Assessment of the risk of gastrointestinal parasite transmission from relocated commensal bonnet macaques to wild animals in southern India

Shanthala Kumar

Final Report of the Project (16567-1)

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Submitted to Rufford Small Grants

By

Shanthala Kumar

Department of Zoology, Bharathiar University, Coimbatore 641046

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Shanthala

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Introduction:

Parasites obtain their nourishment from host organisms. While not all host parasite relationships are damaging, typically those in which the parasite and host have not evolved a close relationship can be the source of severe infections leading to the death of the host, or a significant reduction in host immunity impairing its ability to fight off other diseases. Some endoparasites can cause severe blood loss, tissue damage, spontaneous abortion and congenital malformations in the host organism and or its offspring (Boyce 1990; Chandra and Newberne 1977; Despomsmier et al. 1995). Parasites and the complications caused by them play a major role in altering the host species diversity or a decline in population size, even driving a host species to extinction by altering parasite load and parasite species composition (Harvell et al. 1999, 2002; Jog and Watve 2005). The introduction of exotic parasite species can also be caused by human interventions such as, relocation of commensal animals to wild habitats (Griffith et al. 1993, Woodford and Rossiter 1993). It is believed that animals living commensally with humans and their domestic animals are highly exposed to such exotic parasites species and have a higher degree of parasite species diversity than animals in wild situations with little exposure to human made conditions (Weyher et al. 2006). The bonnet macaque is one such species live commensal with humans.

Across southern India, bonnet macaques *(Macaca radiata*) are one of the most common commensal species, occurring in all possible human habitations, ranging from urban areas to agricultural fields. Bonnet macaques are considered as pests since they often raid and damage crops, causing significant economic loss to farmers. Furthermore, bonnet macaques also often take food directly from people in towns, temples and tourist sites, making them a menace across their natural range. Since, the intensity of damage caused by bonnet macaques is so severe in these habitats, local farmers often employ crude methods to capture and relocate them to distant forested areas outside of their normal range. Also due to high public and

political pressure, the forest department often relocates bonnet macaques from towns, villages, temples and agriculture areas to the interior forests. This is a quite regular practice particularly around the forests of the Western Ghats, an area that harbour many endemic and endangered species. Unusual deaths are reported in many indigenous mammal species from these forests, some of these deaths have been attributed to parasite infection and other diseases. One of the major sources of these infections is considered to be relocated commensal bonnet macaques. The relocation of such commensal macaques is thought to be one of the prime reasons for the spread of exotic parasite species to wildlife populations. Thus, the present study aims to assess the prevalence of endoparasites in bonnet macaque occupying different habitat conditions, and elucidate this situation and provide information to wildlife managers.

Methodology:

Study site: The geographical range of bonnet macaque is entire south India from south of river Tapathi on the west and Godavari on the east. The samples were collected from different parts of state Karnataka and Tamil Nadu (Fig. 1).

Figure 1 Sampling locations for collection of fecal samples of bonnet macaque

Methods:

Spatial Data collection: The Western Ghats region and the adjoining areas were surveyed for the bonnet macaque. Once the macaque group was located, the details of their location, habitat type, forest type, and the rate of exposure to humans and livestock were recorded. The group was followed (one to three days) until a minimum of eight to ten samples per group are collected. About 1-2g of fecal samples were weighed (INFRA DIGI Precision balance - model IN-200), immediately fixed in vials containing 10% formalin solution and stored in room temperature. Each vial was labeled with individual ID, date and sample number and the same was also recorded in a data book.

Temporal Data Collection: One group from Sirsi–Honnavara Range in Karnataka, which was moderately dependent on provisioning, was selected for detailed documentation for a period of one year to understand the impact of season and demography parameters on endoparasite prevalence. Every month, the focal group was followed for three to four days and collected the fecal samples with age-sex identification. Around 1-2g of the fecal sample was collected from each defecation and fixed in vial containing 10%formalin solution and stored in room temperature then each vial was labeled with individual ID, date and sample number. The same was also recorded in a data book.

Fecal sample analysis:

The fecal samples were analyzed in laboratory using sedimentation and flotation concentration methods. A McMaster's counting chamber was used to quantify the number of eggs per gram of feces of each endoparasite species.

Flotation concentration method: 1g of the fecal sample was taken in new labeled 15ml Torson centrifuge tube then 10ml of distilled water was added, homogenized and mixed thoroughly using glass rod and vortex (Remi CM101) for 10min respectively. The mixture was filtered through cheese cloth. The filtrate volume was increased up to 15ml with distilled water and centrifuged (Remi R-8C, 5250 rpm, Centrifuge) at 1800 rpm for about 10 minutes. The supernatant

was discarded and to the sediment 10ml saturated Sucrose solution (1.28 g/ml) was added and thoroughly mixed, again the volume of the mixture was increased up to 14.50 ml with sucrose solution. The mixture was centrifuged at 4000 rpm for about 10 minutes. The upper layer of the mixture was taken and deposited in one of the McMaster's chambers, allowed the eggs to float to the top and then the processed sample was observed under the microscope (LYNX, PH-100, LM-52-1804) for eggs and their counts.

Sedimentation method: 1g of the fecal sample was taken in new labeled 15 ml Torson centrifuge tube then 10ml distilled water was added, homogenized and mixed thoroughly using glass rod and vortex for 10min. The mixture was filtered through cheese cloth. The filtrate volume was increased up to 15ml with distilled water and centrifuged at 1800 rpm for about 10 minutes. The supernatant was discarded and to the sediment 10ml soap solution (dilute soapy water i.e. 2-3 drops /100ml) was added and centrifuged at 5000rpm for five minutes. The supernatant was discarded leaving few drops of suspension on the sediment pellet. This sediment mixture was taken and deposited in one of the McMaster's chambers and was observed under the microscope for eggs and their counts were made.

Identification of eggs and larvae was made based on published identification keys (Arcari et al. 2000; Bowman et al. 1999; Collet et al. 1986; Jessee et al. 1970) with the help of subject experts.

Conspecific density: Based on the groups detected in and around the sample location, the abundance of conspecific was categorized. Considering the space required (two to three sq. km is the home range of a group), if the encounter rate is less than 3 groups per 10 km, 1 to 2 groups per 10km and 1 or less than one group per 10km was considered as high, medium and low density respectively.

Group size: During the sample collection the number of individuals in the group was counted, however, we considered the group count with an error rate of 5. Since the follow of the group was short period, the group count could not be ascertained at 100 percent accuracy.

Altitude: The geocoordinates was recorded using handheld global position system for every group sampled for the fecal samples.

Vegetation and Habitat type: We recorded the major forest type or habitat type was recorded for each samples groups. The major vegetation of the sampling locations include evergreen forest, semi-evergreen forest, deciduous forest and scrub forest, and other habitat type include tourists spot, temple, village and urban.

Exposure to livestock: Visual assessment was made based on livestock encountered and their dung in each of the locations, and degree of exposure was considered 10 to 100 % at ten percent increment.

Analysis:

Spatial data: The mean endoparasite species richness and their abundance were seen against all independent variables. ANOVA was used for the comparison with each of the independent variables except group size, where the Pearson correlation was used to develop a relationship between group size and endoparasite richness and their abundance.

Temporal data: The mean endoparasite species richness and their abundance was computed for each month and plotted on the graph. The data from temporal sampling was also explored to see the mean endoparasite species richness and their abundance according to age-sex category; however, same could not be achieved for every month thus calculated overall.

Results:

Spatial Analysis: A total of 139 fecal samples of bonnet macaque were collected from 20 locations. Out of them a total of 92 spatial samples were processed and analysed for the prevalence of endoparasites. The findings show that eleven nematode species, two trematode species, fourcestodes species and five protozonas (Table 1). Among all the sample sites, the

bonnet macaque in Agumbe (0.0) and Seethanadi (richness: 1.00±0.00; abundance: 5.00±4.61) had negligible number of endoparasites, where groups in Mettupalyam (richness: 6.66±3.14; abundance: 122.66±55.01) and Pachame (richness: 6.33±2.25; abundance: 42.00±28.39) had the highest endoparasite species and their abundance (Fig. 2). Endoparasite species richness and their abundance differed significantly between the sample sites (richness: $F_{11,80} = 7.269$, $p < 0.000$; abundance : $F_{11,80} = 13.097$, $p < 0.000$).

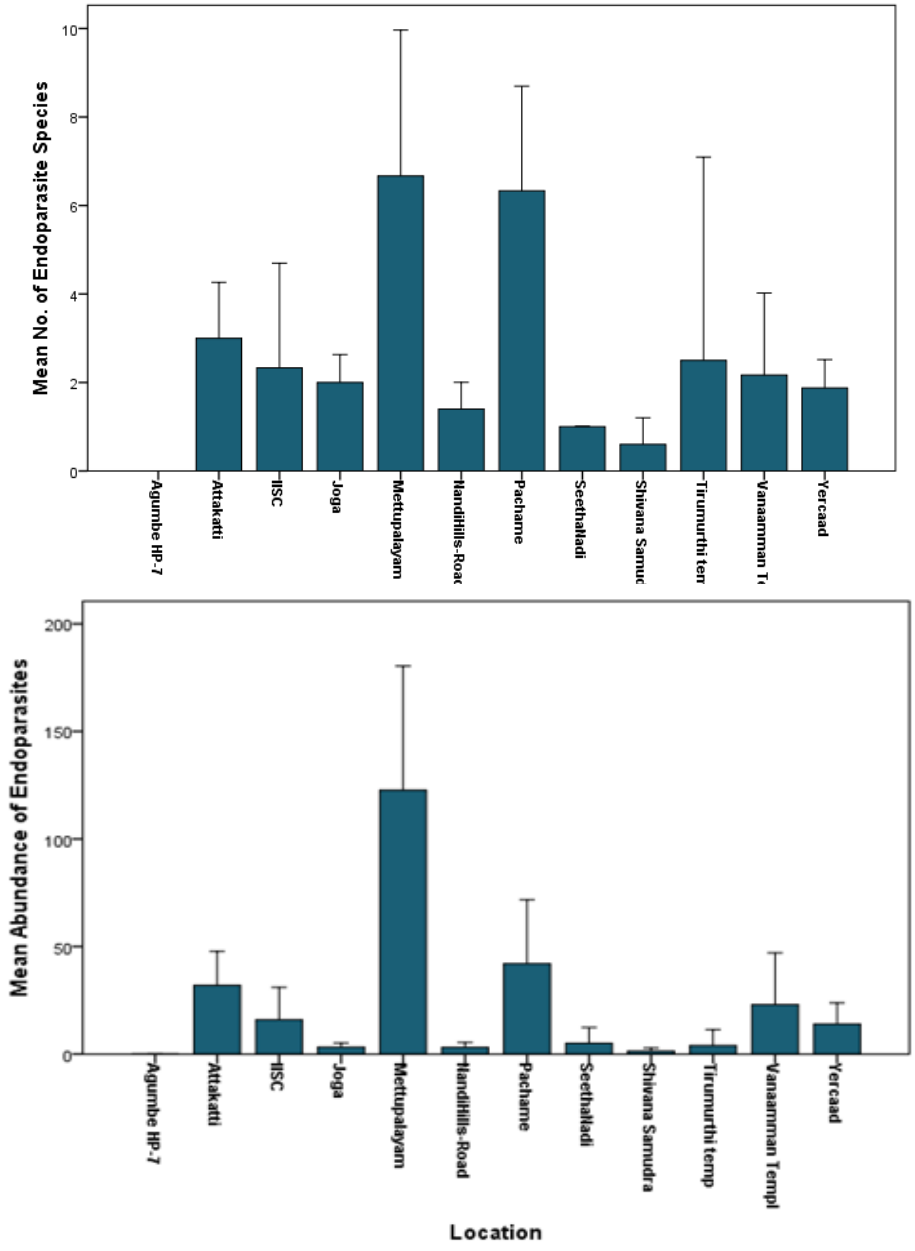


Figure 2Endoparasiterichnessand their abundance in different sampling locations

Table 1 Prevalence of endoparasites in fecal samples from bonnet macaques from different locations

No.	**Parasites**	Prevalence in number of Samples	Mean no. of prevalence
	Nematodes		
1	*Strongylus sp.*	6	18.50±24.74
2	*Strongyloides sp.*	16	5.14±4.74
3	*Trichuris sp.*	4	3.00±1.41
4	*Ancylostoma sp.*	6	5.60±5.68
5	*Bunostomum sp.*	10	6.00±2.44
6	*Oesophagostomum sp.*	6	2.00±1.00
7	*Haemonchus sp.*	2	4.00
8	*Ascaris sp.*	22	12.90±27.00
9	*Taxocara sp.*	4	1.50±0.70
10	*Enterobius vermicularis*	10	6.50±3.78
11	*Trichostrongilodes sp.*	4	5.50±3.53
	Trematodes		
1	*Fasciola hepatica*	1	12.00
2	*Schistosoma mansoni*	6	1.00
	Cestodes		
1	*Diphyllobothrium sp.*	14	7.70±12.57
2	*Moniezia sp.*	12	9.90±7.86
3	*Hymenolepis nana*	8	4.25±2.06
4	*Tenia sp.*	10	1.50±0.57
	Protozoa		
1	*Coccidia sp.*	7	3.33±2.65
2	*Balantidium coli*	12	3.14±1.86
3	*Entamoeba coli*	4	26.00±2.86
4	*Cyclospora sp.*	6	8.00±4.00
5	*Giardia sp.*	4	8.00±5.65

Area with high conspecific density (bonnet macaque) had higher abundance of endoparasites (33.23±48.02) than in sites with lower density ($F_{2, 89} = 4.663$, $p < 0.012$), but the species richness did not differ between the sites with varying bonnet macaque group density ($F_{2, 89} = 2.203$, $p = 0.116$) (Fig. 3).

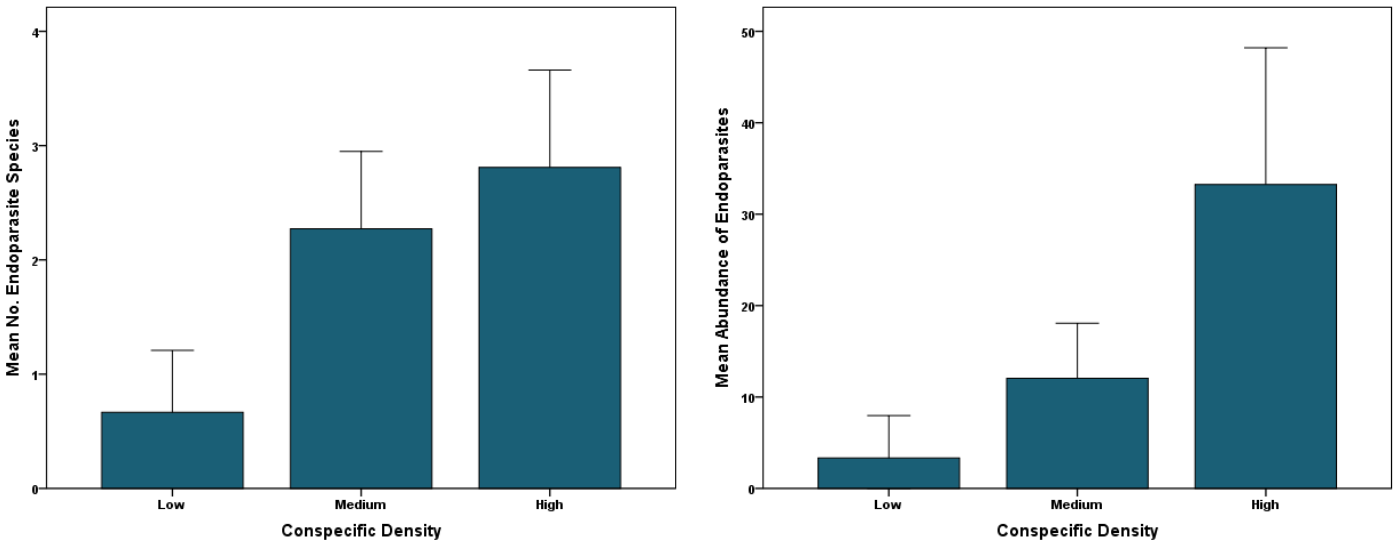


Figure 3Endoparasite richness and their abundance in different degree of conspecific density

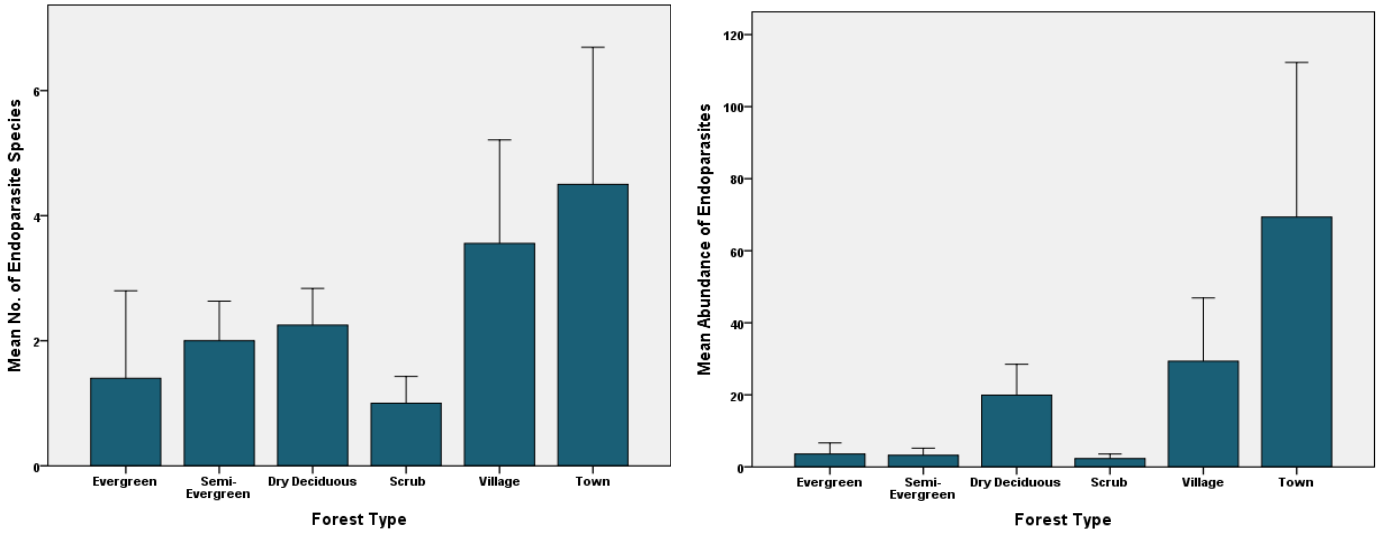


Figure 4 Endoparasite richness and their abundance in different forest and habitat type

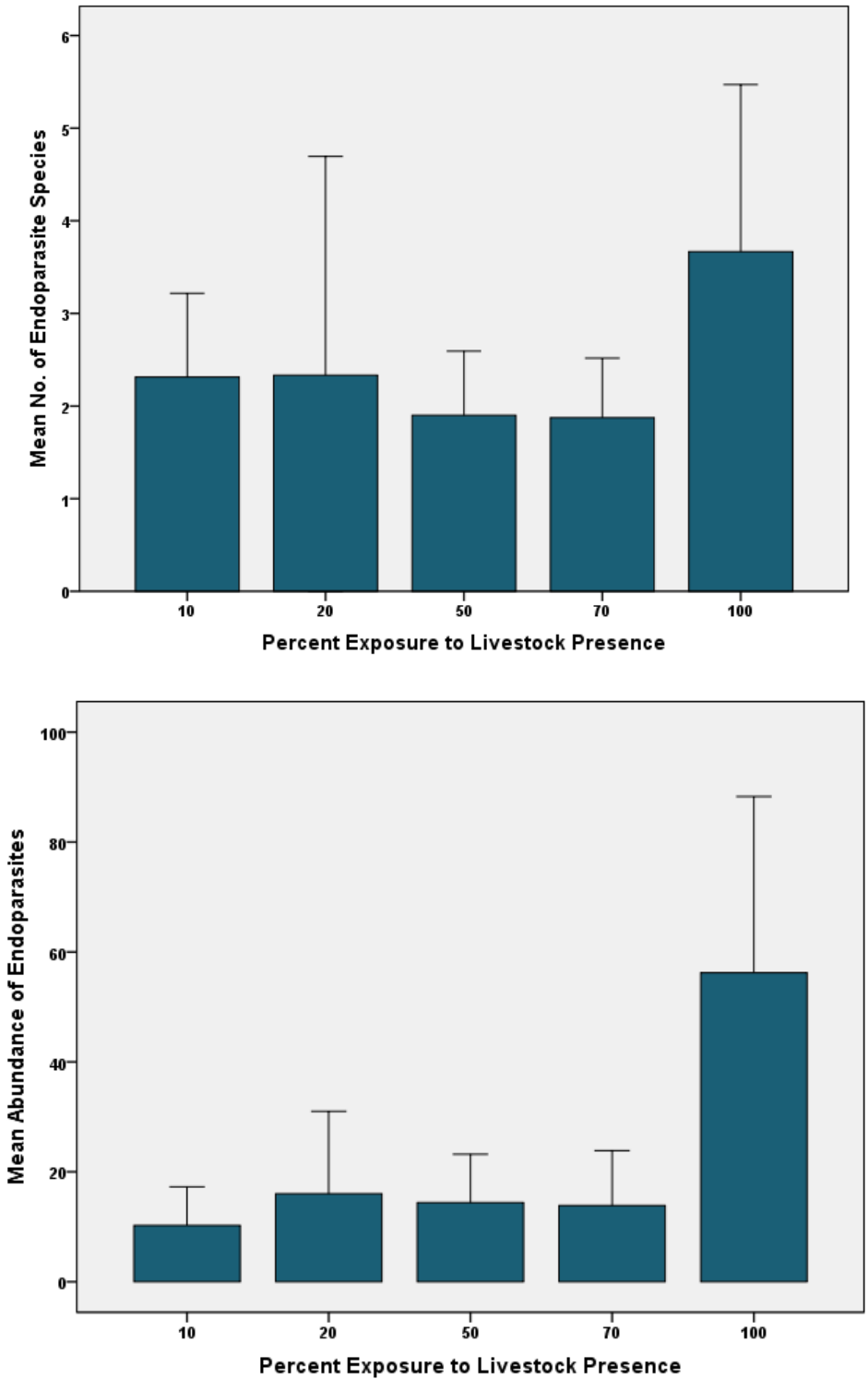


Figure 5 Endoparasite richness and their abundance at different degree exposure to livestock

The town monkeys had the highest endoparasite richness (4.50 ± 3.45) and their abundance (69.33±67.61), and which is followed by village (endoparasite richness: 3.55±3.32 and their abundance: 29.33±35.34) (Fig. 4). Endoparasite richness ($F_{5,\ 86}$ = 5.262, p<0.000) and their abundance ($F_{5,\ 86}$ = 8.846, p<0.000) differed significantly between habitat/ vegetation types. Although endoparasite richness did not differ to the degree of exposure to livestock presence and further the abundance of endoparasites also did not show much variation between the degrees of exposure up to 70% (Fig. 5),however, the abundance of the endoparasites significantly higher in the groups exposed highly to livestock ($F_{4,87}$ = 6.354, p<0,000).

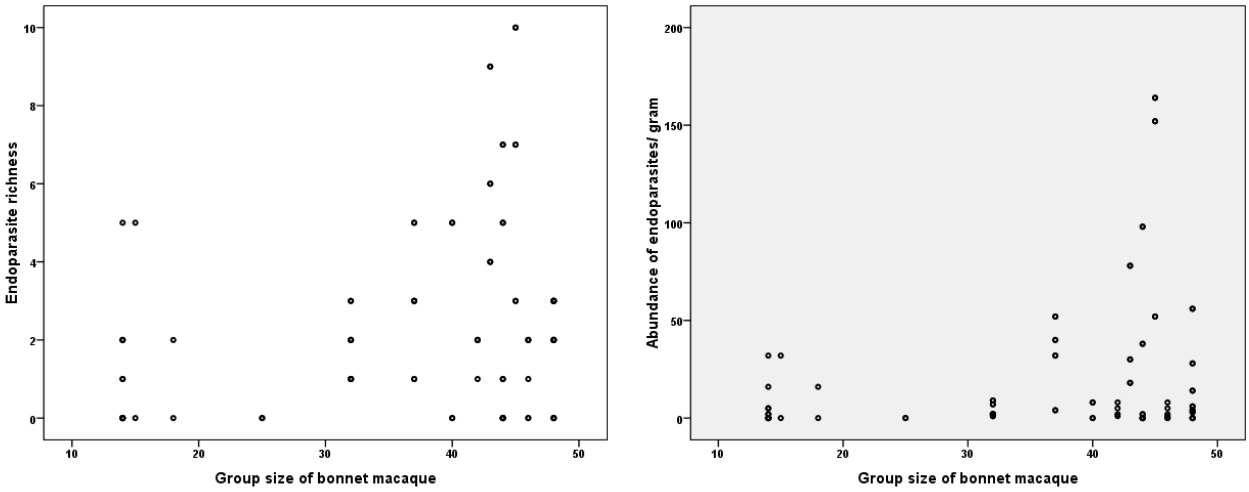


Figure 6 Endoparasite richness and their abundance in varying group sizes of bonnet macaque

Pearson correlation test shows that endoparasite species richness (r^2 =0.258, df = 92, p < 0.013) and their abundance (r^2 =0.247, df = 92, p < 0.018) increases with the increase in group size, but the test shows the strength of relationship is only 25% (Fig. 6). Conversely, endoparasite species richness (r^2 = - 0.276, df = 92, p < -0 .008) and their abundance (r^2 = 0.275, df = 92, p < -0.008) decreases with the increase in altitude (Fig. 7).

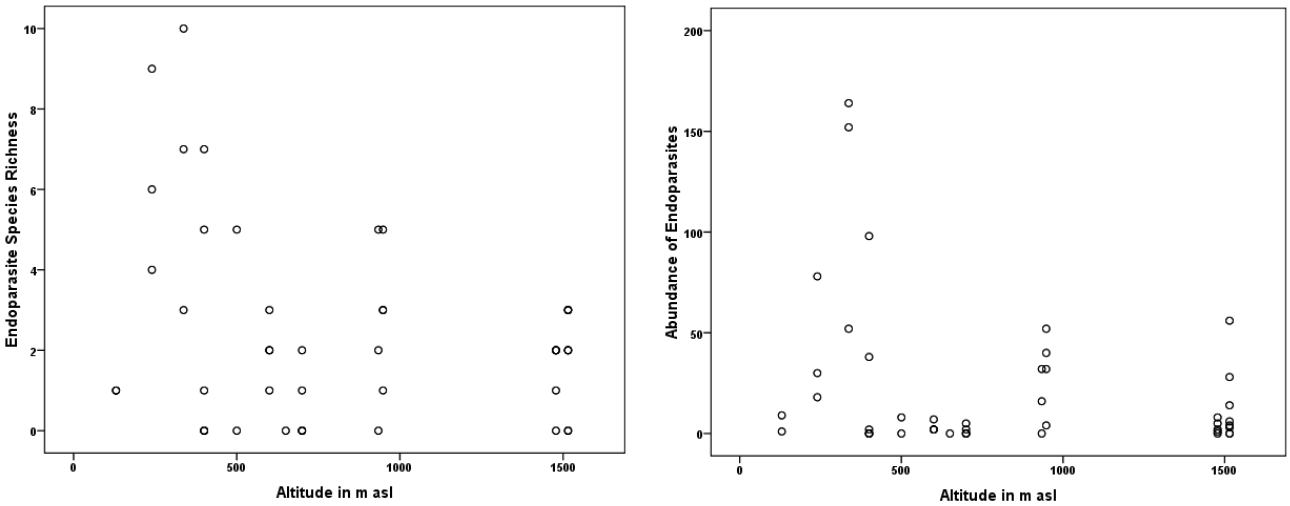


Figure 7Endoparasite richness and their abundance in different altitude

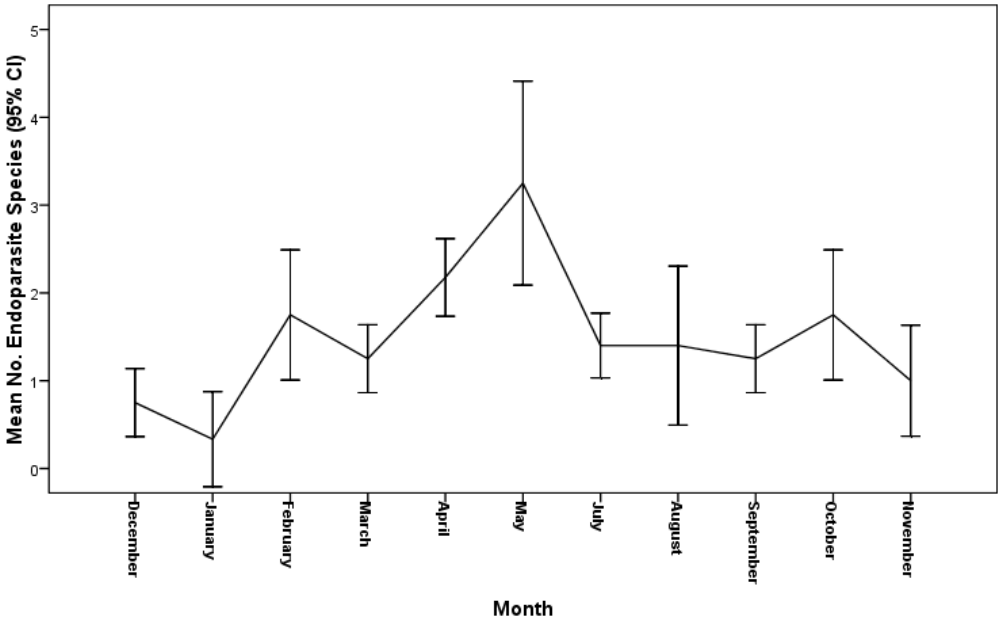
Temporal study: A total of 184 samples were collected from single group monitored for long-term from Sirsi-Honnavara range in Karnataka. Out of them a total of 116 samples were processed and analysed for the prevalence of endoparasites. The findings show that eight nematode species, one trematode species, four cestode species and two protozonas (Table 2).

The study group of bonnet macaque had the highest endoparasite richness (3.25±1.38) and their abundance (30.75±21.21) in the month of May than in other months. Endoparasite richness ($F_{10,\ 105}$ = 5.461, p<0.000) and their abundance ($F_{10,\ 105}$ = 6.402, p<0.000) differed significantly between months (Fig. 8).

Although immature had the highest endoparasite richness (1.78±1.22) and their abundance (9.35±11.82) than adult male and adult female but did not differ significantly (richness: $F_{2,\ 113}$ = 0.226, p = 0.798; abundance: $F_{2,\ 113}$ = 0.192, p = 0.825) (Fig. 9).

Table 2 Prevalence of endoparasites in fecal samples from temporal study group of bonnet macaque

No.	**Parasites**	Prevalence in number of samples	Mean no. of prevalence per sample
	Nematodes		
1	*Strongylus sp.*	4	1.50±0.70
2	*Strongyloides sp.*	19	3.63±3.98
3	*Trichuris sp.*	9	9.11±10.83
4	*Ancylostoma sp.*	2	2.00
5	*Bunostomum sp.*	2	9.00±9.89
6	*Oesophagostomum sp.*	25	5.44±10.57
7	*Ascaris sp.*	11	5.54±8.67
8	*Trichostrongilodes sp.*	1	2.00
	Trematodes		
1	*Schistosoma mansoni*	2	1.00
	Cestodes		
1	*Moniezia sp.*	1	1.00
2	*Hymenolepis diminuta*	1	1.00
3	*Hymenolepis nana*	3	5.00±5.21
4	*Tenia sp.*	2	4.50±3.53
	Protozoa		
1	*Coccidia sp.*	3	4.33±5.77
2	*Entamoeba coli*	3	1.33±0.57



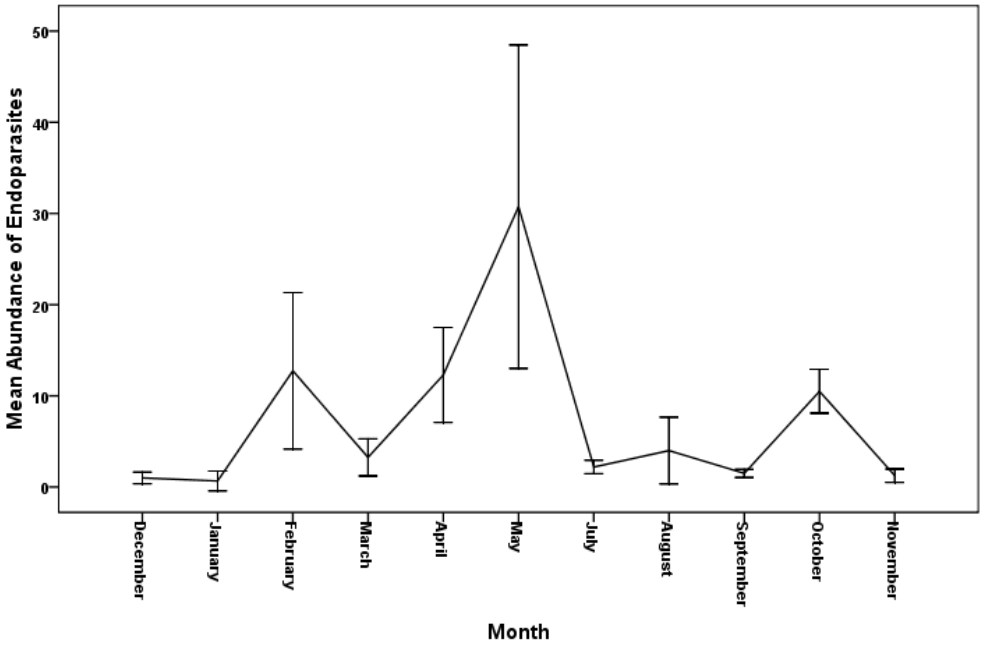


Figure 8 Endoparasite richness and abundance in bonnet macaque group in different months

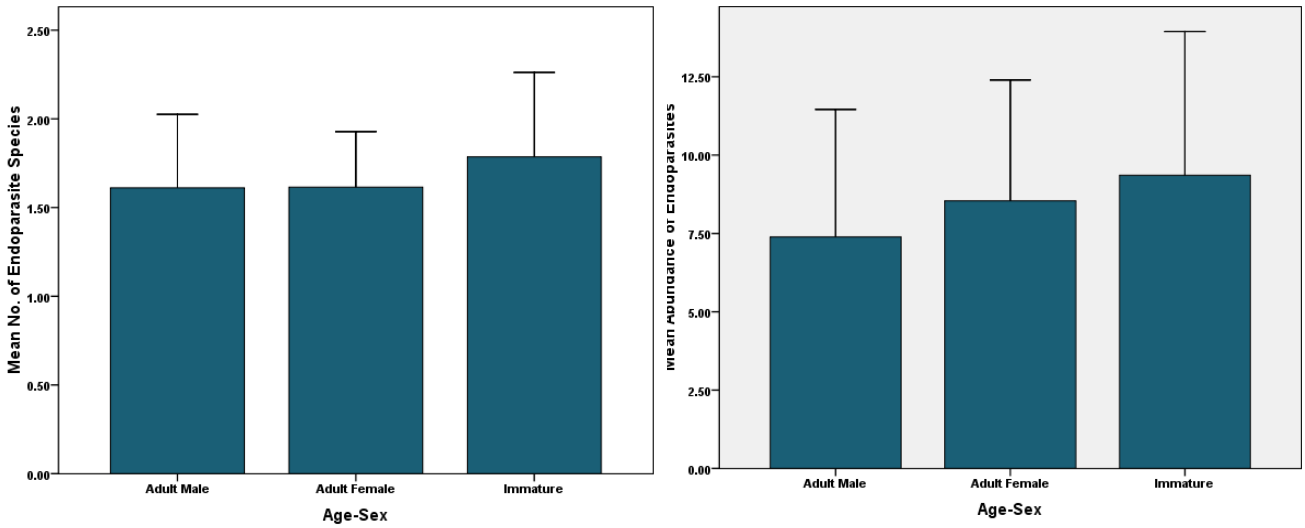


Figure 9 Endoparasite richness and abundance in different age-sex of the bonnet macaque group

Discussion:

The present study reveals that the endoparasite richness and abundance in bonnet macaque differed across different sampling sites. Further, locations with high density of macaques and highly exposed to livestock and macaques in town showed highest endoparasite richness and abundance. Endoparasite richness and abundance increased with increase in group size, but decreased with altitude. Immature had highest endoparasite richness and abundance than adults. The temporal study shows that endoparasite richness and abundance is high during the peak summer (April and May month of the year).

Directly transmitted parasites in primates was positively affected by host population size, density, and group size (Mbora and McPeek 2009; Hussain 2013), and it is shown that there will be more diverse in the infection in accordance with the host population size (Freeland 1979; Stuart et al. 1993; Cote and Poulin 1995; Arneberg et al. 1998; Morand 2000; Roberts et al. 2002; Poulin and Morand 2004; Bagge et al. 2004; Chapman et al. 2005a, b; Snaith et al. 2008). This pattern is common in group living primate species (Fuentes 2007) which enables the

transmission of endoparasites from individual to individual within the population very rapidly (Brown and Brown 1986; Moller 1993). The parasite richness and density increases with increase in the density of conspecifics. Similarly, endoparasite richness and abundance increased with increase in conspecifics density in bonnet macaque.

Altitude is expected to have negative influence on endoparasite prevalence. Ejima et al. (2011) reported decrease in endoparasite prevalence in increase in altitude in Japanese macaques in Yakushima *Macaca fuscata yakui.* In contrast to this, collective effects of anthropogenic activity, climate change, and expansion in the ranges of many vectors are reported to occur in many vector borne pathogens of wildlife and humans in higher altitude (Gratz 1999; Mellor et al. 2000). However, yet bonnet macaque shows higher endoparasite prevalence only in lower altitude than in higher altitude.

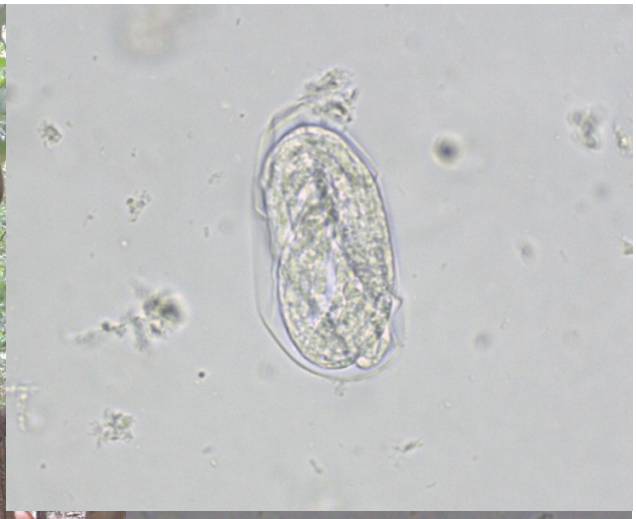
Endoparasite richness and their abundance were higher in the dry season compared to other seasons, which have also been reported in elephants *Elephas maximus* of south India (Watve 1992; Vidya et al. 2002). In contrast to this, endoparasite prevalence in mandrill *Mandrillus sphinx* was lower in the dry season than in the other seasons (Setchell et al. 2006). The endoparasite prevalence in Chimpazees *Pan troglodytes schweinfurthii* in Mahale mountains was higher in wet season (Haffman et al 1997), where there was no seasonal variation in Savanna Chimpanzees *Pan troglodytes schweinfurthii* (Kalousová 2014). Differential impact of season on endoparasite prevalence is reported in different species. In tropical forests, the variation in food availability may have influence on habitat use and exploration for food resources in bonnet macaques might have lead to higher endoparasite prevalence than in the other seasons.

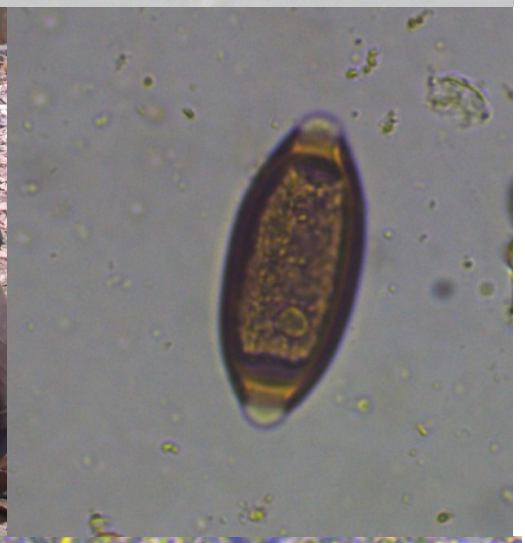
The life style of each primate species is expected to have different degree of endoparasite infection. For e.g. adult females had higher endoparasite prevalence than in other age-sex individuals in baboons *Papio sp*. (Hausfater and Watson 1976) and Sumatran Orangutans *Pongo abelii* (Mul et al. 2007), and it was attributed to stress and lack of nutritional values in females (Gulland 1992; Milton 1996). In contrast, adult males had higher endoparasite prevalence in howler monkeys *Aloutta palliata mexicana* and *A. pigra* (Trejo-Macias et al. 2007), red colobus

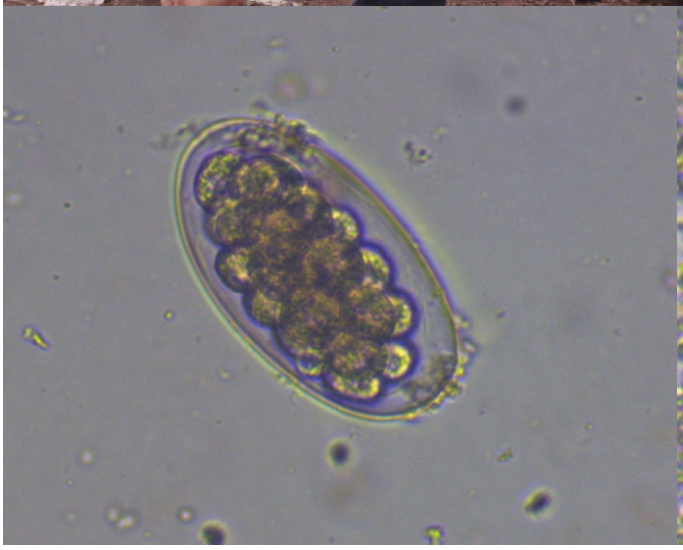
monkeys *Procobus rufomitratus* (Mbora and Munene 2006) and rhesus macaques *Macaca mullata* (Knezevich 1998), but there was no variation in parasite prevalence among different age-sex individuals in mandrills *Mandrillus sphinx* (Setchell et al. 2007). Where, endoparasite prevalence was more in younger individuals than in adults in olive baboons *Papio cynanocephalus anubis* (Muller-Graf et al. 1996), Japanese macaques *Macaca fuscata fuscata* (Horii et al. 1982) and rhesus macaque in Cayo Santiago (File and Kessler 1989; Knezevich 1998), similarly in bonnet macaque also. The probable reason for higher endoparasite prevalence among younger individuals may be due to their higher explorative nature.

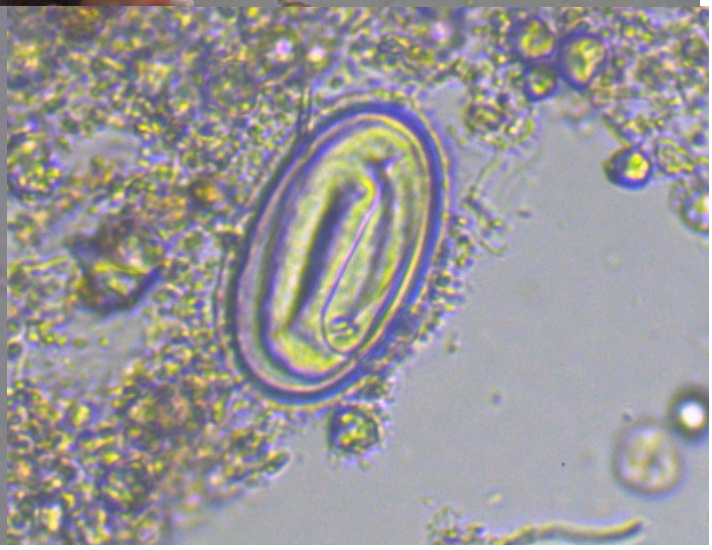
In the present study *Ascaris sp*., *Strogyloides sp., Oesophagostomum sp., Trichuris sp. and Diphyllobothrium sp*. were the most widely spread and abundant in the samples. The definitive host of all these endoparasites includes humans, many livestock species and other wild mammals. Thus, any wild animal living in the human and livestock dominated landscape are much more prone to having high infection of many endoparasite (Mbora and McPeek 2009; Hussain 2013). As shown in the current study, the monkeys living in such human-livestock dominated landscape like town monkeys had higher parasite prevalence than in the other habitat types. Similarly, parasite richness was higher in the crop raiding howler monkeys which has higher interface between human and livestock than the wild foraging monkeys (Weyher et al. 2006).

Higher endoparasite prevalence and their load in the animals are always the concern of their health and transmission of the same to other animals, which can be fatal to the animal. Further, relocation of most commensal bonnet macaque to wild habitat without understanding their health and infections is another issue in the management and conservation of wild animals. It is apparent that the endoparasite prevalence is higher in groups in certain environmental conditions that require to be considered while relocating them to any other habitat. Further, it is suggested to treat them before releasing of them to the new habitat and avoid the relocation programme during the dry season.









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