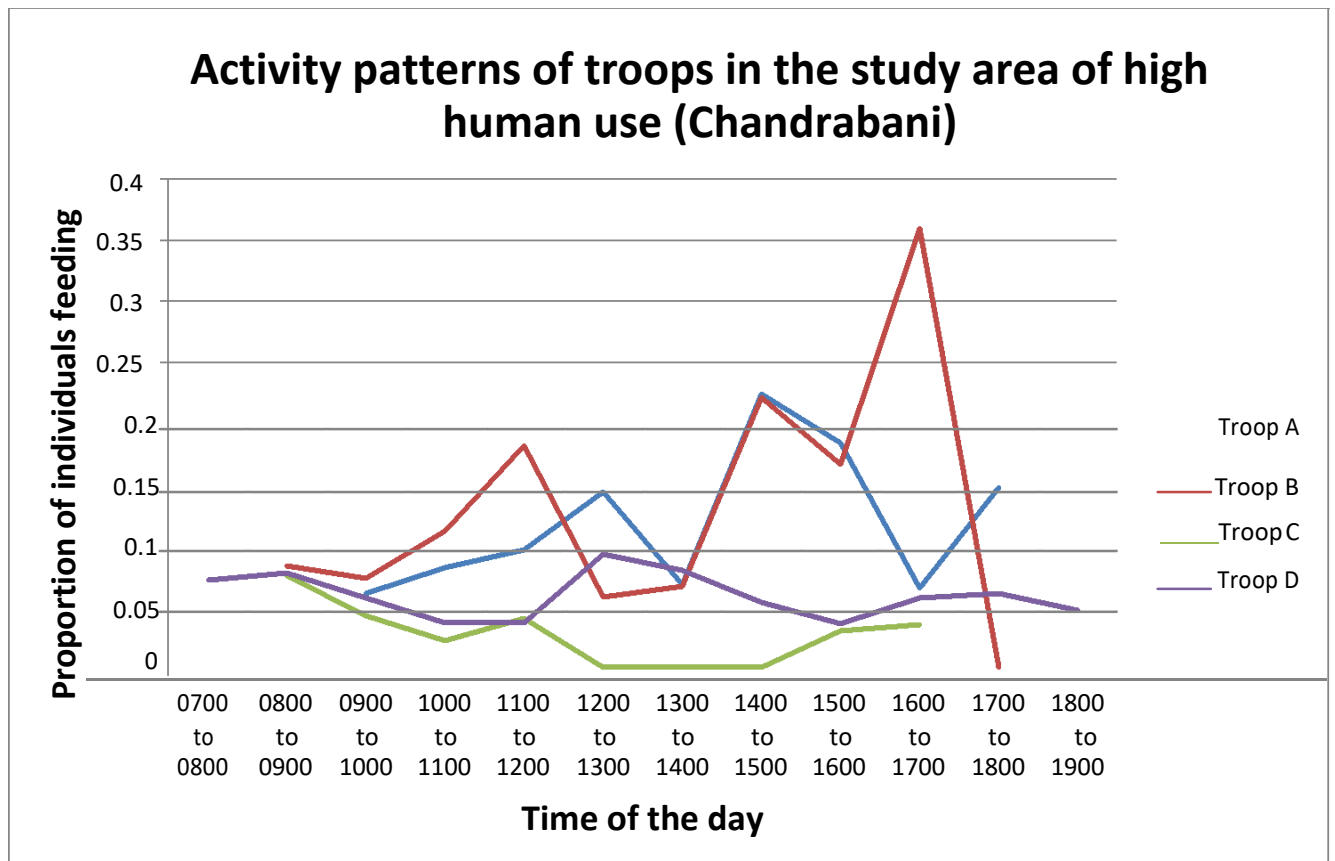


Figure 6: Troop-wise activity pattern for Troops A, B, C and D ranging in different areas of area of high human use (Chandrabani).

5.1.1 Comparison of percentages of food types across different troops

The percentage of foraging on three main food types: Kitchen waste from garbage dumps, leaves and grasses is shown (Figure 7).

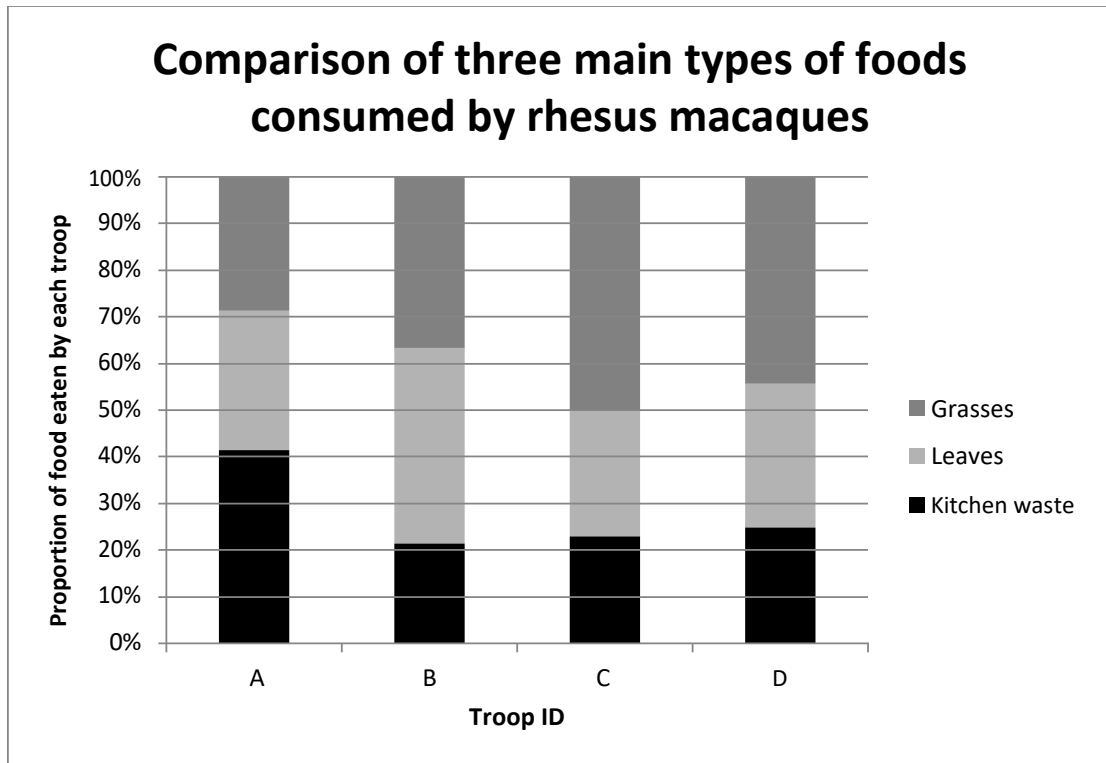


Figure 7: Percentages of food types across troops A, B, C and D

5.2. Percentage of single and mixed infections

A higher percentage of mixed infections with *Strongyles*, *Strongyloides* and *Trichuris* was found in Chandrabani (high human use) in comparison with Rajaji-Chidiyapur (low human use). All three parasite genera were detected individually in the single parasite infections. (Table 2)

Table 2: Single and mixed infection percentage

Sr. No	Study area	Samples with single parasite genera in same individual	Samples with mixed infection (>1 parasite genera in same individual)	Samples negative for the presence of parasites
1	Chandrabani	16.66% (11)	12.12% (8)	71.21% (47)
2	Rajaji + Chidiyapur	3.89% (3)	5.19% (4)	90.90% (70)

5.3. Percentage prevalence of parasites in the two study areas

The number of individuals infected amongst a population is higher in Chandrabani (high human use) than in Rajaji (low human use).

For *Strongyle* and *Strongyloides* there is a significant difference in the percentage prevalence of infected individuals in the two areas (Table 3). However, for *Trichuris*, the difference in prevalence between the two areas is not significant.

Table 3: The number of infected individuals in a population (parasite prevalence).

Sr. No	Parasite	Chandrabani (% prevalence)	Rajaji Chidiyapur (% prevalence)	p-value (Difference of prevalence between areas)
1	<i>Strongyles</i>	21.21%	6.49%	0.019
2	<i>Strongyloides</i>	21.21%	7.79%	0.038
3	<i>Trichuris</i>	7.57%	1.29%	0.147
4	All three parasite genera	21.21%	7.79%	0.038

5.4. Group size versus Prevalence

The group size of macaques observed in the study area was regressed against the parasite prevalence of the samples in the troop (assuming that the collection of the faecal samples was proportional to the troop size, and a representative of the population). It was expected that as the troop size increases, the number of individuals infected in a population should increase. An R^2 value of 0.51 (Figure 8a) indicates some relationship that as the mean group size increases, the parasite prevalence also increases. The test also gave a p-value of 0.02. The largest group size (n=80) was removed from the data set to check if that point influenced the relationship shown in Figure 8a. The relationship (excluding largest group size) is shown in Figure 8b, and a weak relationship was seen ($R^2 = 0.11$, p-value = 0.2). However, a larger sample size with wider variability in group sizes may provide better information.

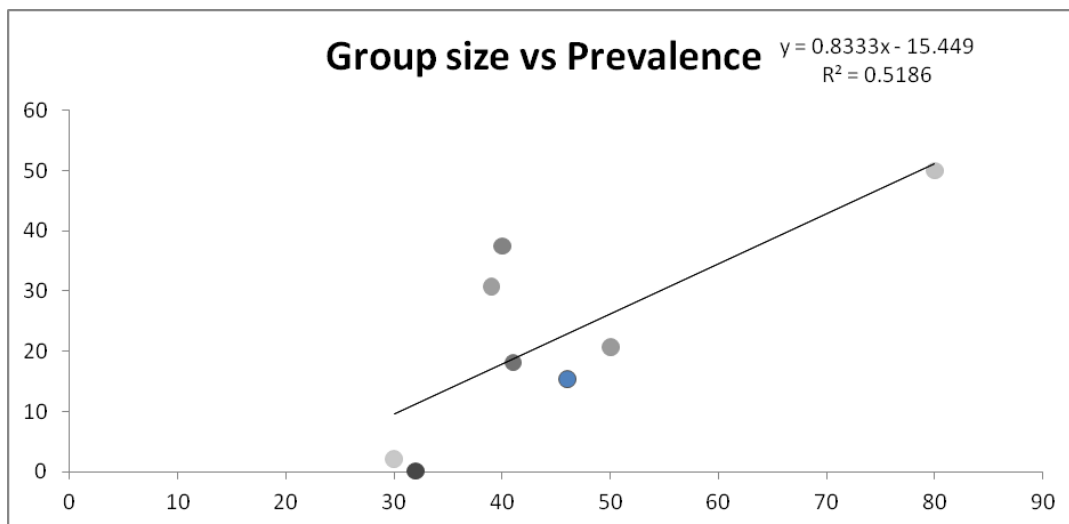


Figure 8a: Relationship between group size and parasite prevalence (with largest group size (n=80) included)

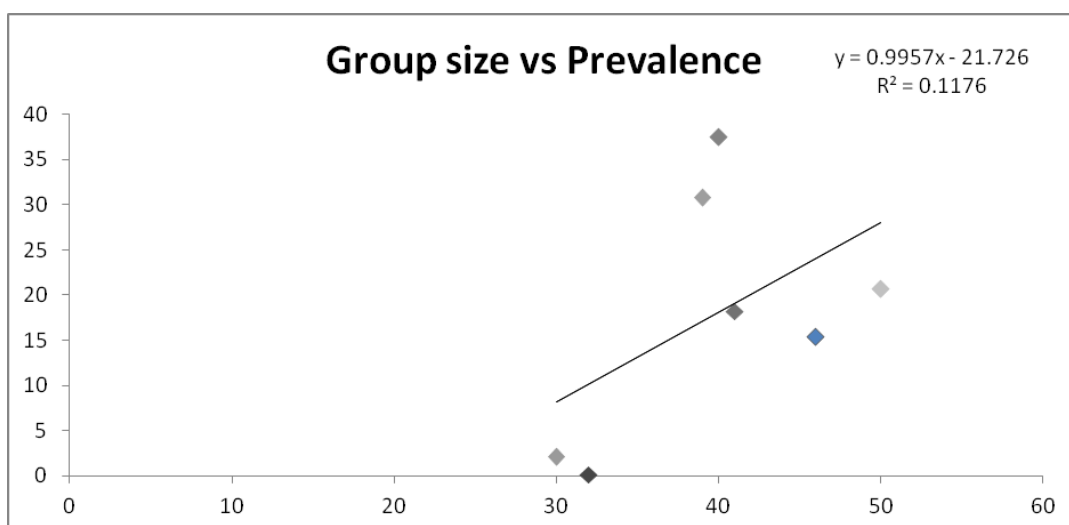


Figure 8b: Relationship between group size and parasite prevalence (with largest largest group size (n=80) excluded)

5.5. Eggs per gram count (for determining parasite load)

	Mean
High use	189.28 ± 43.59
Low use	58.33 ± 8.33

Table 4: Mean parasite load (with standard errors) across high and low human use areas.

The EPG count shows that the mean EPG in area of high human presence is significantly higher than that in area of low human presence (Mann-Whitney U test, p-value = 0.04), thus Chandrabani area has a higher parasite load than Rajaji-Chidiyapur area. (Table 5)

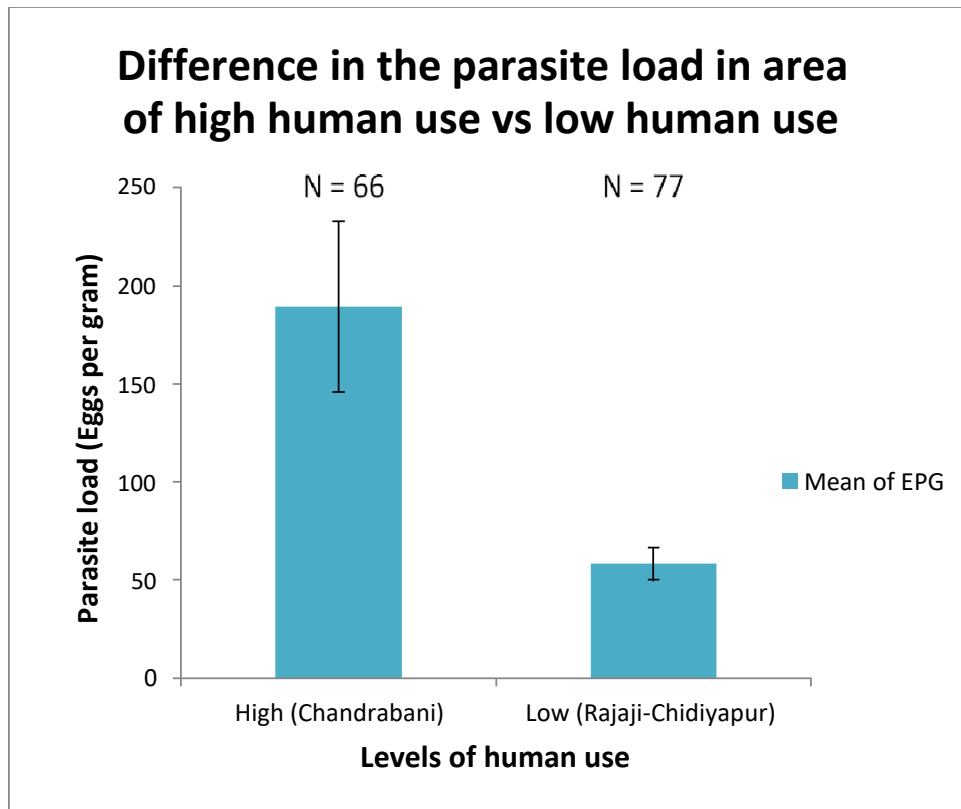


Figure 9: Comparative graph of parasite load across areas of high vs low human use.

5.6. Blood hematology and biochemistry

Table 5: Mean hematological and blood biochemical parameters

Blood parameter	Mean value \pm SE (across all samples)	Reference values (Hom et al., 1999)
Haemoglobin	12.11 \pm 0.22	12.55 \pm 0.54
RBC count	5.26 \pm 0.086	5.32 \pm 0.26
Neutrophils	65.53 \pm 1.73	39.54 \pm 12.94
Lymphocytes	29.14 \pm 1.71	55.44 \pm 16.16
Monocytes	2.30 \pm 0.10	2.91 \pm 1.41
Eosinophils	3.02 \pm 0.24	2.18 \pm 1.74
Platelet count	349.55 \pm 15.75	310.49 \pm 50.04
Total bilirubin	0.093 \pm 0.0096	0.19 \pm 0.04
Total protein	5.72 \pm 0.43	6.68 \pm 0.31
Albumin	3.02 \pm 0.23	4.28 \pm 0.20
A:G ratio	0.95 \pm 0.08	1.82 \pm 0.23

Representation of blood parameters with their reference values (represented in a red horizontal line through the graph).

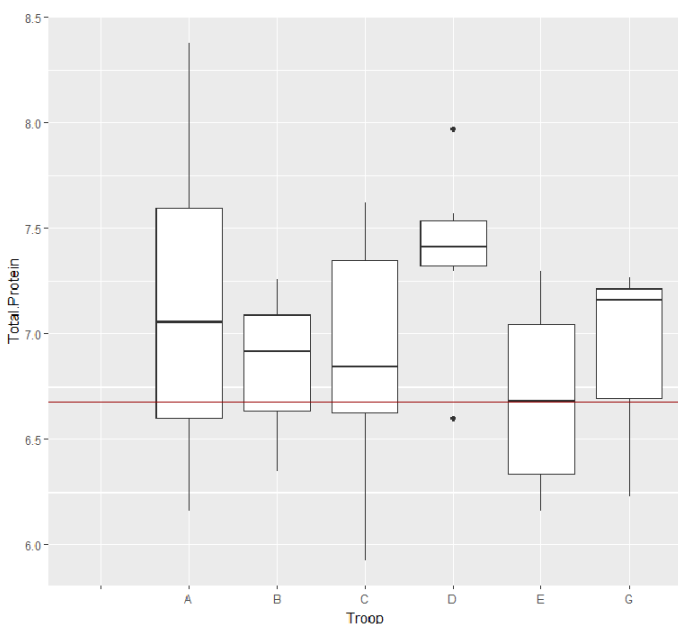


Figure 10: Total protein (g/dL) across troops

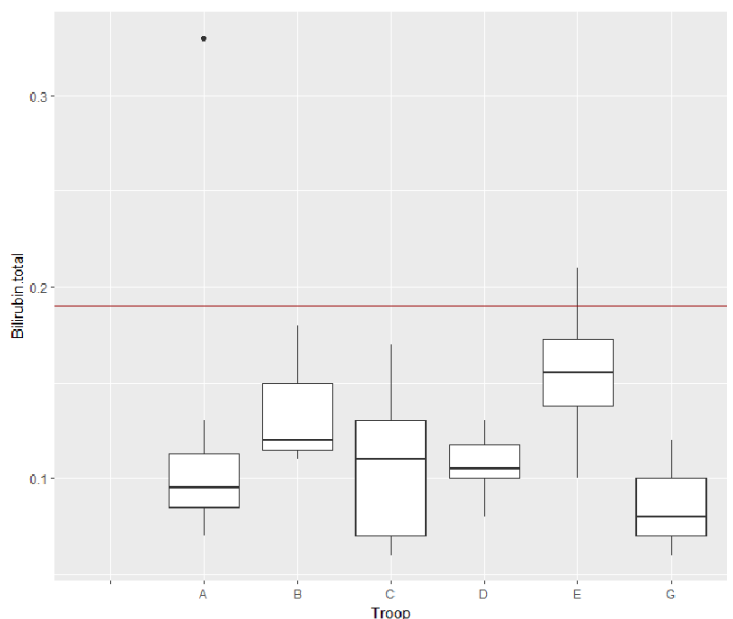


Figure 11: Total Bilirubin (mg/dL) across troops

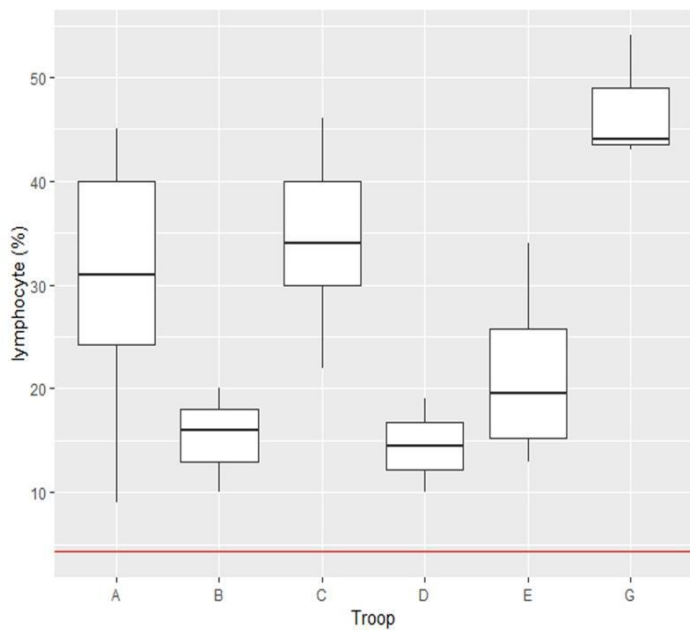


Figure 12: Lymphocyte (%) across troops

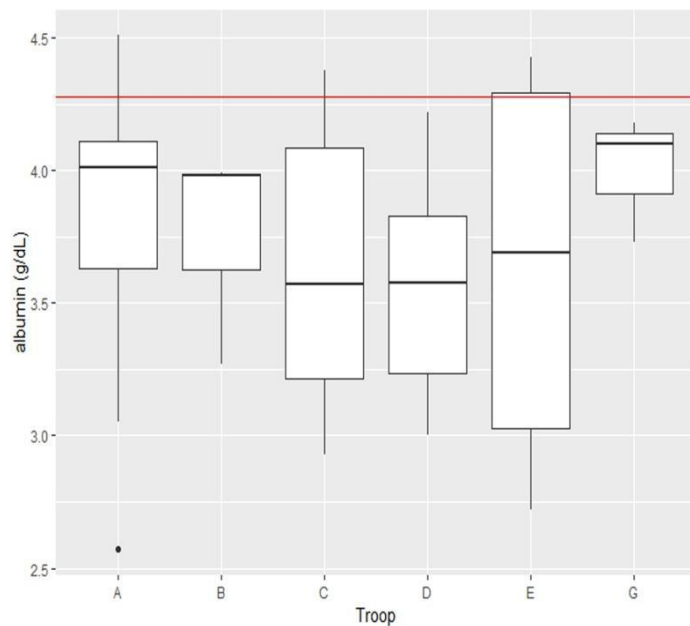


Figure 13: Albumin (%) across troops

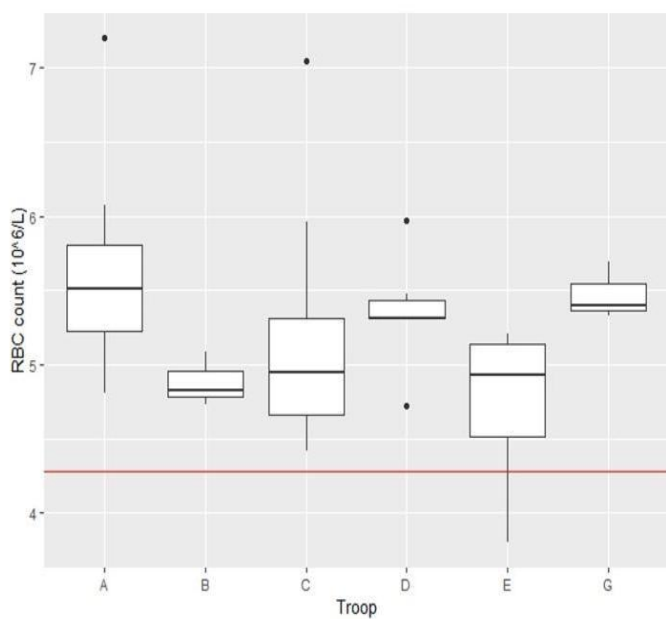


Figure 14: RBC count ($10^6 / L$) across troops

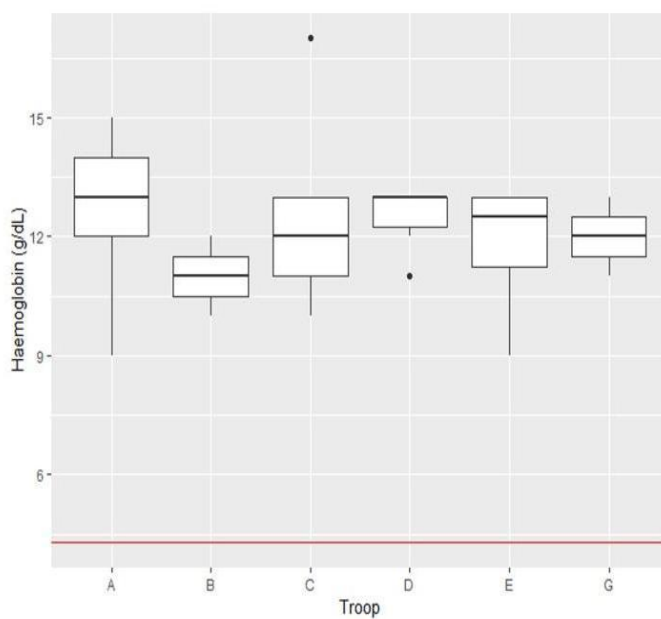


Figure 15: Haemoglobin (g/dL) across troops

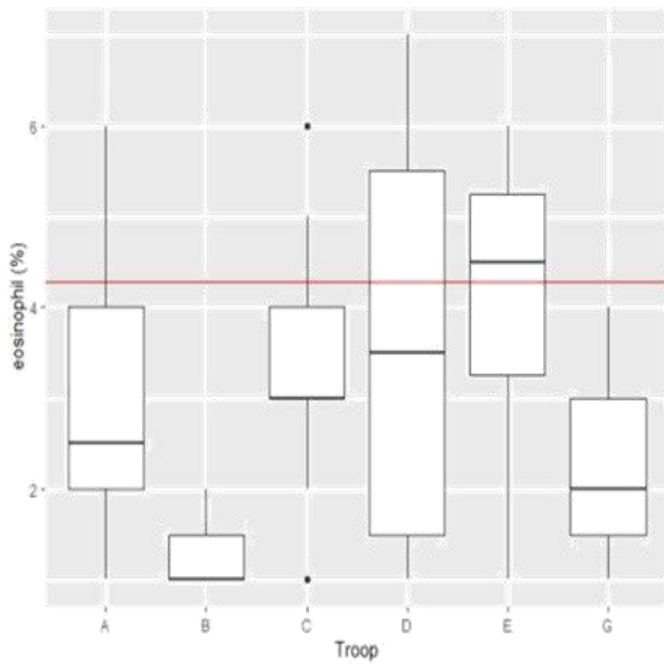


Figure 16: Eosinophil (%) across troops

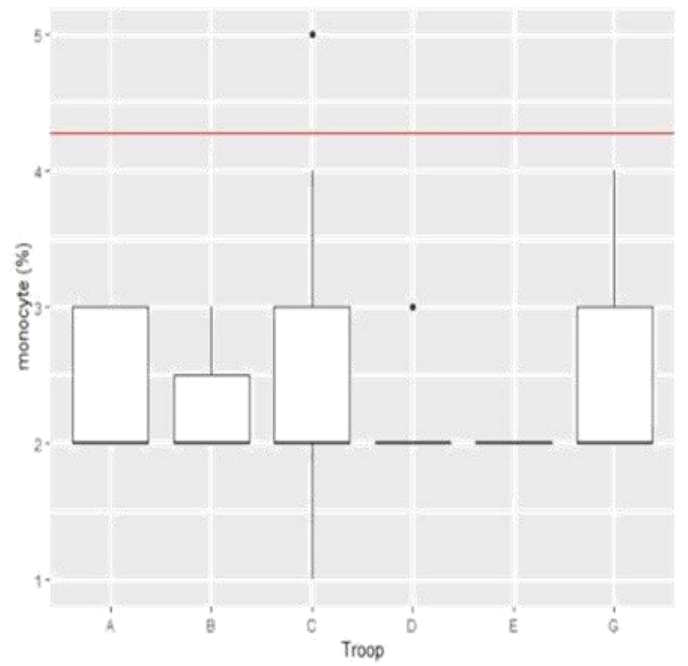


Figure 17: Monocytes (%) across troops

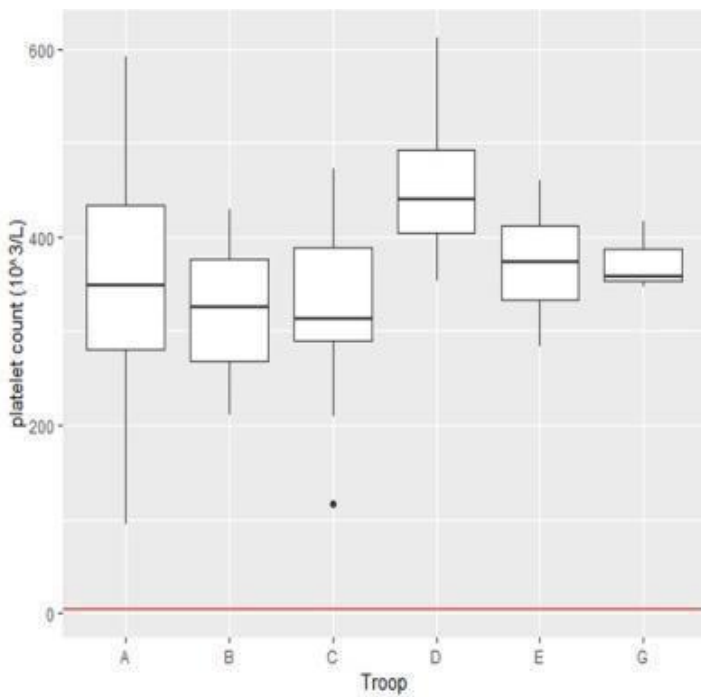


Figure 18: Platelet counts (10³/ L) across troops

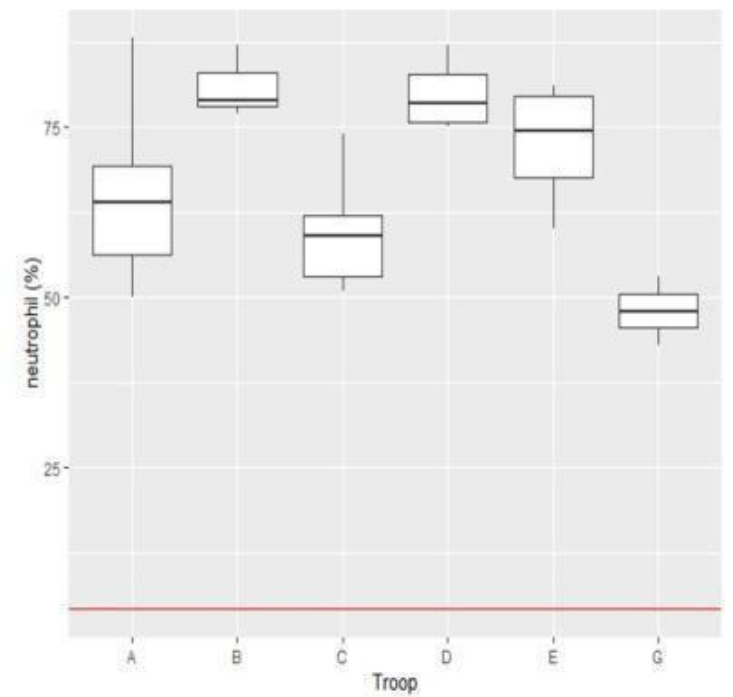


Figure 19: Neutrophils (%) across troops

5.7. Blood parameter values vs Prevalence.

Blood profile was analysed to assess the overall health status of Rhesus Macaques.

Table 6: Relationship between blood parameters and parasite prevalence. (Pearson's correlation test results)

Correlations	Correlation coefficient	p-value
Prevalence vs total bilirubin	0.96	0.007
Prevalence vs RBC count	-0.93	0.018
Prevalence vs total protein	-0.80	0.09
Prevalence vs Hemoglobin	-0.77	0.12
Prevalence vs monocytes	-0.53	0.34
Prevalence vs A:G Ratio	0.54	0.34
Prevalence vs platelets	-0.20	0.736
Prevalence vs albumin	-0.00077	0.99
Prevalence vs neutrophils	0.37	0.53
Prevalence vs lymphocytes	-0.36	0.54
Prevalence vs eosinophils	-0.13	0.82

Graphical representation of the relationship between parasite prevalence and blood parameters.

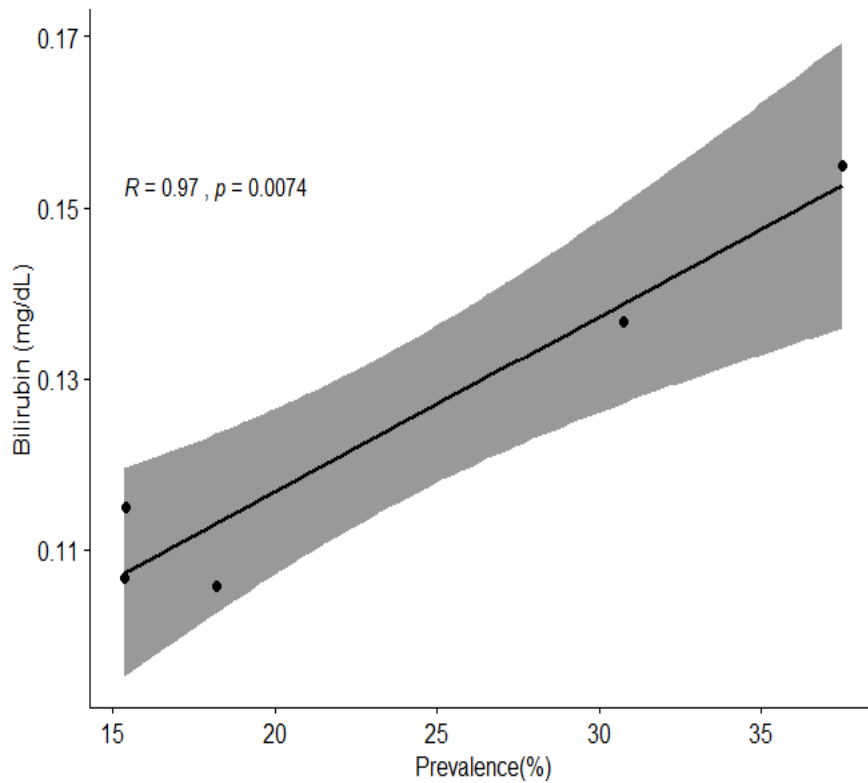


Figure 20: There is a strong positive relationship of bilirubin (mg/dL) and parasite prevalence across different troops.

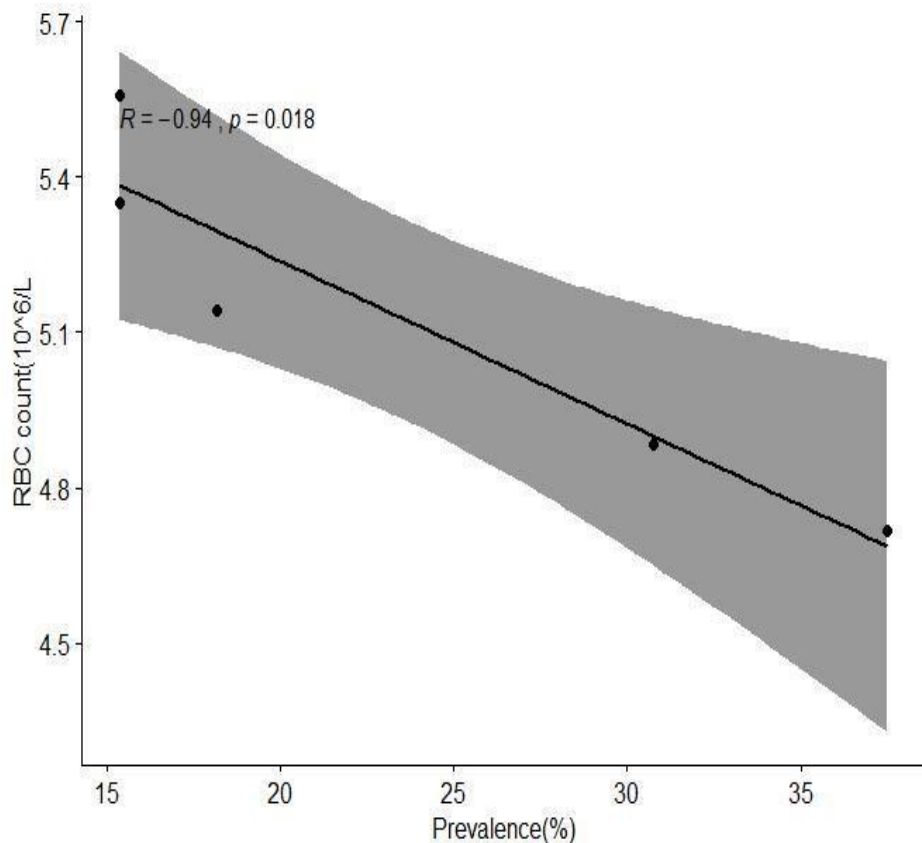


Figure 21: There is a strong negative relationship between RBC count (10⁶/L) and parasite prevalence across different troops

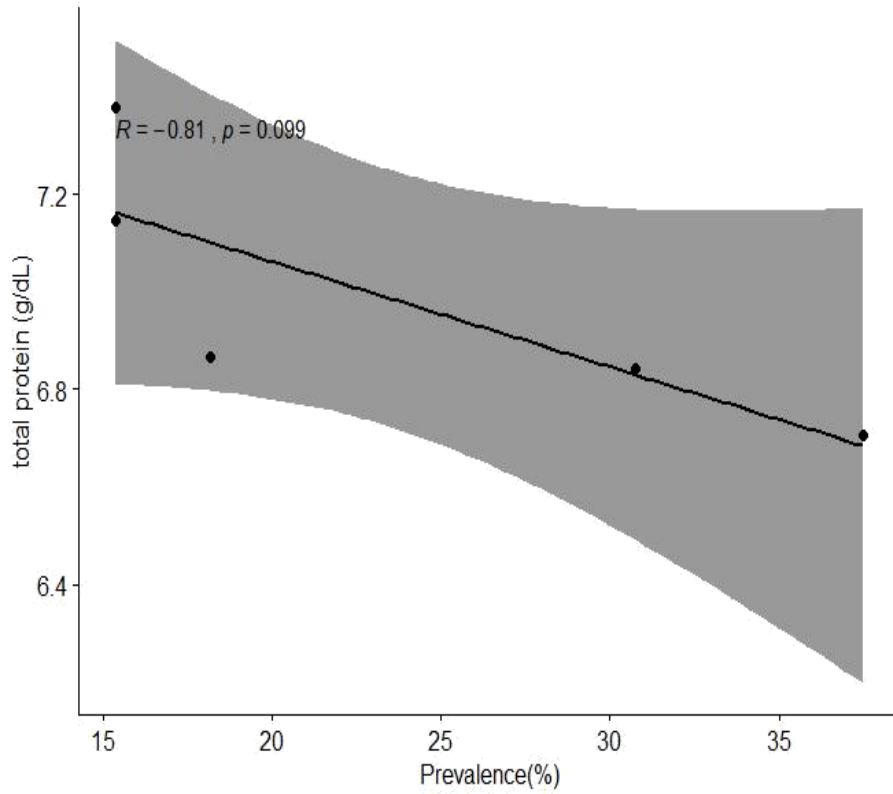


Figure 22: There is a negative relationship between total protein (g/dL) and parasite prevalence across different troops.

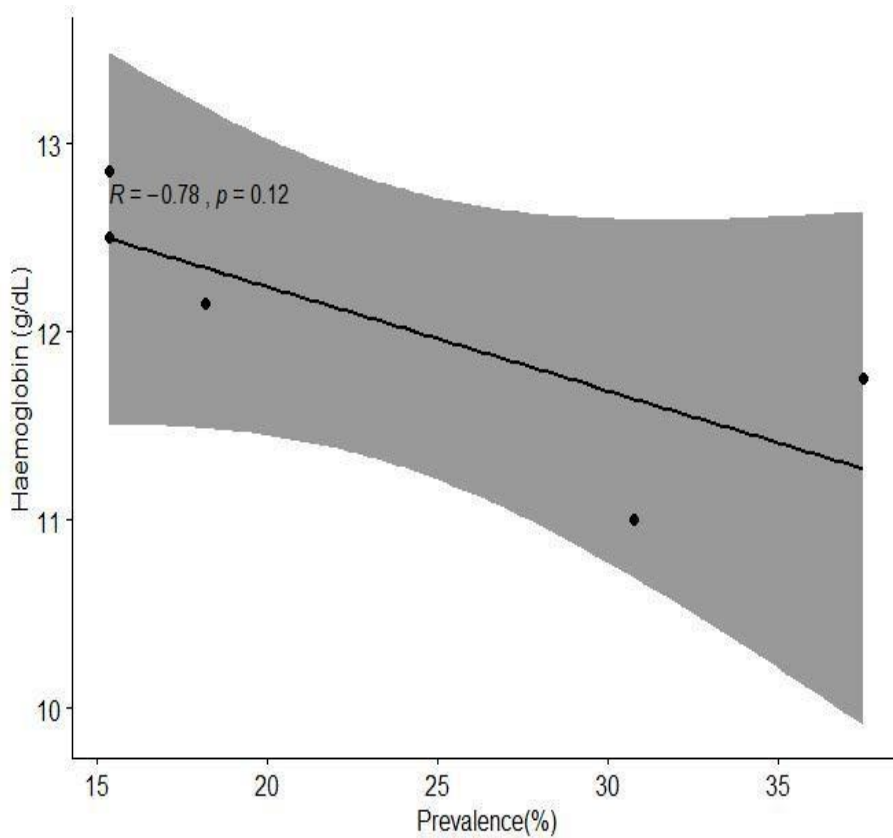


Figure 23: There is a negative relationship between Haemoglobin (g/dL) and parasite prevalence across different troops

6. Discussion

Diseases and parasitic infections are associated with the health and nutrition of the hosts. There is a significant association between nutrition and immune status of an individual, thus influencing the parasitic infection rate for animals in a population. The infection rates may be influenced by nutritional availability to the macaques in a population where exposure to parasites is more (Plaza and Lambertucci, 2017). Healthy and well-nourished animals are able to cope with infections and parasite load, and may not show clinical signs of disease even while harbouring parasites.(Coop and Holmes, 1996). The area of Chandrabani was chosen with this information in mind, since macaques here are known to forage on garbage dumps and are in close indirect contact with humans.

Previously garbage dumps have been studied with considering that it functions as additional food resources for several animals. Animals congregating at garbage dumps are likely to acquire zoonotic diseases and also spread it to humans (Dobson and Foufopoulos, 2001). Firstly, if a garbage dump is a highly attractive source of forage for animals, they congregate in large numbers, thus enabling direct and easy transmission of parasites and other transmissible diseases due to close proximity. For example, when the scavenger bird populations in India drastically declined, the dogs congregating on these abundant carcasses showed a higher prevalence of rabies and leptospirosis. These infections spread from dog to dog, but also from dog to human, thus indicating an increase in transmissions due to direct and indirect contact (Markandya et al., 2008). Large congregations also increase human-wildlife conflict. Secondly, garbage dumps can be the hotspots for exposure to diseases since many animals come to defecate on garbage dumps. This observation is especially evident in the Chandrabani area where pigs and dogs are regularly seen defecating. These dumps may contain either infective parasite eggs or infective, free-living parasite larvae. Thirdly, garbage dumps may attract many types of insects and flies which may act as the vectors for many infectious diseases.

The feeding activity pattern of the four troops A, B, C and D peak at different hours of the day. The number of garbage dumps in the ranging area of the troop may have an impact on this pattern. In the study area, a total of 58 garbage dumps fall within the ranging areas of the troops. Although the macaques did not forage solely on garbage, this pattern may be an artefact of food availability of the dump at a given duration of the day. It is worth

mentioning here that garbage dumps are continually refilled on a daily basis given the large human population of this area.

Exposure to garbage dumps, high use of the area by humans and other potential hosts/ intermediate hosts (domestic cattle, dogs and pigs) may be the governing factors for a higher percentage of mixed infections in Chandrabani area (Hussain et al., 2013). Mixed infections are likely to occur in areas of high exposure to parasites. Parasite infections go through seasonal changes, and winter months generally do not exhibit peak infection periods in a seasonal cycle. Thus, if mixed infections are observed even in a low-prevalence season, it can be speculated that these families of parasites must have high exposure rates regardless of the season. If the parasite species is capable of infecting multiple hosts, its population in the environment may be sustained by the presence of several co-hosts thus allowing it to maintain high exposure rate in the environment.

The proportion of infected individuals in the populations is significantly different in the two areas. Since parasite prevalence is a population level measure, it can be indicative of the general health of a host population. Parasite prevalence is a natural phenomenon and may not necessarily cause mortality but causes morbidity within a population. Parasite prevalence in a population depends on the route of exposure available in the environment. The parasite genera observed in this study can gain entry into the host through two major routes namely, direct ingestion of fertilized egg, or by skin penetration by an infective, free-living larva.

Strongyloides has both free-living and parasitic life cycles. The third stage larvae (L3) penetrates the skin or oral mucosa to enter the host and infection is common among human and non-human primates and other animals including cattle (Soulsby 1968). *Strongyloides* might cause diarrhoea and weight loss if present in numbers above the threshold of infectivity (Soulsby, 1968).

Trichuris is another infective parasite common in humans and in primates as well as other mammals. *Trichuris* has a direct life cycle. It is transmitted through ingestion of first-stage infective larva (Soulsby, 1968). This larvae is still unhatched at the time of ingestion and eggs hatch when ingested by a suitable host. In a study by Hussain et al., 2013, it was found that *Strongyloides* and *Trichuris* prevalence was significantly higher in the lion-tailed macaques

found near human settlements compared to forest interior indicating a high rate of transmission between human/cattle and lion-tailed macaque.

All species of Strongyles have a direct life cycle. The eggs fall out with the excreta of the infected animals, and they hatch under suitable conditions in the environment. The larval stages undergo 3 stages, and the L3 infective stage can penetrate the oral mucosa of animals ingesting the larva (Soulsby, 1968).

In the present study, the mean group size showed a positive relationship with its parasite prevalence, which is a known phenomenon in published literature (Anderson and May, 1982). It is known that the risk of direct transmission increases with higher contact rates between the animals of a bigger troop. This supports the hypothesis that high host density is one of the factors that mediate increased parasite prevalence (Gillespie et al., 2006; Nunn et al., 2003). However, the relationship shown in this study will benefit from a larger sample size with wider group size variability. Thus, to obtain a conclusive result on this relationship, a larger sample size will be required.

Hematological and biochemical analyses:

The blood parameters of 49 individuals from the Chandrabani area were analysed. These parameters were selected to see their relationship with the parasitic prevalence.

It was observed that haemoglobin and RBC count strongly correlates to the parasitic prevalence and decreases with the increase in parasite prevalence across troops. Literature shows that all the helminth genera that have been observed from the present study are capable of causing anaemia in their hosts. These gastrointestinal parasites, especially Strongyles are active blood-suckers (Burden et al., 2010) while Trichuris colonizes the caecum and ascending colon causing anaemia and bloody diarrhoea. Strongyloides has also been reported to be associated with anaemia in the human and animal hosts in acute cases (Dey-Hazra et al., 1979).

The mean total protein levels strongly correlate with parasite prevalence and a decreasing trend in total protein level with an increase in parasite prevalence was observed. Gastrointestinal parasites are usually accompanied by increased intestinal loss or leakage of plasma proteins through the injured gut caused by the parasites also known as malabsorption of protein through damage intestinal mucosa which is reflected occasionally

as hypoproteinaemia (Radostits et al., 1994; Enigk and Dey-Hazra, 1975; Pandey et al., 2010).

The bilirubin level strongly correlates with parasite prevalence and increases with an increase in parasite prevalence. Although all the helminth genera detected in this study area are extra-hepatic, several pathological conditions of extra-hepatic origin are responsible for having an effect on the liver function (Kumar S et al., 2015).

Other blood parameters assessed, which showed weak correlations with the parasite prevalence data were eosinophils, neutrophils, lymphocytes, platelets and albumin. These parameters are known to show changes with parasitic infections in previous studies, but they did not show any trends in this study.

The data for the haematological and biochemical parameters of free-ranging Rhesus Macaques is the first report for free-ranging Rhesus Macaques and will be useful for building reference values for future studies (Appendix 1).

6.1 Implications of the study

This study has important implications for public health because it indicates that macaques do act as a host reservoir for several parasitic genera which are able to cause infections in humans. There is a high risk of spread of zoonoses to children, especially in the Chandrabani area. Within Chandrabani, there are a few settlements of people of lower incomes. They do not have proper sanitation, toilets and most often do not maintain personal hygiene. Children are very often not in proper clothes or footwear and seen playing outdoors in the presence of garbage dumps and macaques. Such a situation is ideal for the transmission of parasites amongst not only humans but also domestic animals, pets and livestock. Parasitic infections in livestock cause economic losses in the form of lower production of milk, lowering the fecundity rates etc. Even though parasitic infections may not be fatal, they can deteriorate the quality of life severely. Thus hygiene should be maintained and deworming practised every few months for the people living in this area.

Moreover, the macaques in Chandrabani also frequent the forest patches where they may come in direct or indirect contact with other wildlife. The risk of transmission of multi-host

parasites and novel parasites or pathogens by macaques into wildlife cannot be undermined. Thus, such studies about the infectious agents and health of wildlife are important with a conservation perspective.

There is scope for similar studies to be carried out across seasons to understand the seasonal trends of parasitic infections in macaques. A larger sample size will also be favourable to answer detailed questions about the relationships between the blood parameters and the parasite prevalences and intensities of infection.

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APPENDIX (Blood parameter values of free-ranging Rhesus Macaques in the area of high human use, Chandrabani, Uttarakhand, India)

Sr. No	Sex	Age group	Hb	PCV	RBC count	MCV	MCH	MCHC	TLC	Neutro	Lympho	Mono	Eosino	DLC baso	Platelet count	Bilirubin total	BUN	Chol total	Creat	Total Protein	Albumin	A:G ratio	SGOT	SGPT
1	M	Adult	14	42	5.44	77	25.1	32.5	14.1	51	42	3	4	0	404	0.13	NA	131	NA	6.66	3.95	1.46	NA	NA
2	M	Adult	15	46.7	5.89	79	24.9	31.5	10.3	73	22	3	2	0	287	0.07	NA	110	NA	7.52	4.21	1.27	NA	NA
3	M	Adult	14	42.7	5.44	79	25.2	32	12.1	67	25	3	5	0	382	0.11	NA	144	NA	7.83	4.51	1.36	NA	NA
4	M	Adult	14	46.5	7.2	65	19.5	30.2	13.8	56	40	2	2	0	443	0.12	NA	120	NA	8.03	4.07	1.03	NA	NA
5	M	Adult	13	42.2	5.67	75	23.6	31.7	18.4	64	32	2	2	0	128	0.33	NA	133	1.07	7.16	4.17	1.39	25	16
6	M	Adult	13	40.4	5.58	72	22.6	31.2	14.5	67	24	3	6	0	291	0.11	NA	110	NA	7.3	4.08	1.27	NA	NA
7	M	Adult	9	29.5	4.81	61	18.5	30.1	9	88	9	2	1	0	189	0.07	18	131	0.58	8.38	2.57	0.44	39.5	13.6
8	F	Adult	14	44.8	6.08	74	22.8	31	13.4	70	26	2	2	0	342	0.09	15	135	0.84	6.9	4.09	1.46	40.3	18.5
9	M	Infant	11	34.6	4.83	72	22	30.7	5.7	57	40	2	1	0	95	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	M	Adult	13	40.6	5.35	76	23.9	31.6	14.3	64	30	2	4	0	279	0.09	16	128	1.02	6.43	3.76	1.41	67	35.7
11	M	Juvenile	12	37.1	5.18	72	22.6	31.5	12.1	52	44	2	2	0	591	0.1	20	126	0.63	6.95	3.05	0.78	50.3	25.4
12	M	Adult	15	48.1	5.85	82	24.9	30.3	10.2	75	20	2	3	0	355	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	M	Juvenile	12	39.4	5.64	70	25.1	31.5	8.3	50	45	2	3	0	472	0.09	12	110	0.67	6.16	3.92	1.75	34	14.6
14	F	Adult	11	34.1	4.83	72.4	22.3	31.6	10.7	57	36	3	4	0	454	0.07	14	68	0.62	6.42	3.25	1.03	39.4	25.7
15	F	Adult	10	34.5	4.73	73	21.8	29.9	14.6	79	16	3	2	0	325	0.12	NA	128	NA	7.26	3.99	1.22	NA	NA
16	F	Adult	12	39.1	5.09	77	23.9	31.2	15.3	87	10	2	1	0	211	0.11	NA	93	NA	6.35	3.27	1.06	NA	NA
17	F	Adult	11	36.1	4.83	75	23.1	30.9	12.9	77	20	2	1	0	429	0.18	NA	129	NA	6.92	3.98	1.35	NA	NA
18	M	Adult	17	54	7.05	77	23.3	30.4	15.9	58	37	2	3	0	209	0.17	14	104	1.24	7.62	4.07	1.15	50.6	23
19	F	Adult	12	36.4	4.98	73	23.1	31.6	22.6	68	23	5	4	0	116	0.11	11	94	0.83	6.87	3.5	1.04	33.5	16.2
20	F	Juvenile	13	42.8	5.96	72	22.2	30.9	15.8	59	34	4	3	0	306	0.13	13	76	0.65	5.93	3.26	1.22	42.4	21.8
21	M	Juvenile	11	35.8	4.66	77	23.9	31.2	24.4	74	22	2	2	0	362	0.12	16	122	0.63	7.33	4.38	1.48	44.5	21.8
22	M	Infant	12	40.1	5.51	73	21.6	29.6	13.1	52	40	3	5	0	282	NA	NA	NA	NA	NA	NA	NA	NA	NA
23	M	Juvenile	12	39.6	5.31	75	23.2	31.1	8.7	62	30	2	6	0	388	0.07	13	120	0.57	6.95	4.13	1.46	35.2	21.7
24	M	Juvenile	13	39.8	4.95	80	25.4	31.5	15.3	66	28	3	3	0	473	0.13	22	148	0.54	6.44	3.64	1.3	48.5	23.2
25	F	Adult	10	33.9	4.42	77	23.5	30.7	12.9	52	39	3	6	0	289	0.13	15	78	0.63	6.69	3.46	1.07	38.6	15.9
26	M	Juvenile	11	36.5	4.74	77	23.5	30.5	12.2	51	46	2	1	0	290	0.11	18	125	0.72	7.4	4.15	1.28	48.5	41.4

27	F	Juvenile	11	36.5	4.62	79	24.2	30.7	23	60	34	2	4	0	328	0.1	14	137	0.64	7.42	3.91	1.11	70.6	64.8
28	F	Adult	13	39.7	5.31	75	23.6	31.6	10.3	53	44	1	2	0	313	0.06	8	78	0.55	6.18	2.93	0.9	53.2	20.3
29	F	Adult	12	35.8	4.49	80	25.7	32.3	18.4	53	41	3	3	0	396	0.07	11	62	0.48	6.76	3.08	0.84	52.6	22.4
30	F	Adult	11	35.4	4.85	73	22.8	31.3	14.9	61	33	2	4	0	389	0.07	11.76	62	0.51	6.82	3.05	0.81	44.4	15.5
31	M	Adult	13	40.8	5.31	77	24	31.2	13.2	75	16	2	7	0	398	0.11	16	125	1.06	7.57	3.87	1.06	43.1	26.2
32	M	Juvenile	13	40.9	5.48	75	23.2	31	12.6	78	17	2	3	0	503	0.12	15	107	0.41	7.97	4.22	1.13	102.5	28.1
33	F	Adult	13	40.1	5.31	75	24.1	31.9	14.5	75	19	2	4	0	611	0.13	8	72	0.65	7.44	3.17	0.74	42	14.2
34	F	Adult	11	35.1	4.72	74	22.6	30.4	13.7	84	12	3	1	0	353	0.1	9	77	0.54	7.3	3.44	0.89	29	16.8
35	F	Adult	13	42.7	5.97	72	21.4	29.9	10.1	79	13	2	6	0	460	0.08	20	143	0.8	7.39	3.71	1.01	51	24.4
36	M	Juvenile	12	39.1	5.31	74	23	31.2	18.6	87	10	2	1	0	421	0.1	13	79	0.66	6.6	3	0.83	55.7	23.6
37	F	Adult	12	38.7	4.75	82	25.7	31.5	7	70	23	2	5	0	395	0.21	17	71	0.81	6.4	3.13	0.96	53.5	16.9
38	F	Adult	9	30.1	3.8	79	24.3	30.7	7.2	60	34	2	4	0	284	0.16	8	55	0.53	6.16	2.72	0.79	59.5	34
39	F	Infant	13	41.7	5.21	80	25	31.2	23.1	79	13	2	6	0	350	0.15	15	99	0.43	6.96	4.43	1.75	76.6	18.4
40	M	Juvenile	13	39.8	5.11	78	24.4	31.3	18.9	81	16	2	1	0	460	0.1	16	98	0.47	7.3	4.25	1.39	75.5	28.5
41	M	Adult	12	37.4	5.33	70	21.8	31	12.8	48	44	4	4	0	416	0.06	14	128	0.51	7.27	4.1	1.29	28.9	24.7
42	M	Juvenile	13	39.6	5.69	70	22.2	31.8	10.3	43	54	2	1	0	347	0.12	15	136	1.86	7.16	4.18	1.4	37.8	16.7
43	M	Juvenile	11	36	5.4	67	20.5	30.7	5	53	43	2	2	0	358	0.08	12	131	0.7	6.23	3.73	1.49	49.3	22.1
44	F	Adult	10.4	35.4	4.93	71.8	21.1	29.4	15.8	65	28	2	5	0	152	NA	NA	NA	NA	NA	NA	NA	NA	NA
45	M	Adult	11.7	40.2	5.68	70.8	20.6	29.1	13.8	76	20	2	2	0	408	NA	NA	NA	NA	NA	NA	NA	NA	NA
46	F	Adult	9.6	33.9	4.84	70	19.8	28.3	11.5	48	48	2	2	0	281	NA	NA	NA	NA	NA	NA	NA	NA	NA
47	F	Adult	10.1	35.3	4.81	73.4	21	28.6	12.1	53	44	2	1	0	449	NA	NA	NA	NA	NA	NA	NA	NA	NA
48	M	Adult	12.3	43.9	5.72	76.7	21.5	28	15.9	81	17	1	1	0	387	NA	NA	NA	NA	NA	NA	NA	NA	NA
49	M	Adult	10.6	38	5.37	70.8	19.7	27.9	11.6	73	25	1	1	0	277	NA	NA	NA	NA	NA	NA	NA	NA	NA