

**Macro (helminth) and Micro (parvoviral) parasites in the
Asiatic lions, *Panthera leo persica* of Gir National Park and
Wildlife Sanctuary, India.**

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EXECUTIVE SUMMARY

The critically endangered Asiatic lion (*Panthera leo persica*) is now restricted to a single, small population in the wild. Thus, its relict demographic status, restricted geographical range, low genetic variability and increased anthropogenic pressures make it vulnerable to stochastic events such as epidemics and newly emerging diseases. Further, co-evolved diseases, both endemic and epidemic, are known to have an effect on the hosts ecology. Thus, monitoring of diseases and disease-causing parasites in this endangered population has huge ecological, as well as conservation implications. Due to the paucity of such critical information, I attempted this study to provide baseline data on selected disease-causing parasites in the free-living Asiatic lion populations of Gir, in the state of Gujarat, India. The study concentrated on endoparasitic helminth communities (macro parasites), as well as parvoviruses (micro parasites) in the wild Asiatic lions. In the first part of this study, I looked at the ecology of helminth communities; further, I tried to quantify the levels of infection in order to determine parasitic loads, as well as to compare different regions of the study area. In the second part of the study, I attempted to detect the presence of parvovirus in lions and subsequently, determine its relationships with other strains of parvovirus from around the world. The field-based component of the study, which involved non-invasive sampling methods, was conducted at the Gir National Park and Wildlife Sanctuary, in the state of Gujarat, India, from 1st December 2008 to 20th May 2008. This was followed by a laboratory component, which I conducted till the 25th of July 2008 at the National Centre for Biological Sciences, Bangalore. Simultaneously, certain parts of the

laboratory analysis were done at *Indian Immunologicals Limited*, Hyderabad. The study established the presence of 9 helminth OTUs (Operational Taxonomical Units) and a median parasitic load of 7 parasite propagules per gram of faeces (per individual). As expected, the parasite community had a frequency distribution approximating a negative binomial curve. Further, a trend in the geographical distribution of parasites was noticed, where the scat samples from the eastern region of the protected area had a significantly lower parasite load when compared to those from the western region. This could be due to the different anthropogenic factors existing between different parts of the protected area or due to the intrinsic biology of the helminth community.

The second part of the study established the presence of parvovirus in about 15% of the total scat samples analysed (n=105). Preliminary analysis of this particular strain showed that, contrary to expectations, the parvovirus found in lions was closer to the canine parvovirus than the feline panleucopenia virus. Even though this study couldn't throw light on the origin of this particular strain, it could have important conservation and management implications.

Both these results provide important baseline information for future studies on the role of these parasites in the Asiatic lion population.

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INTRODUCTION

The critically endangered Asiatic lion (*Panthera leo persica*) is now restricted to a single population in the wild, present in the Gir National Park, Gir Wildlife Sanctuary and its surrounding forests. Its restricted geographical location, small size (Gujarat Forest Department Census 2005), lack of genetic variability and the presence of increasing anthropogenic pressures (Chellam 1993) makes it vulnerable to extinction pressures and stresses the importance of its conservation.

Diseases, both endemic and epidemic, are important considerations in such situations and thus, have to be monitored with the necessary management interventions when and if required; in order to ensure the survival of this critically endangered Asiatic lion population over time. Epidemic diseases are responsible for discrete epidemic bursts (Anderson & May 1976, Packer *et al.* 1999), which may be responsible for extinctions, especially in critically endangered populations (Thorne & Williams 1988). On the other hand, endemic diseases are known to have a significant effect on the ecology of the host species. Diseases, in general, are known to regulate host population numbers (Anderson 1978, Scott & Dobson 1989); affect the genetic diversity in a population, as well as have significant effects on host health, behaviour; reproductive success, sexual selection, cub mortality; host competition and host predation strategies (Freeland 1983, Lafferty 1992, Jog & Watve 2005a, Jog & Watve 2005b, Jog & Watve 2005c). Another important scenario deals with the emergence of new diseases in a population. When small and restricted populations are exposed to such emerging diseases for which they

haven't developed any form of immunity, the possibility of extinction or a near-extinction event cannot be ruled out.

Diseases are caused by both macro and micro parasites. In this study, I looked at one macro-parasitic community, namely endoparasitic helminths and another micro parasite, namely the lion parvovirus. Whereas the former is an endemic parasite, the latter generally falls under the epidemic category.

There have been very few studies, which have looked at the prevailing endoparasitic patterns in free ranging lions. However, some studies have documented the endemic parasites of lions, particularly in Africa. Endoparasites such as *Spirometra* spp. (Muller-Graf 1995, Muller-Graf 1998), taenids (Muller-Graf 1995), *Toxocara* spp., *Trichuris* spp., *Ancylostoma* sp. (Muller-Graf 1995), trichostrongylids, spirurids, *Physaloptera* spp., coccidians (Muller-Graf 1995), sarcocystis and *Giardia* spp., have been described from free-ranging African lions. Endemic parasites of free-ranging Asiatic lions, on the other hand, has received very little attention. Watve (pers comm., unpublished data) has demonstrated the presence of diphyllbothrid, taenid, ascarid, strongyle, hookworm and isosporid eggs from the scat of wild lions in Gir. In captive Asiatic lions, there are accounts of *Ascaris* spp. (Sabapara 2002), *Toxocara* spp. (Hase *et al.* 2007, Ravindran *et al.* 2006), *Toxoscaris* spp. (Ravindran *et al.* 2006), *Strongyloides* spp. (Ravindran *et al.* 2006), *Ancylostoma* spp. (Sabapara 2002), *Spirometra* spp. (Sabapara 2002), *Taenia* spp. (Sabapara 2002), *Schistosoma* spp. (Ravindran *et al.* 2006), *Balantidium* spp. (Hase *et al.* 2007) and *Isospora* spp. (Ravindran *et al.* 2006, Sabapara 2002). However, parasitic communities in captive lions do not reliably reflect those in free-living lions

(Muller-Graf 1995).

On the other hand, no study has looked at the presence of parvoviral infections in the Asiatic lion population. This infection, which is characterised by severe gastroenteritis, fever, anorexia, vomiting, diarrhoea and marked leucopenia, leading to the death of the animal (Carter & Wise 2005), has, however, been documented in free living lion populations of Africa (Packer *et al.* 1999). It has also been documented in captive lions, of Asian and African sub-species, as well as in Afro-Asian hybrids (Ramanathan *et al.* 2007). The study of this particular virus in wild Asiatic lions is important because parvoviruses, in addition to having the capability of causing discrete epidemics are fast evolving organisms and hence, may be possible ancestors for an emergent, potentially pathogenic strain in the future.

The first part of my study looked at helminth communities in the Asiatic lion populations, its frequency distribution patterns across the landscape and the presence of any possible differences in parasitic loads and diversity in different regions of the park. In the second part of the study, by employing molecular methods, I looked at the presence of lion parvovirus in this population and tried to determine its relationships with other parvoviral strains found with across the world.

I conducted the study in Gir National Park and Gir Wildlife Sanctuary (20° 57' N to 21° 20' latitude and 70° 27' to 71° 13' E longitude), located in the Gujarat State of India. The study involved a field component, which was conducted from 1st December 2007 till 20th May 2008, followed by a laboratory component, which continued till 26th June

2008. I used non-invasive methods to collect field samples; the sedimentation-floatation (zinc sulphate) method and McMasters methods for parasitology laboratory work and molecular genetic methods for the virology laboratory work.

The thesis comprises two manuscripts, the first regarding the helminth communities in the Asiatic lion population and the second regarding the lion parvovirus in the same population. Both these manuscripts are targeted for the *Journal of Tropical Ecology*.

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The magnificent Asiatic lion (*Panthera leo persica*)

Helminth communities in free-living Asiatic lions in Gir National Park, India

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ABSTRACT

Helminth (endoparasitic) communities in the critically endangered wild Asiatic lions (*Panthera leo persica*), from Gir Wildlife Sanctuary and National Park, Gujarat, India were studied, using non-invasive, scat-based sampling methods. Nine different types of helminth parasites were identified. The median intensity of parasite propagules was found to be 7 propagules per gram of faeces. When a median test, followed by a chi-square test was performed to compare the parasitic loads present in various subdivisions of Gir, it was found that those from the eastern part of the wildlife sanctuary had a significantly lower level of helminth load when compared to the western part.

Keywords: Asiatic lion (*Panthera leo persica*), endoparasite, helminths, diphyllbothriidae, abundance, diversity, patterns, population, scat, non-invasive faecal, stress indicators.

INTRODUCTION

The Asiatic lion (*Panthera leo persica*), whose historic range extended across the Asian continent from Palestine to India (Divyabhanusinh 2005), is now restricted to a single population in the wild, present in the Gir National Park, Gir Wildlife Sanctuary and the surrounding forests. Further, it is the largest carnivore of this region. Thus, conservation of the Asiatic lion is extremely vital, which is reflected in the IUCN Red List classification, where it is mentioned under the ‘critically endangered species’ category.

The Asiatic lion, like all wild populations, is susceptible to both epidemic and endemic diseases. While epidemic diseases in wild populations have received the most attention, especially after the canine distemper epidemic in the Serengeti lion population (Roelke-Parker *et al.*1996), endemic diseases are important too. An increasing volume of information indicates the importance of endemic parasites in the ecology of the biology of host ecosystems. Endemic parasites, which have co-evolved with the host, are important in regulating host population densities (Anderson 1978, Scott & Dobson 1989) and maintaining genetic diversity. Further, they are known to have significant influences on host health, behaviour; reproductive success, sexual selection, cub mortality; host competition and host predation strategies (Freeland 1983, Lafferty 1992, Jog and Watve 2005-a, Jog and Watve 2005-b, Jog and Watve 2005-c).

None of the studies which have indicated a casual relationship between endemic infections and ecological parameters have looked at the relationship of parasites and lion hosts. However, some studies have documented the endemic parasites of lions,

particularly in Africa. Endoparasites such as *Spirometra* spp. (Muller-Graf 1995, Muller-Graf *et al.* 1998), taenids (Muller-Graf 1995), *Toxocara* spp., *Trichuris* spp., *Ancylostoma* spp. (Muller-Graf 1995), trichostrongylids, spirurids, *Physaloptera* spp., coccidians (Muller-Graf 1995), sarcocystis and *Giardia* spp., have been described from free-ranging African lions. Of these, certain species like *Spirometra* spp., Taeniids, *Ancylostoma* spp. and coccidians are prevalent in higher proportions of the lion population (Muller-Graf 1995). For lions of the Serengeti and the Ngorongoro Crater, the median intensity of infection (for *Spirometra* spp.) was found to be 975 propagules per gram of processed faecal pellet (Muller-Graf *et al.* 1998).

Endemic parasites of free-ranging Asiatic lions have been much more poorly documented. Watve (pers. com, unpublished) has demonstrated the presence of diphyllbothrid, taenid, ascarid, strongyle, hookworm and isosporid eggs from the scat of wild lions in Gir. In captive Asiatic lions, there are accounts of *Ascaris* spp. (Sabapara 2002), *Toxocara* spp. (Hase *et al.* 2007, Ravindran 2006), *Toxoscaris* spp. (Ravindran 2006), *Strongyloides* spp. (Ravindran 2006), *Ancylostoma* spp. (Sabapara 2002), *Spirometra* spp. (Sabapara 2002), *Taenia* spp. (Sabapara 2002), *Schistosoma* spp. (Ravindran 2006), *Balantidium* spp. (Hase *et al.* 2007) and *Isospora* spp. (Ravindran 2006, Sabapara 2002). However, parasitic communities in captive lions do not reliably reflect those in free-living lions (Muller-Graf 1995).

My study aims to determine the existing helminthic endoparasite communities, and the intensity of infection, in the only surviving, free ranging population of Asiatic lions. The three sections of the Gir Protected Area also provide an opportunity to compare the

nature of the endoparasitic community across the Gir protected landscape.

STUDY AREA

This study was carried out in the Gir Wildlife Sanctuary and National Park (20° 57' N to 21° 20' latitude and 70° 27' to 71° 13' E longitude), Saurashtra region, Gujarat State, India. Spread across an area of 1412 km², this is the only remaining habitat for the wild Asiatic lion (*Panthera leo persica*). For the purposes of this study, this contiguous region can be classified into 3 sub-divisions – Gir West, Gir National Park Area and Gir East. These sub-divisions roughly correspond to the three management regimes – Gir West, Gir National Park and Gir East. These sub-divisions differ in terms of geo-climatic attributes (vegetation, topography, water availability, and rainfall), predator-prey densities and anthropogenic pressures (human settlement densities, tourist pressure; Khan *et al.* 1996, Meena 2008).

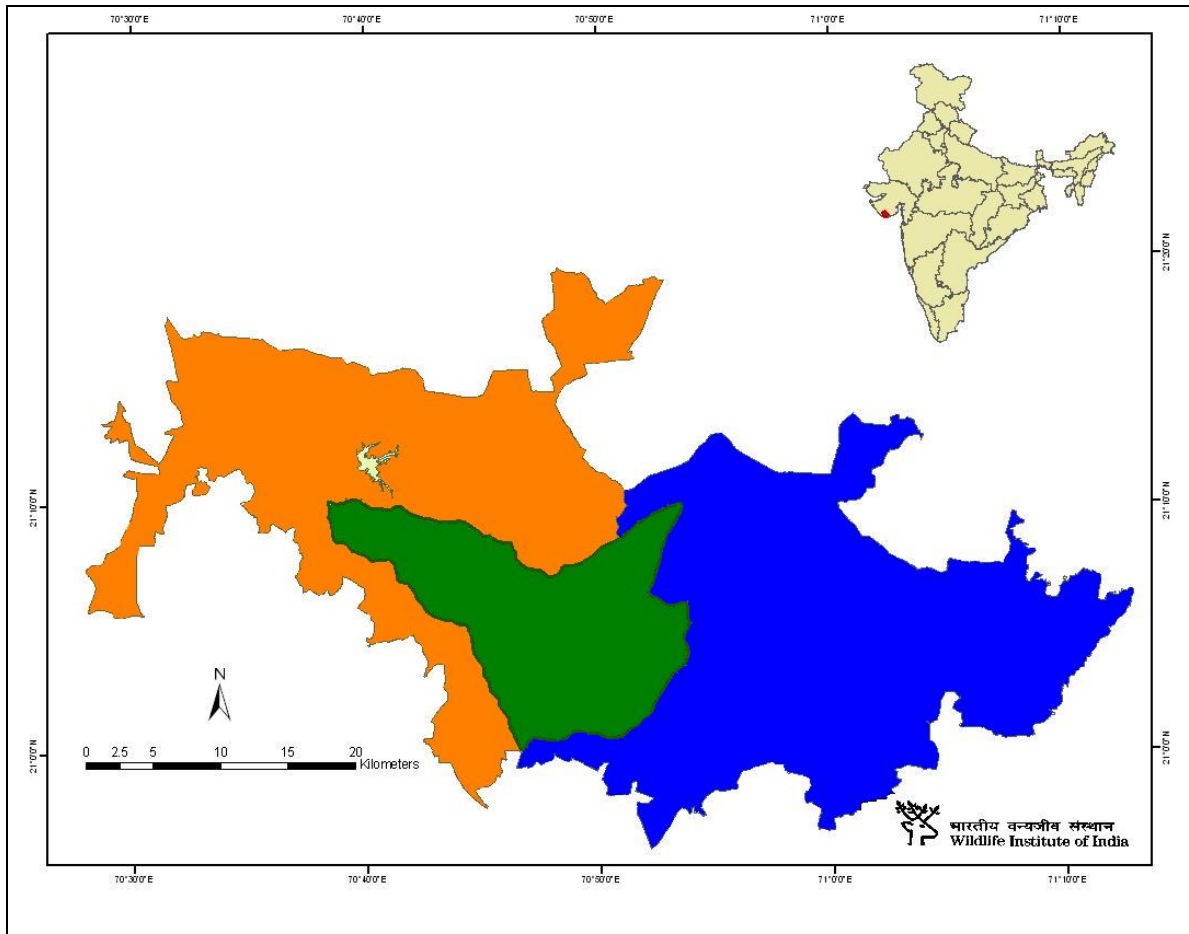


Figure 1: Map showing Gir National Park (colored green) and Gir Wildlife sanctuary. The three study regions are marked accordingly: Gir west (orange), Gir National Park (green) and Gir east (blue).

The vegetation of Gir National Park and Wildlife Sanctuary is mostly tropical dry deciduous forests, interspersed with tropical thorn forests (Champion & Seth 1968). Within this protected area, the moderately wooded Gir West and the densely wooded National Park Area are dominated by teak forests whereas Gir East is dominated by open wooded grassland and thorny *Anogeissus* spp. - dominated forests (Khan *et al.* 1996). The rainfall levels and water availability are the highest in Gir West, moderate in the National Park area and the lowest in Gir East.

This diverse region supports an impressive assemblage of large mammals such as the Asiatic lion (*Panthera leo persica*), leopard (*Panthera pardus*), sambar (*Rucervus unicolor*), chital (*Axis axis*) and nilgai (*Boselaphus tragocamelus*), with the lions being the top predators. The lion population densities are the highest in Gir East, followed by Gir West and then, the National Park area. The domestic prey population densities follow a similar pattern. They are the highest in Gir East, followed by Gir West and are negligible in the National Park area. However, the wild prey population densities are the highest in the National Park Area, followed by Gir West and the lowest in Gir East (Khan *et al.* 1996).

The human settlement densities are the highest in Gir West, lower in Gir East and negligible in the National Park Area. However, most wildlife tourists visit Gir West and most pilgrim tourists visit Gir East.

Table 1. Characteristics of the three sites in the study area

	Gir West	Gir National Park Area	Gir East	Reference
Rainfall	High	Moderate	Low	Meena 2008
Vegetation	Teak-dominated forests	Teak-dominated forests (more dense than gir west)	Dry, savannah (thorny) forests	Meena 2008 Khan <i>et al.</i> 1996
Canopy Cover	Moderately closed	Relatively closed	Relatively open	Meena 2008
Lion population density	Relatively* moderate	Relatively low*	Relatively high	* Meena 2008
Cattle (domestic) density	Moderate	Low	High	Chellam 1993
Livestock in scat	14 % *	14 % *	14 % *	*Meena 2008
Prey (wild) density	Moderate	High	Low	Khan <i>et al.</i> 1996
Wild prey in scat	86 % *	86 % *	86 % *	*Meena 2008
Wildlife Tourist Pressure	High	Nil	Low	Gujarat Forest Dept.
Pilgrim Tourist Pressure	Low	Nil	High	Gujarat Forest Dept.
Settlers and maldhari population	High	Nil	Moderate	Khan <i>et al.</i> 1996, Chellam 1993

This protected area was selected for the study since it is the only region with a surviving

population of wild Asiatic lions and thus, has a high conservation value.

METHODS

Field methods

Lions prefer to use roads and trails as travel routes (Chellam, R. pers. com. unpub.) and are likely to leave scats and tracks on such routes. Thus, to maximise the collection of fresh scats, sampling was conducted periodically on all available (motorable) roads and tracks within the protected area. In addition to these roads and tracks, dry river beds were also used to collect fresh scat samples. Individual lions were also followed, wherever possible, to collect fresh faecal samples.

Lions are also known to defecate near kills. Thus, whenever a kill was noticed, the individual lions (which were feasting at the kill) were intensively observed and scat samples were collected immediately after defecation. If the lion was not present near a kill, sampling for scat samples was carried out in an area (of about 50 meter radius) around the kill.

Sampling was done from 1st December, 2007 up till 20th May, 2008. Sampling was done by two teams; each of two members. These teams moved along predetermined routes, followed individual animals or made observations at kill-sites throughout the day. Only fresh scats (deposited approximately within the past 12 hours) were collected. The fresh state of the scat was determined by its appearance and the presence of insect activity.

The faecal material was collected using standardised collection protocols. Ten grams of each scat sample was measured and collected with the help of a *pesola spring balance*. The material was taken from 2-3 different regions of the faecal sample. Adequate precautions were taken to prevent contamination of the samples, as well as to prevent the spread of zoonotic diseases. This was done by using disposable gloves, face masks and sterilised collection kits. These samples were transferred into sterile containers, containing 30 ml of 10% formalin. Then, the collected sample was mixed thoroughly until it was a consistent mixture. Each fresh scat sample was labelled on the spot with the sample number, date of collection and the GPS location. Then, the samples were sealed in a labelled plastic zip lock bag.

At each scat collection event, the following details were recorded: date, time of collection, GPS location, locality, approximate distance on the road transect, species, presence of solitary lion/pride, presence of other faeces (old/new) in its vicinity, presence of indirect marks, presence of shade, diameter of the largest faecal bolus, scat colour, scat consistency and the scat odour.

Laboratory detection and quantification of parasites

The detection and quantification of parasites was made at a laboratory in Bangalore, by employing the (zinc sulphate) sedimentation-floatation concentration method, followed by an analysis under a McMaster's counting chamber.

Each faecal sample (in the container) was thoroughly mixed, in order to get a consistent solution. Then, three ml of the solution (containing approximately one gram of the

faecal material) was taken and mixed with about 10 ml of water. This mixture was filtered through a cotton sieve to remove the coarse debris found in faecal matter. The filtrate was centrifuged at 2000 rpm for about 5 minutes. The supernatant was discarded and the sediment was taken. Subsequently, five ml of saturated zinc sulphate solution (specific gravity = 1.18) was added to the sediment and mixed well. Further, the mixture was again centrifuged at 2000 rpm for about 5 minutes. The upper layer of the mixture was taken and deposited in one of the McMaster's chamber. About 2 minutes were allowed for the eggs to float up and then, the processed sample was observed under the microscope for eggs and larval forms.

All parasite propagules under the chamber were recorded. Sizes were measured to the nearest micron by means of a pre-calibrated micrometer. These propagules were photographed and classified as *Operational Taxonomic Units* (OTUs), based on qualitative as well as quantitative features (Watve 1992, Watve & Sukumar 1995). The OTU represents the genus or a higher taxonomic classification, e.g. family. Due to the difficulties involved in identifying endoparasitic species from scat-based egg forms, coupled with the absence of any previous reference studies, it was difficult to identify the species level of the individual parasites. Hence, the concept of an OTU was employed.

Analytical methods

The frequency distribution of helminthic endoparasites in the Asiatic lions was graphically plotted.

The software EstimateS 8.0.0 was used to compute species richness and to plot a species accumulation curve. This was done in order to compare the parasite communities across the three regions of Gir and to verify adequacy of sampling effort. The Jackknife-1 estimator of species richness (Magurran 1986, Chao 1990) was used here.

A median test, followed by a chi-square test was performed to determine whether the median parasitic loads in the national park area and Gir east varied from that found in the West, where the maximum number of samples were collected.

The statistical packages employed were SPSS/PC 11.5 (Norusis, 1990) and R (Version 2.7.1) (R Development Core Team 2007).

RESULTS

Parasitic diversity

At least 9 OTU's were identified from 105 scat samples that were collected from across the landscape (Table 2).

Table 2: Helminth Endoparasites in the Asiatic lion (*Panthera leo persica*) were identified. The Diphyllbothridae OTU was found to infect almost three-fourths of all scats sampled.

No.	OTU (Parasite)	Percentage of lions Infected
1	Diphyllbothriidae	73.33 %
2	<i>Taenia spp.</i>	5.71 %
3	Round worm larvae	22.85 %
4	Ascarid – I	18.09 %
5	Ascarid – II	4.76 %
6	Trichuridae -1	
7	Trichuridae – 2	2.85 %
8	Unidentified OTU – 1 (Strongyle)	0.95 %
9	Unidentified OTU – 2 (Trematode)	6.66 %
10	Unidentified OTU - 3	3.80 %
	Total	84.76 %

Parasite frequency distribution

The parasite frequency distributions were plotted (Figure 1). The plotted curve approximated to a negative binomial distribution.

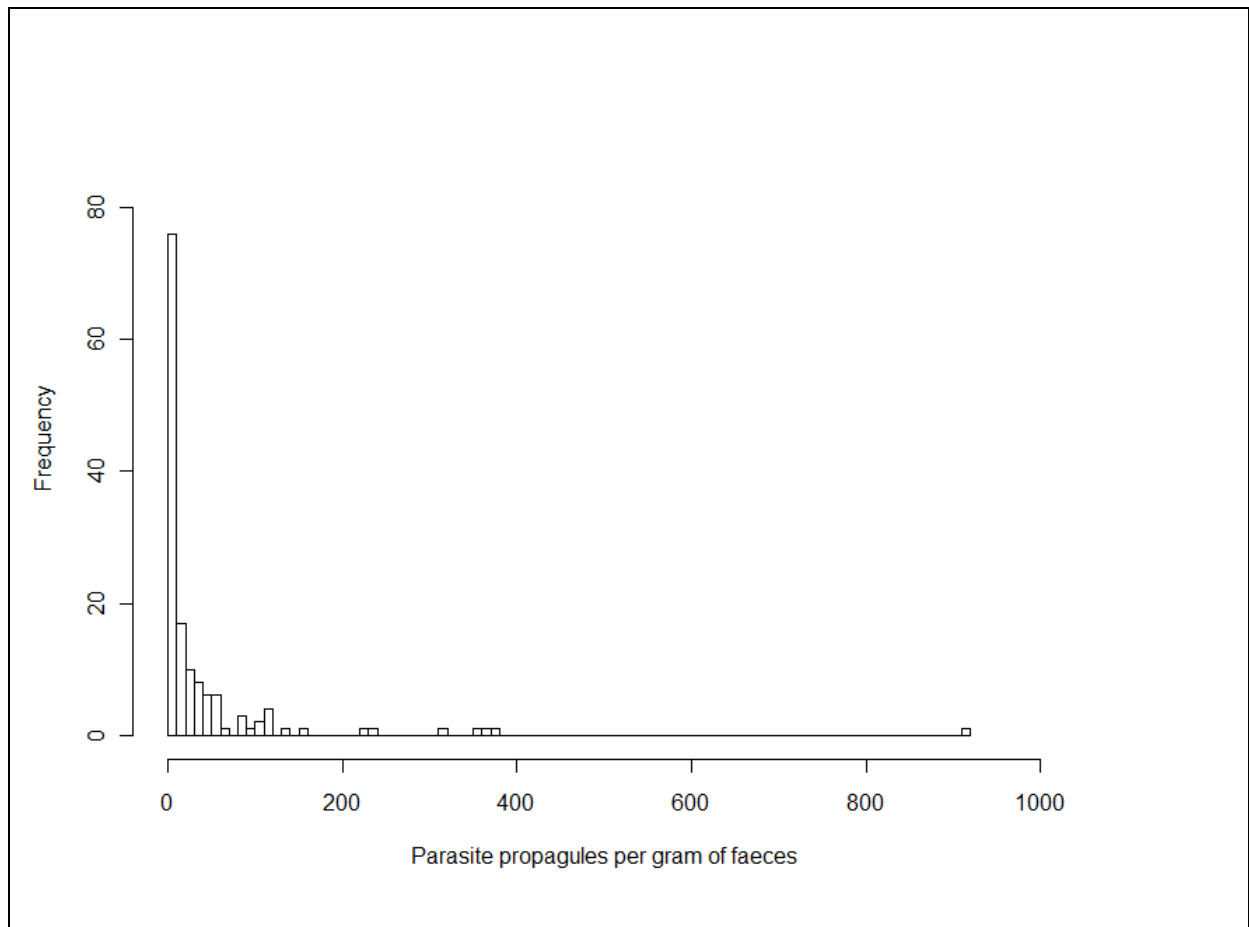


Figure 1. The parasite frequency distribution approximated to a negative binomial curve.

When the rarefied species accumulation curve was plotted, the curve tended towards an asymptote, which indicates that the sampling effort was adequate (Figure 2).

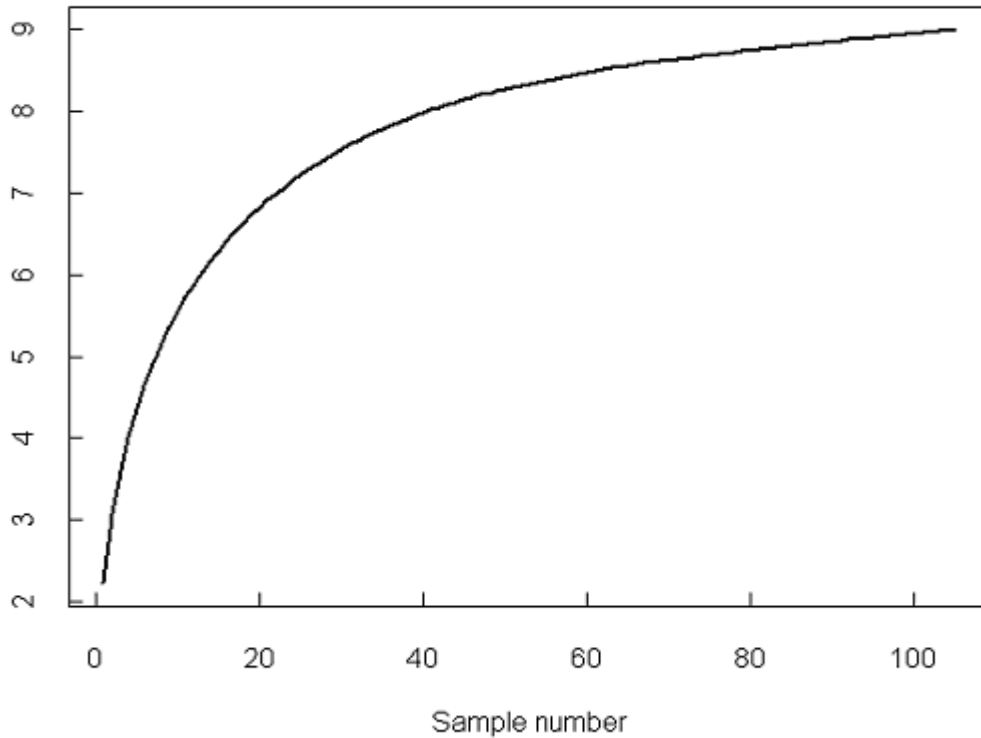


Figure 2. The rarefied species accumulation curve from 106 lion scat samples showed that the sampling effort was adequate. The cumulative species richness is provided on the y-axis.

The species richness was also estimated using Jackknife – 1 species estimator (Figure 3). This was done by employing EstimateS to simulate 99 sample order randomisations. The total species estimated was found to be 9.99 with a standard deviation of 0.99.

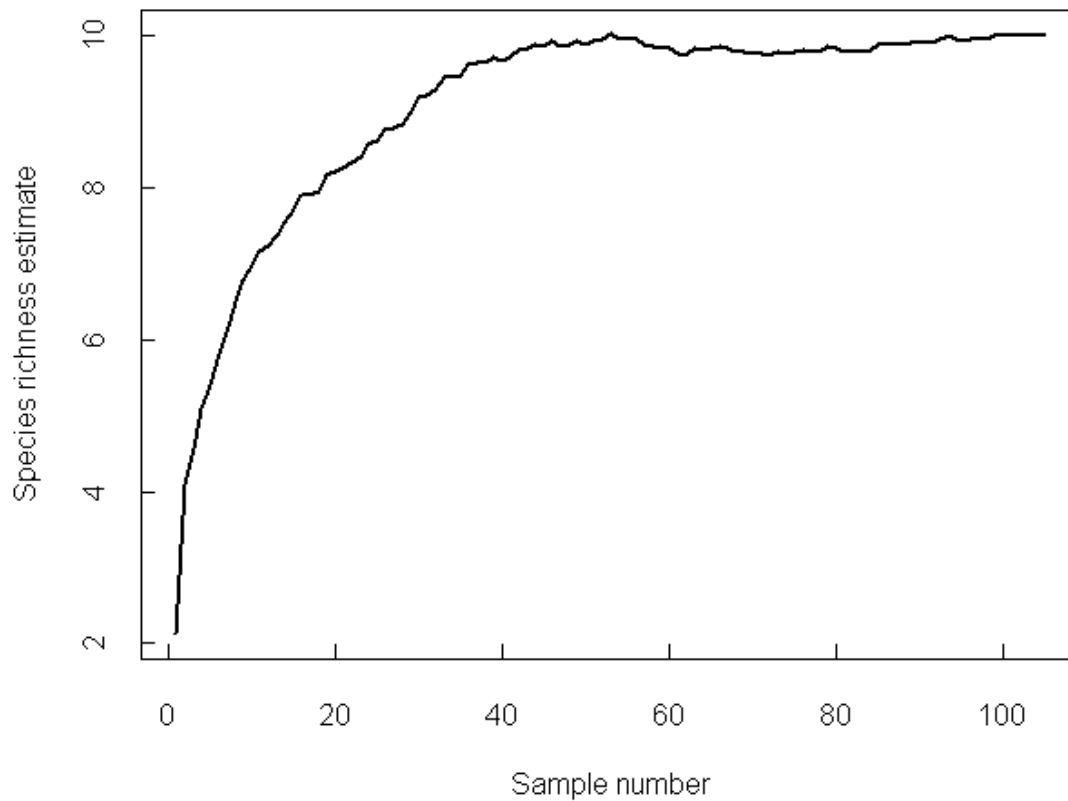


Figure 3. The species richness, which was estimated using Jackknife-1, was found to be 9.99 with a standard deviation of 0.99.

Further, the species richness estimates (from Jackknife-1) across the three regions (Gir West, Gir National Park area and Gir East) were compared (Figure 4). It was found that Gir West had the maximum number of species, followed by Gir National Park region; the lowest estimate was observed in Gir East.

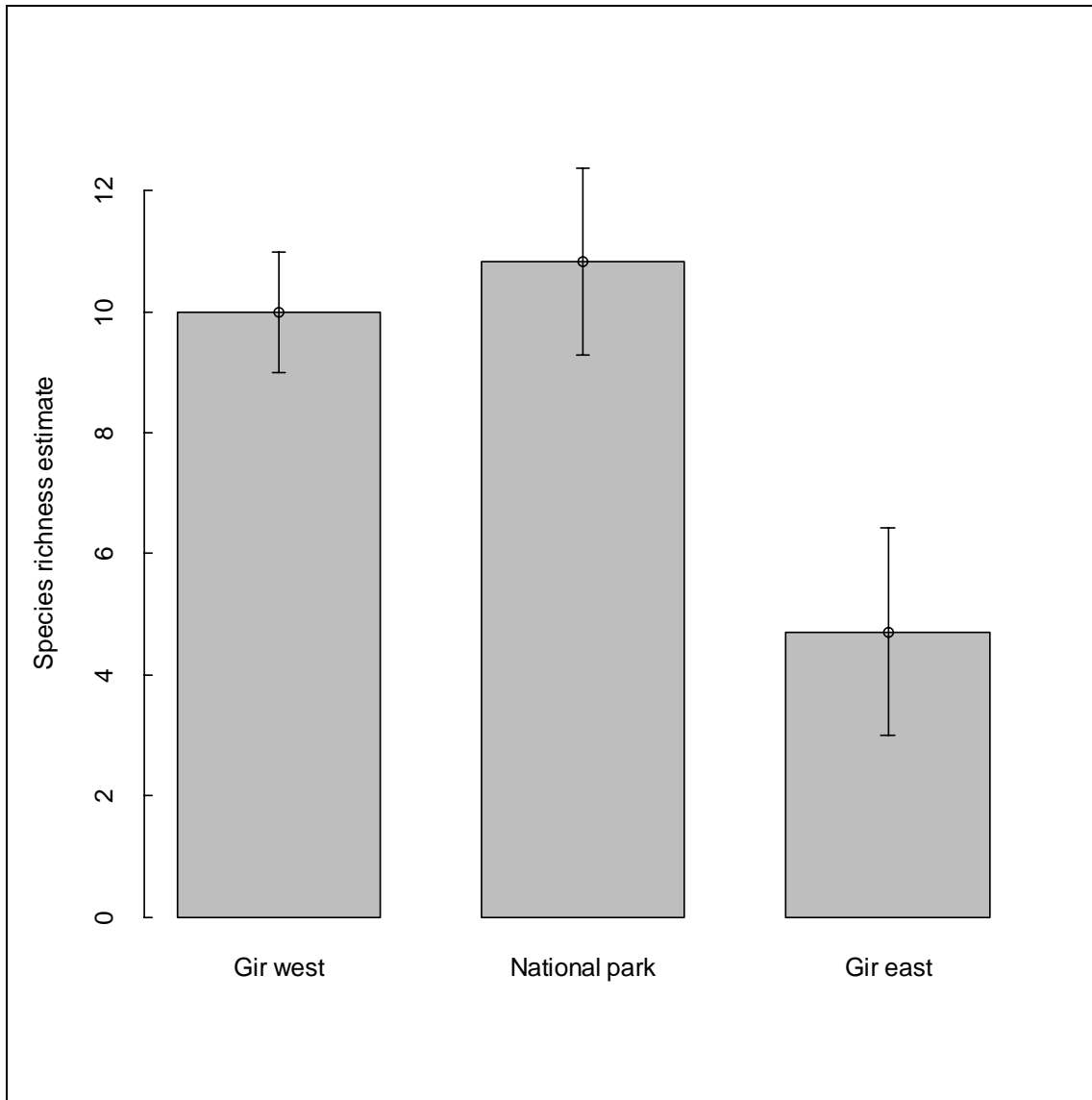


Figure 4. Variations in species richness estimates (using Jackknife -1 estimator) across the three sub-divisions showed that Gir West had the highest number of parasites and Gir East had the lowest number.

Intensity of infection

The intensity of infection (i.e. number of propagules per gram of faeces) was found to range from 0 to 912 propagules/gram of faeces/individual. The median intensity of infection was calculated to be about 7 propagules per gram of faecal sample.

Almost two-thirds of the sampled lion population were infected with the Diphylobothriidae OTU. Further, this OTU also constituted most of the eggs observed per sample.

Differences between Gir east, Gir National Park area and Gir west

A median test, followed by a chi-square test was employed to detect the presence of any statistically significant differences between the populations of Gir West, National Park area and Gir East. Although the scat collected from Gir National Park Area did not show any significant difference from that of Gir West, those collected from Gir East showed a significantly lower load of parasites than that collected from Gir east.

DISCUSSION

In this study, I aimed at documenting the parasites present in the only free ranging population of the Asiatic lion and to assess the levels of infection across the Gir protected landscape.

The parasite frequency distribution obtained was similar to that observed in earlier studies on parasites. Parasites have been known to follow the negative binomial distribution in nature (Schmid & Robinson 1972, Guyatt *et al.* 1990).

The Diphylobothriidae OTU was found in about two-thirds of the sampled population. A similar trend was also observed in the lions of Serengeti and Ngorongoro Crater (Muller-Graf 1995). Further, about 92 % of all the propagules (pooled samples) were found to be of the Diphylobothriidae OTU. The genus of this OTU is most likely to be *Spirometra* spp. This is based on earlier reports of *Spirometra* spp. identified in wild African lions (Muller-Graf 1995, Muller-Graf *et al.* 1998) and captive Asiatic lions (Sabapara 2002). No other diphylobothriid has been reported from lions. However, this cannot be confirmed as only ova were used for identification and it is not possible to identify this species based on the macroscopic structure of ova alone.

The parasite loads were found to range from 0 to 912 parasite propagules gram⁻¹ of faecal material (median = 7). These figures are low when compared to other studies in lions (Muller-Graf 1998) and could be attributed to three factors. Firstly, different methods were used to quantify parasites in earlier studies. The second factor could be that I sampled during the dry season, when the parasite loads are known to be low. Further, my site is in a predominantly dry region and parasite loads are known to be lower in dry regions (Watve M., pers. com. unpub.).

Differences in the species richness estimates and parasitic loads were observed between samples from Gir West and those from Gir East. This could be attributed to either anthropogenic pressures (Landsberg *et al.* 1998) or the biology of the parasite communities; or both. The anthropogenic pressures in Gir West, especially in the tourist area where most of my sampling was done, are higher since it is the only region where tourists are allowed (Gujarat forest Dept., pers. com.). However, the density of

Maldharis in the tourist area of Gir west is comparable to that found in Gir east. Though pilgrims are allowed into a few areas in Gir east, it is highly restricted (in area and time) and thus, may contribute to lower anthropogenic pressure. The biology of the species may also play an important role here. A high proportion of the parasites found in this study were from the Diphyllbothriidae OTU. Thus, the biology of this species may be responsible for the differences in parasitic loads between the two regions. It is a well established fact that Gir east receives significantly lesser amounts of rainfall when compared to Gir west. Since the life cycle of Diphyllbothriidae involves a stage (egg stage) which is water-dependent (Muller-Graf *et al.* 1998), water may be the limiting factor in the eastern region. The Diphyllbothriidae is known to have an indirect life cycle (Muller-Graf *et al.* 1998). It involves two intermediate hosts, the second being a large mammal in wild populations (Muller-Graf *et al.* 1998), which could potentially be the prey species of the lion. Thus, this particular parasite may have intricate relationships with the predator, which need to be further investigated. Further, another study (Dave 2008) had reported that the body condition score of chital, the main prey base of lions, was better in Gir east than Gir west. Thus, this confirms the trends reported in my study.

However, the sampling effort was very low in Gir east (n=7) and the species accumulation curve for this particular area was still in its ascendancy. Thus, these results may only point towards a trend in the geographical distribution of helminth communities in the Asiatic lions of Gir. Another reason is that sampling was performed at the beginning of winter in Gir east but towards the end of winter (and the beginning of summer) in Gir west. This could also be one of the reasons for the possible

differences in parasitic loads across the two study regions.

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Feline Parvovirus in free-living Asiatic lions (*Panthera leo persica*) of Gir National Park and Wildlife Sanctuary, India.

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ABSTRACT

The critically endangered Asiatic lion (*Panthera leo persica*) is now restricted to a single, small population in the wild. It is thus, highly susceptible to rapidly evolving, disease-causing organisms such as parvoviruses. The study of parvoviruses in the free ranging population of Asiatic lions, therefore, has important conservation implications. This study, which made use of non-invasive sampling techniques, detected the presence of parvovirus infection in about 15 % of the total scats sampled (n=106). In addition to this, one leopard scat sample was also found to be infected. The parvoviral strain detected from this study was sequenced and analysed. Phylogenetic relationships and genetic distances were calculated to determine the position of this parvoviral strain, in relation to other strains found around the world. Preliminary results suggest that the parvoviral strain detected in this study was closer to the canine parvoviral viral strains rather than the feline panleucopenia virus.

Key words: Asiatic lion, *Panthera leo persica*, canine parvovirus, disease, feline panleucopenia virus, non-invasive, parvovirus, scat, viral phylogeny.

INTRODUCTION

The critically endangered Asiatic lion (*Panthera leo persica*), which once ranged in Asia from Palestine in the west to Palamau (Bihar, India) in the east (Divyabhanusinh 2005) are presently restricted to a single, free-living population in the forests of Gir, Gujarat, India. Further, the population is extremely small and consists of only about 359 individuals (Gujarat Forest Department 2005). Also, this population is said to have encountered a genetic bottleneck in the early 20th century, during which their numbers reportedly fell to about 20 individuals (Wynter-Blyth & Dharmakumarsinhji 1950). Thus, the relict distributional status (Chellam & Johnsingh 1993), small population size and the possible lack of genetic variability predispose them to stochastic demographic threats such as epizootics, cyclones or large forest fires (Ramanathan *et al.* 2007).

The role that diseases play in the survival of such critically endangered populations is important for two reasons. Firstly, they could cause near-extinction events (Ramanathan 2007) when they occur as outbreaks or epizootics. This could be in the form of demographic crashes, modification of the population structures, or the loss of genetic diversity (O'Brien & Evermann 1988). The devastating epidemics of avian malaria in the endemic birds of Hawaii (Riper *et al.* 1986), canine distemper in the lions of Serengeti and Mara ecosystems (Roelke-Parker *et al.* 1996), canine distemper in black-footed ferrets (Thorne & Williams 1988) and chytridiomycosis in amphibians (Berger *et al.* 1998) are examples of the possible population crashes and extinctions that diseases can induce. Secondly, diseases which have co-evolved with the host eco-system play an important role in the ecology of the host animal. They are known to have various effects

on the host population, especially on health, behaviour, reproductive success, sexual selection, neonatal mortality, host competition, predation strategies and host population regulation (Freeland 1983).

Parvoviruses

Feline panleucopenia is one of the most important viral diseases in felines. It is also known as feline distemper, feline infectious enteritis, malignant panleucopenia and spontaneous agranulocytosis (Steinel 2001). The disease is characterised by severe gastroenteritis accompanied by fever, anorexia, vomiting, diarrhoea and marked leucopenia, leading to the death of the animal (Legeay 1988). It has a short incubation period of about four days (Carter & Wise 2005, Legeay 1988) and once infection has occurred, it lasts for about two weeks. Infected cats are viremic for a few days and excrete viral particles in their faeces, urine, saliva, vomitus, nasal discharges and ocular discharges (Carter & Wise 2005, Csiza *et al.* 1971, Reif 1976). Susceptible felines may be infected by one of the three following methods: direct contact with an infected individual, indirect exposure to the viral particles present in the environment (Bertier *et al.* 2000, Carter & Wise 2005) or by means of vertical transmission, i.e. from mother to offspring. The virus is able to cross the placental barrier, leading to neonatal deaths or cerebellar hypoplasia. (Carter & Wise 2005).

Feline panleucopenia is a member of the family Parvoviridae (Murphy *et al.* 1995). It is hierarchically placed under the sub-family Parvovirinae in the feline parvovirus sub-group (Steinel 2000). Other members of this sub-group are the canine parvovirus,

canine minute virus, blue fox virus, raccoon parvovirus, raccoon dog parvovirus, porcine parvovirus, mink enteritis virus (MEV) and goose parvovirus (Carter & Wise 2005, Siegl *et al.* 1985, Balak & Ballagi-Pordany 1993, Pfeffer *et al.* 1995, Steinel 2000). In this chapter, the term 'parvoviruses' denotes all members of the feline parvovirus group; further, feline panleucopenia is abbreviated as FPLV and canine parvovirus is abbreviated as CPV.

Parvoviruses are extremely small (20-22 nm capsid with a 5 kb genome), non-enveloped, single stranded DNA viruses, with a structure exhibiting icosahedral symmetry (Tsao *et al.* 1991, Carter & Wise 2005). The virions replicate in the nuclei of rapidly dividing cells (Carter & Wise 2005) since the genome lacks the genetic code required to produce the enzyme 'DNA polymerase', which is essential during the initial step of parvoviral DNA replication (Steinel 2001). Since cellular DNA-polymerase is expressed only during the mitosis stage of the host cell, this virus requires dividing cells for its replication (Steinel 2000). Thus, this virus is relatively more fatal in young individuals, due to the presence of a higher number of rapidly dividing cells. The mortality level in young animals is as high as 80% whereas it is only about 15% in adults (Legeay 1988). There does not seem to be any sex-based difference in the receptivity to this particular virus (Reig 1976).

A unique character of this virus is that it can resist extremes of physical conditions such as heat and desiccation (Carter & Wise 2005, Riser 1943) and thus may survive in the environment for long periods of time (MacPherson 1956, Poole 1972). Due to this feature, it is easier to use non-invasive methods to detect this virus from biological

samples (such as faecal material), which are collected from the field rather than directly from the host.

Parvoviruses in wild populations

Parvoviruses have been detected from different wild hosts around the world. The incidence of FPLV has been confirmed in Siberian tigers *Panthera tigris altaica* (Steinel 2000, Cockburn 1947), cheetahs *Acinonyx jubatus* (Steinel 2000, Cockburn 1947), leopards *Panthera pardus* (Cockburn 1947), snow leopards *Uncia uncia* (Bieniek *et al.* 1968), mountain lions *Puma concolor*, (Roelke *et al.* 1993), African wild cats *Felis silvestris lybica* (Steinel 2000, Cockburn 1947), leopard cats *Prionailurus bengalensis* (Ikeda *et al.* 2000), lynx (Cockburn 1947), servals *Leptailurus serval* (Cockburn 1947), clouded leopards *Neofelis nebulosa*, bat-eared foxes *Otocyon megalotis* (Steinel 2000), honey-badgers *Mellivora capensis* (Steinel 2000), red pandas *Ailurus fulgens* and ocelots *Leopardus pardalis* (Cockburn 1947).

Parvoviral antibodies have been detected in wolves *Canis lupus* (Goyal *et al.* 1986, Fletcher *et al.* 1979), red foxes *Vulpes vulpes* (Barker *et al.* 1983, Davidson *et al.* 1992, Truyen *et al.* 1998), gray foxes *Urocyon cinereoargenteus* (Davidson *et al.* 1992), blue foxes *Alopex lagopus* (Veijaleinen 1986), coyotes *Canis latrans* (Davidson *et al.* 1992) and jackals *Canis aureus* (Alexander *et al.* 1994).

FPLV is the oldest member of the feline parvoviral subgroup and was the first to be identified (Verge & Christoforoni 1928). The first outbreaks occurred during the 1930s and 1940s (Hindle and Findlay 1932, Goss 1942). Subsequently, the first outbreak in

captivity was reported from the “*Zoological Society of London*” (Cockburn 1947) in 1947. It affected various feline species such as tigers, leopards, cheetahs, lynx, servals, tiger cats and ocelots. Lions were the only cats which were not infected during this outbreak. Thus, they were thought to possess a natural resistance to this virus. However, in 1973, FPLV was detected from captive lions (Studdert *et al.* 1973), disproving this notion.

Since then, a number of studies have studied parvoviral infections in lion populations around the world. Its dynamics have been studied in the lion populations of Serengeti as well as in the Ngorongoro crater (Packer *et al.* 1999) region. In Asia, two serological studies have looked at parvoviruses in captive lions (Asiatic, African, Afro-Asian hybrids) with contrasting results. While Ramanathan *et al.* (2007) found that 100% of samples were serologically positive for parvovirus, Sabapara (2002), which attempted to detect parvoviral particles from faecal material, did not find any evidence of parvovirus in the captive lions. CPV has also been demonstrated from domestic dogs in the state of Gujarat state, India (Sabapara 2002), the location of this study.

Conservation implications of parvoviruses

The study of parvoviruses in critically endangered wild populations is important due to several reasons. Firstly, the fast-evolving nature of this virus could lead to the appearance of new strains and possibly, new viruses which could have dire pathogenic consequences (Ikeda *et al.* 2000) for the only surviving Asiatic lion population. This argument is buttressed by the fact that parvoviruses, ever since they were identified at

the beginning of the 20th Century (Verge & Christoforoni 1928), have displayed high levels of plasticity in evolving into new strains and viruses. The first identified member of the feline parvovirus sub-group was FPLV (Verge & Christoforoni 1928). Known to affect only felines (Horuchi *et al.* 1992), this virus strain is believed to have evolved in the 1940s to affect minks too (Parrish *et al.* 1984). Further, during the late 1970s, a new virus, CPV-type-2 (Ikeda *et al.* 2000, Truyen & Parrish 1992, Truyen *et al.* 1995) was identified, which was shown to be antigenically and genetically very similar to FPLV (Appel *et al.* 1979), sharing almost 98% of its genome (Carter & Wise 2005, Steinel 2000). Thus, CPV-2 was hypothesised to have evolved from FPLV (Ikeda *et al.* 2002). It has also been suggested that FPLV and CPV-2 should be classified as host range variants of the feline parvovirus (Ikeda *et al.* 1998).

However, the hypothesis that CPV-2 evolved from FPLV was further reinforced when a wild strain with characteristics intermediate between those of CPV-2 and FPV was found in a free ranging European red fox (Steinel 2000). CPV-2, unlike FPLV, can only infect canids. Within a very short time, CPV-2 spread rapidly throughout the world, initially killing thousands of unprotected dogs (Carmichael & Binn 1981). Subsequently, CPV-2 further evolved to give rise to two strains, CPV-2a and CPV-2b, which have almost completely replaced the CPV strain worldwide (Parrish *et al.* 1985). Thus, this set FPLV as a significant model for natural viral evolution (Parrish *et al.* 1985). While CPV-2 did not have an in-vivo feline host range (Truyen *et al.* 1996, Mochizuki *et al.* 1993), CPV-2a and CPV-2b were found to have gained a feline host range, in addition to the canine host range (Truyen *et al.* 1996, Mochizuki *et al.* 1993, Mochizuki *et al.* 1996, Mochizuki *et al.* 1996).

Genetic studies have demonstrated that very few changes in the parvoviral genome are responsible for these evolutionary patterns and changing host ranges (Reed *et al.* 1988). The host range variability between the parvoviruses has been attributed to changes in the important capsid proteins (VP1 and VP2). Within the region encoding for these two important structural proteins, only 6 nucleotides differ between FPV and CPV-2; and a further 5 between CPV and its two antigenic variants, CPV-2a and CPV-2b.

A study conducted in Vietnam and Taiwan showed that more than 80% of isolates from domestic cats and leopard cats were CPV, rather than FPLV. Also, parvovirus isolates from three Vietnamese leopard cats showed higher genetic relatedness to CPV rather than FPV and showed a natural mutation of VP2 residue 300 Gly to an Asp., resulting in remarkable changes to their antigenic properties. Additionally, another study (Truyen *et al.* 1996) showed that in Germany and the United States of America, about 5 % of all cases of reported feline parvoviral infections in domestic cats were caused by either CPV 2a or CPV 2b. Further, CPV 2a was detected from a captive Siberian tiger (Steinel 2000) and CPV 2b was detected from 5 captive cheetahs and a bat-eared fox (Steinel 2000). Thus, there exists a possibility that CPV-2a and CPV-2b can spread in felines more efficiently than conventional FPLV under natural conditions and that CPV-2a and CPV-2b viruses are further evolving in cats (Ikeda *et al.* 2000). Further, a recent study (Ikeda *et al.* 1999, Ikeda *et al.* 2000) has demonstrated a new variant of the CPV-2 strain, which has been called the CPV-2c strain. This has only been detected in a wild population of leopard cats and has not been found in adjoining populations of domestic dogs, wild canines, domestic cats or any other wild felines till now. Thus, this particular

strain might have evolved in the wild leopard cat only. This may indicate the continued evolution of the CPV strains in cats, as was hypothesised (Ikeda *et al.* 2000).

The rapid emergence of new strains of CPV, which have the potential to cause pathogenic effects on wild felines, is a matter of concern, especially for critically endangered populations such as that of the Asiatic lion. This view is further reinforced when the CPV-2a and CPV-2b strains were identified from cheetahs with chronic diarrhoea and enteritis or a tiger with anorexia and diarrhoea (Steinel 2000). Further, another study (Nakamura *et al.* 2001) found that the newly identified strain, CPV-2c caused diseases in experimentally infected cats.

The domestic dog populations which harbour CPV may act as sources of infection to the Asiatic lions. A study (Battilani *et al.* 2001) has shown that similar strains of CPV were circulating between the dogs and wolves in Italy. Thus, the spread of this disease between wild and domestic populations, albeit canine populations, has been established. This, coupled with the fact that CPV-2a, CPV-2b and CPV-2c are found in wild felines, makes the domestic dog populations (surrounding Gir) sources for the spread of CPV to felines like lions. The constant monitoring of this virus and its evolutionary and pathogenic patterns in the wild Asiatic lion population has extremely important conservation implications. The possibility that discrete epidemics (Anderson and May 1976, Packer *et al.* 1999) of panleucopenia may occur in the small Asiatic lion population further adds urgency to this.

Other than the potential pathogenic problems associated with newly emerging strains,

parvoviruses have important conservation implications in controlling host population densities. A modelling study found that, under certain conditions, the virus was able to control the host population at a low density (Berthier 2000). Further, the disease caused by parvoviruses drastically reduces the immune status of the host, which makes it susceptible to other diseases (Mochizuki 1999). Given the conservation relevance of parvovirus, the single, isolated Asian lion population in Gir and the presence of domestic dogs outside the park, the initial aim of my study was to detect the presence of parvoviruses in the free-living Asiatic lion population of Gir. Second, I characterized the strain of parvoviruses present in the population and finally, I investigated the phylogenetic affinities of the extant parvoviral strain in Gir lions to parvoviral strains found in other geographical regions.

STUDY AREA

This study was carried out in the 1412.13 km² Gir Wildlife Sanctuary and National Park (20° 57' N to 21° 20' latitude and 70° 27' to 71° 13' E longitude), located in the state of Gujarat, India. This protected area, along with its surrounding forests, constitutes the sole remaining habitat for the critically endangered Asiatic lion (*Panthera leo persica*).

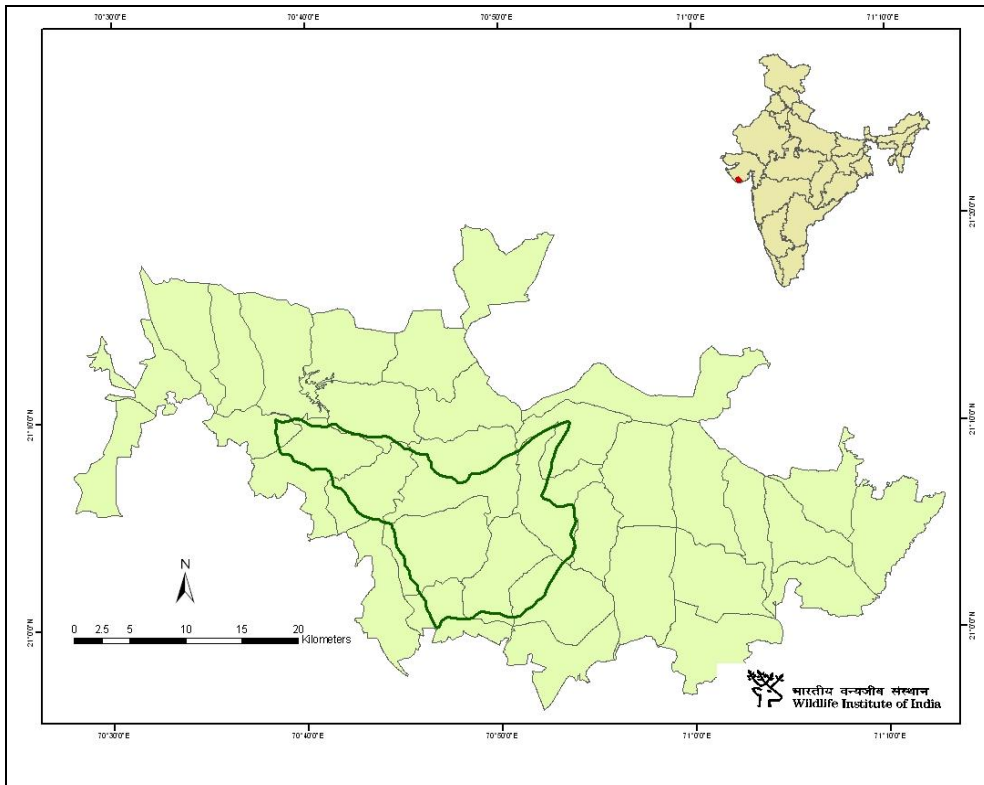


Figure 1: A map showing the study area; the outlined region encloses the Gir National Park, whereas other shaded regions constitute Gir Wildlife Sanctuary.

The Gir National Park and Wildlife Sanctuary is comprised of tropical dry deciduous forests, interspersed with tropical thorn forests (Champion & Seth, 1968). This diverse region supports an impressive assemblage of large mammals such as the Asiatic lion, leopard *Panthera pardus*, sambar *Rucervus unicolor*, chital *Axis axis* and nilgai *Boselaphus tragocamelus*, with the lions being the top predators. This protected area was selected for the study since it is the only region with a surviving population of wild Asiatic lions and thus, has a high conservation value.

METHODS

Field methods

Lions prefer to use roads and trails (pers. com. Chellam, Ravi) as travel routes and are likely to leave scats and tracks along them. Thus, to maximise the collection of fresh scats, sampling was conducted periodically on all motorable roads and tracks within the protected area. In addition to these roads and tracks, dry river beds were also used to collect fresh scat samples. Individuals were also followed, wherever possible, to collect fresh faecal samples.

Lions are also known to defecate near kills. Whenever a kill was noticed, the individual lions (feeding at the kill) were intensively observed and scat samples were collected immediately after defecation. If the lion was not present near a kill, sampling was carried out in an area (of about 50 meter radius) around the kill.

Sampling was done from 1st December 2007 up till 20th May 2008. Sampling was done by two teams; each team consisting of two members. These teams moved along predetermined routes, followed individual animals or made observations at kill-sites throughout the day. Only fresh scats (deposited within the past 12 hours) were collected. The fresh state of the scat was determined by its appearance and the presence of insect activity. The faecal material was collected using standardised collection protocols. Each sample was taken from 2-3 different regions of the faecal sample. Adequate precautions were taken to prevent contamination of the samples, as well as to prevent the spread of

zoonotic diseases. This was done by using disposable gloves, face masks and sterilised collection kits. Each sample was collected in duplicate for this particular study – one in ‘Hank’s Balanced Salt Solution’ and the other in absolute alcohol. The collected sample was then shaken vigorously to obtain a consistent mixture. Each fresh scat sample, which was collected in sterile containers, was labelled on the spot with the sample number, date of collection, species and the GPS location. The samples were then sealed in a labelled plastic zip-lock bag individually.

At each scat collection event, details such as date, time of collection, GPS location, locality, approximate distance on the road transect, species, presence of solitary lion/pride, presence of other faeces (old/new) in its vicinity, presence of indirect marks, presence of shade, diameter of the largest faecal bolus, scat colour, scat consistency and the scat odour were recorded.

Laboratory methods

I analysed one set of samples at the National Centre for Biological Sciences (TIFR), Bangalore. The second set of samples, which were collected in HBSS (Hanks Balanced Salt Solution), was analysed at Indian Immunologicals Limited (IIL), Hyderabad.

Parvoviruses can be detected using many methods, both direct and indirect. Direct methods such as electron microscopy (Carter & Wise 2005), ELISA (Carter & Wise 2005), virus isolation (Desario *et al.* 2004, Carter & Wise 2005), Haemagglutination Test (Desario *et al.* 2004), Haemagglutination-inhibiting (HI) Test (Carter & Wise

2005), Virus Neutralising Test (Ikeda *et al.* 1998), Southern Blot Test (Truyen & Parish 1992), Immunochromatography (Desario *et al.* 2004) and PCR (Desario *et al.* 2004, Schunk *et al.* 1995, Belak & Bellagi-Pordany 1993) are used to screen viral particles directly. Indirect methods usually involve the detection of antibodies and this can be done through methods such as ELISA and FAT (Carter & Wise 2005). Comparative methodology studies (Desario *et al.* 2004, Schunck *et al.* 1995) have demonstrated that the ‘Polymerase Chain Reaction’ (PCR) method yielded the best and most accurate diagnostic results. Therefore, this method was chosen to detect parvoviruses from my study samples.

The initial screening was performed at IIL, Hyderabad. DNA was extracted from all the 150 samples that were collected from the field site. A polymerase chain reaction was used to amplify a portion of the VP 2 (Capsid Protein 2) gene of the viral genome. Previously designed parvovirus-specific primers (Chinchkar *et al.* 2006) were employed for this purpose (Table 1).

Table 1: Forward and reverse primers that were used to detect parvoviruses from scat samples of wild Asiatic lions. These primers amplified a segment of the VP-2 gene, from the 2949 to 3840 nucleotide regions of the viral genome.

Primers	Primer sequence (5' – 3')
Forward Primer - 1	5'-AGCTGTCGACGAAAACGGATGGGTGGAAAT-3'
Reverse Primer – 1	5'-GCCCTTGTGTAGACGC-3'

A 25 µl reaction containing 1 µl of dNTP mix (10 mM), 1 µl of *Thermus aquaticus*

DNA polymerase (Invitrogen) (3 units/ μ l), 1 μ l of $MgCl_2$ (25 mM), 1.5 μ l of Taq buffer (10X), 16.5 μ l of DNase/RNase-free water and 1 μ l each of forward and reverse primers were used in each PCR reaction, along with 2 μ l of the DNA extract. The optimised PCR conditions were as follows: 94°C for 3 minutes; denaturation at 94°C for 30 seconds, primer annealing at 50°C for 40 seconds and extension at 72°C for 90 seconds, respectively repeated for 30 cycles; 72°C for 10 minutes and hold at 4°C. The obtained PCR products were electrophoresed on 2 % agarose gel in 1X Tris-Borate-EDTA buffer (pH = 8.3) and visualised under UV light after staining with ethidium bromide.

Further processing was carried out at NCBS, Bangalore. All those samples which tested positive, along with a random set of samples which tested negative, were used. DNA from these samples was extracted using the 'QIAGEN protocol for isolation of DNA from stool for pathogen detection'. PCRs were then repeated to confirm the results. A negative control was used to check for cross contamination, both during extractions and polymerase chain reactions. A positive control was used as a reference.

In order to construct phylogenetic trees, a different set of primers (Horiuchi *et al.* 1998), which amplified 451 base pairs of the VP2 region from the viral genome, was used (Table 2). This was done since the previously amplified fragment was found to be too large to attempt a sequencing reaction (in the automatic sequencer present at NCBS, Bangalore).

Table 2: Forward (V1) and reverse (V52) primers that were used to amplify 451 base pairs from the parvoviral VP2 gene, in order to sequence the region and construct phylogenetic trees. These primers were used to sequence the viral genome from the 3029 to the 3480 region.

Primers	Primer sequence (5' – 3')
V1	5' – GTACATTTAAATATGCCAGA – 3'
V52	5' – ATTAATGTTCTATCCCATTG – 3'

A 15 µl reaction containing 7.5 µl of *Qiagen PCR Multiplex kit reagent*, 1.5 µl of Bovine Serum Albumin (4 mg/ml) and 0.75 µl each of forward and reverse primers (4 uM) were used in each PCR reaction, along with 4 uL of the DNA extract. The optimised PCR conditions were as follows: 94°C for 10 minutes; denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds, respectively repeated for 45 cycles; 72°C for 10 minutes and hold at 4°C. A negative control was used to check for any cross-contaminations.

The PCR products were electrophoresed on 2 % agarose gel in 1X Tris-Borate-EDTA buffer(pH = 8.3) and visualised under UV light after staining with ethidium bromide. The remaining PCR products were purified using EXO-SAP-IT (USB Corporation) and sequenced using an automated sequencer.

Genetic variation and phylogenetic tree construction

Once the respective sequences were obtained, they were edited using Finch TV (Version 1.4.0, Geospiza) and aligned using the ClustalW algorithm in the software, Molecular Evolutionary Genetics Analysis, MEGA (Kumar *et al.* 2007, Version 4.1). The sequences were analysed at the National Centre for Biotechnology Information (NCBI) and compared to other nucleotides of somewhat similar sequences, by using the algorithm *nblast*. Each of the four sequences was BLASTed against the NCBI database and the virus identity determined by examining the relative e-values and the maximum identity scores of each sample.

Then, the samples sequenced from this study were compared to 87 other samples from around the world by calculating genetic distances and constructing phylogenetic trees. These samples were downloaded from the NCBI database and constituted the following: four samples of FPLV from other wild felines, 22 samples of domestic cat with FPLV strains, one sample of domestic dog with a CPV-2 strain, 30 samples with CPV-2a from around the world, one sample of CPV-2a from India, 19 samples of CPV-2b from around the world, four samples of CPV (undetermined strain) from India and six samples of Mink enteritis virus (Appendix 1).

The pair-wise distances were computed using MEGA (Kumar *et al.* 2007, Version 4.1). Subsequently, all the sequences were classified into ten groups – lion, FPLV (domestic), FPLV(wild), CPV 2, CPV 2a, CPV 2a (India), CPV 2b, CPV unidentified strain (from Hyderabad 1), CPV unidentified strain (from Hyderabad 2), CPV unidentified strain

(from Uttar Pradesh and Tamil Nadu). Both inter-group and intra-group distances were also calculated. Distance between samples, as well as between and among pre-formed groups were calculated using pair wise deletion of gaps/missing data, maximum composite likelihood model for nucleotides, heterogeneous lineage patterns and gamma distributed rates among the various sites (gamma parameter of 0.5).

Phylogenetic trees were constructed using the software MEGA (Kumar *et al.* 2007, Version 4.1). The method used was the neighbour-joining method with a phylogeny test conducted by bootstrapping it by 1000 replicates. A pair-wise deletion for gaps was used. A maximum composite likelihood model, with heterogeneous patterns following a gamma distributed rates for sites (gamma parameter = 0.5) was employed.

RESULTS

A total of 106 fresh lion scat samples, randomly collected from various regions of the Gir protected area were analysed for the presence of parvovirus. In addition to this, 21 leopard scat samples, 12 large carnivore scat samples (which could have been either lion or leopard), two jungle cat samples and three jackal samples, which were found during the course of this study, were analysed for this particular virus. Of these samples, 16 lion scat samples (n=106; percentage of scats infected = 15%) and one leopard scat sample (n = 21; percentage of scats infected = 4%) tested positive for parvovirus.

A 423 base pair region in the highly conserved VP2 region of the lion parvovirus was sequenced. A total of four different samples, which were detected to be positive, were sequenced for this study. All the four different samples were found to have identical nucleotide sequences for the given region. Thus, they were taken as one group while calculating the genetic distances and constructing a tree.

The samples sequenced from this study were compared to other parvoviral sequences, obtained from the NCBI database (Appendix 1). Eighty-seven sequences were used for the comparison; in order to determine the genetic distances, the sequences were grouped into 10 classes, which were as follows: lion, FPLV (domestic), FPLV (wild), CPV-2, CPV-2a, CPV-2a (India), CPV-2b, CPV-unknown strain (Hyd-1), CPV-unknown strain (Hyd-2), CPV-unknown strains (from Uttar Pradesh and Tamil Nadu).

The inter-group and intra-group genetic distances were also calculated (Table 3).

Table 3: Intra-group and inter-group genetic distances for the 10 parvoviral groups were calculated for the 423 base pair region of the VP2 gene.

	Lion Parvo virus	FPLV (dom)	FPLV (wild)	CPV 2	CPV 2a	CPV 2a (India)	CPV 2b	CPV (Unid) India (Hyd1)	CPV (Unid) India (Hyd2)	CPV (Unid) India (UP & TN)	MEV
Lion parvo virus	0.000										
FPLV (dom)	0.015	0.007									
FPLV (wild)	0.012	0.007	0.004								
CPV 2	0.005	0.017	0.013	-							
CPV 2a	0.008	0.020	0.016	0.003	0.003						
CPV 2a (India)	0.010	0.022	0.018	0.005	0.008	-					
CPV 2b	0.006	0.018	0.014	0.001	0.004	0.006	0.003				
CPV (Unid) India (Hyd1)	0.010	0.022	0.018	0.005	0.008	0.010	0.006	-			
CPV (Unid) India (Hyd2)	0.007	0.019	0.015	0.002	0.005	0.007	0.004	0.002	-		
CPV (Unid) India (UP & TN)	0.005	0.015	0.012	0.010	0.013	0.015	0.011	0.015	0.012	-	
MEV	0.013	0.008	0.006	0.013	0.015	0.018	0.014	0.018	0.015	0.014	0.007

The genetic distances show that the lion parvovirus is closest to the CPV-2 strain (genetic distance = 0.05), as well as the CPV-unknown strain from Uttar Pradesh and Tamil Nadu, India (genetic distance = 0.05); also, it was found to be far away from the FPLV (wild) and FPLV (domestic) strains (Table 3).

Further, I constructed a phylogenetic tree using the above strains, in order to determine the existence of any genetic relationships (Figure 2).

Figure 2. Phylogenetic tree showing the relationships between the samples sequenced from this study and those from previous studies (Appendix 1). The lion parvovirus samples clade with the canine parvovirus group rather than the feline panleucopenia group.

The phylogenetic trees confirm the fact that the lion parvovirus is closer to the CPV than the FPLV.

DISCUSSION

The results revealed that almost 15% (16 scat samples, n=106) of the lion scats sampled were positive for parvovirus. Subsequently, a 423 base pair region of the VP2 gene in the parvoviral genome was sequenced. This was used to construct a phylogenetic tree, along with sequences of other parvoviruses, taken from the NCBI database. It was observed that the parvovirus strain identified in this study, contrary to expectations (since the host is a feline), grouped together with canine parvoviruses rather than with the feline panleucopenia virus. The inter-group distances also verified this fact, where it was seen that the genetic distances between the lion parvovirus and the CPV strains (genetic distance = 0.05 to 0.10) were shorter than those exhibited between the lion parvoviruses and the FPLV strain (genetic distance = 0.012 to 0.015). The lion parvoviruses and the CPV were found to share the MRCA (Most Recent Common Ancestor).

On further scrutiny, within the parvoviral clade, the genetic distances between the established CPV strains and the lion parvovirus strain (genetic distance = 0.05) was found to be closer than those between the established CPV strains and the undetermined CPV strains from Uttar Pradesh and Tamil Nadu, India (genetic distance range = 0.10 to 0.15). This further supports the argument that the lion parvovirus is within the CPV clade.

Further, of the various CPV strains present, the lion parvovirus sample was found to be closest to CPV-2 (genetic distance = 0.05), as well as the two undetermined CPV strains from Uttar Pradesh and Tamil Nadu (genetic distance = 0.05). Compared to these two strains of CPV, the lion parvovirus was found to be genetically further away from CPV-2a (genetic distance range = 0.08 to 0.10) and CPV-2b (genetic distance = 0.06).

Thus, based on this study, we can conclude that:

- 1) The lion parvovirus is distinct from the FPLV strains and thus, cannot be considered to be FPLV.
- 2) The lion parvovirus clades together with the CPV; further, it is nested between the established strains of CPV and the unidentified strains of CPV from India. Thus, it can be considered to be a new strain of CPV.
- 3) There are many more strains of CPV than was previously thought.

However, this study is of a preliminary nature and further investigations are required to get a more detailed picture. The sample sizes have to be improved since very few were considered in this study, especially while looking for phylogenetic relationships.

Further, the number of genes considered for analysis should also be increases since only one gene may not portray the actual patterns clearly. Thirdly, the length of the sequence in this particular VP2 gene has to be increased, since it will help in getting more robust inferences. The fact that the VP2 gene is highly conserved and very little differences, of the order of five nucleotide changes (in a region of 3000 nucleotides), separate the FPLV from the CPV-2 strain, further reinforces this argument. Thus, the small size of my fragment may point towards a trend; but this has to be confirmed by sequencing longer regions.

However, my study points out to the importance of monitoring lion parvoviruses in the critically endangered Asiatic lion population. The highly contagious, fast-evolving nature of the virus makes it a larger threat to the lion population than other diseases.

Further, this virus may have come either from the surrounding domestic dog population or evolved as a separate strain in the wild Asiatic lion population itself, without entering the canine population in the intermediate time. Thus, despite the host dynamics remaining unknown, preventing the potential spread of such diseases from domestic carnivores to the lion population is justified and of paramount importance. Ring vaccination of the surrounding domestic dog population against this disease is one such precautionary measure. Maybe the example of distemper and how it was already present for many years in lions and yet suddenly broke out is appropriate here

Thus, for any conservation program involving the Asiatic lion to be successful, it should incorporate a disease monitoring strategy in order to safeguard the only surviving

population from the threat of rapidly evolving diseases such as parvoviruses. Further, the ecology of host-disease relationships should be studied, which will throw more light into the disease dynamics of this sole surviving Asiatic lion wild population.

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APPENDIX

Appendix 1: Details of the sample sequences taken from the NCBI website in order to build a phylogenetic tree; the tree was constructed to determine the phylogenetic association with samples from around the world

Sl. No.	STRAIN	ACCESSION NUMBER	GEOG. REGION	HOST
1	FPLV	EF418569.1	Portugal	Lion
2	FPLV	EF418568.1	Portugal	Tiger
3	FPLV	EU659113.1	USA	Mountain Lion
4	FPLV	EU659114.1	USA	Mountain Lion
5	FPLV	AF015223.1	Taiwan	Cat
6	FPLV	X55115.1	Australia	Cat
7	FPLV	EF988660.1	China	Cat
8	FPLV	EU018144	Argentina	Cat
9	FPLV	EU018145	Argentina	Cat
10	FPLV	DQ099431	China	Cat
11	FPLV	D88287.1	Japan	Cat
12	FPLV	AY606131.1	France	Cat
13	FPLV	AB054226.1	Japan	Cat
14	FPLV	D78584.1	Japan	Cat
15	FPLV	M24002	Unknown	Cat
16	FPLV	AY665655.1	Russia	Cat

17	FPLV	M24004	Unknown	Cat
18	FPLV	M38246.1	USA	Cat
19	FPLV	M10824	Unknown	Cat
20	FPLV	EU018143	Argentina	Cat
21	FPLV	EU018142	Argentina	Cat
22	FPLV	DQ099431	China	Cat
23	FPLV	DQ099430	China	Cat
24	FPLV	AB054227	Japan	Cat
25	FPLV	AB054225	Japan	Cat
26	FPLV	D88286	Japan	Cat
27	CPV-2	AY380577.1	Italy	Dog
28	CPV – 2a	EF666068.1	China	Dog
29	CPV – 2a	AB054214.1	Japan	Cat
30	CPV – 2a	EU009201.1	South Korea	Dog
31	CPV – 2a	EF666061.1	China	Dog
32	CPV – 2a	DQ354068.1	China	Red Panda
33	CPV – 2a	EF592511.1	Taiwan	Dog
34	CPV – 2a	EF666065.2	China	Dog
35	CPV – 2a	AB054217	Japan	Cat
36	CPV – 2a	AB054213.1	Japan	Cat
37	CPV – 2a	AJ564427.2	India (UP)	Dog
38	CPV – 2a	EU009201	S. Korea	Dog
39	CPV – 2a	EU009200	S.Korea	Dog

40	CPV – 2a	EF666069	China	Dog
41	CPV – 2a	EF666067	China	Dog
42	CPV – 2a	EF666066	China	Dog
43	CPV – 2a	EF666064	China	Dog
44	CPV – 2a	EF666063	China	Dog
45	CPV – 2a	EF666062	China	Dog
46	CPV – 2a	EF666060	China	Dog
47	CPV – 2a	EF666059	China	Dog
48	CPV – 2a	EF599096	S.Korea	Dog
49	CPV – 2a	EU009204	S.Korea	Dog
50	CPV – 2a	EU009203	S.Korea	Dog
51	CPV – 2a	EU009202	S.Korea	Dog
52	CPV – 2a	AB128923	S.Korea	Dog
53	CPV – 2a	AB054216	Japan	Cat
54	CPV – 2a	AB054215	Japan	Cat
55	CPV – 2a	CPU72698	Taiwan	Dog
56	CPV – 2a	U72697	Taiwan	Dog
57	CPV – 2a	U72695	Taiwan	Dog
58	CPV – 2b	AB115504.1	Japan	Dog
59	CPV – 2b	EF599097.1	South Korea	Dog
60	CPV – 2b	EU009206.1	South Korea	Dog
61	CPV – 2b	DQ120515.1	China	Dog
	CPV – 2b	U72696.1	Taiwan	Dog

62				
63	CPV – 2b	AB120723.1	Vietnam	Dog
64	CPV – 2b	AB054219.1	Japan	Cat
65	CPV – 2b	AB054220.1	Japan	Cat
66	CPV – 2b	AY900660	Italy	Dog
67	CPV – 2b	EU009205	S.Korea	Dog
68	CPV – 2b	AB120728	Vietnam	Dog
69	CPV – 2b	AB120727	Vietnam	Dog
70	CPV – 2b	AB120726	Vietnam	Dog
71	CPV – 2b	AB120725	Vietnam	Dog
72	CPV – 2b	AB120724	Vietnam	Dog
73	CPV – 2b	AB120722	Vietnam	Dog
74	CPV – 2b	AB120721	Vietnam	Dog
75	CPV – 2b	AB120720	Vietnam	Dog
76	CPV – 2b	AB054221	Japan	Cat
77	CPV – 2b	AB054218	Japan	Cat
78	CPV-unid	DQ182624.1	India (Hyd)	Dog
79	CPV-unid	DQ182627.1	India (Hyd)	Dog
80	CPV-unid	EU430518.1	India (TN)	Dog
81	CPV-unid	AJ698134.1	India (UP)	Dog
82	MEV	D00765	Japan	Mink
83	MEV	EU137663.1	China	Mink
84	MEV	AY665657.1	Russia	Mink

85	MEV	EF428258.1	China	Mink
86	MEV	AY665656.1	Russia	Mink
87	MEV	AF469009.1	Russia	Mink

CONCLUSION

I studied helminth communities and the parvovirus present in the free ranging population of the critically endangered Asiatic lion (*Panthera leo persica*), using non-invasive sampling methods. The first part of my study showed that the lion population had at least 9 helminth OTUs, which were distributed across the population in a negative binomial fashion. Further, a geographical trend was visible in the parasitic loads across the protected landscape, with the western parts showing higher loads when compared to the eastern parts of the protected area. This could either be due to the differential anthropogenic factors present in these regions or the intrinsic ecology of the parasite community. During the second part of the study, I detected the presence of parvoviruses in 15% of the samples analysed (n=105). This particular strain of parvovirus was found to be closer to the existing canine parvoviral strains than to the feline panleucopenia virus. Even though the study could make no interpretations about the origin of this particular lion parvoviral strain, I would, as a precautionary measure, suggest ring-vaccination of the domestic carnivore population surrounding the park as part of conservation measures for the lion population. Further, this particular virus, due to its fast-evolving nature, will have to be monitored in the future.

This study provides baseline information for future studies and disease-monitoring programmes in the only surviving population of wild Asiatic lions. Further studies will be able to throw light on the ecological interactions of the helminth communities and parvovirus with their host, whereas disease monitoring programmes will ensure that future conservation measures are more holistically designed and thus, more successful.