TAMIL NADU FOREST DEPARTMENT
ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(RESEARCH, TRAINING & EDUCATION)
VANDALUR

PROJECT COMPLETION REPORT

HEALTH MONITORING OF FREE-RANGING WILD ANIMALS IN SELECTED HABITATS WITHIN TAMIL NADU

Annual Plan of Operations (APO)
(2020-21)

ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
GOVERNMENT OF TAMIL NADU
VANDALUR, CHENNAI- 600048

APO PROJECT REPORT-2020-21

HEALTH MONITORING OF FREE RANGING WILD ANIMALS IN SELECTED HABITATS WITHIN TAMIL NADU.

Research theme 2: Monitoring of wildlife health and disease management.

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CONTENTS

I. ACKNOWLEDGEMENTS

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This project received much support from the forest offices and field staff of tiger reserves of ATR and KMTR of Tamil Nadu. We are deeply thankful to them for the timely support and co-operation. We also grateful to all officials & staff of Arignar Anna Zoological Park, Vandalur, Chennai for the timely help and support during the time of sample collection.

1. Introduction

Growing human population, globalization, climate change and a number of ecological perturbations have resulted in an increasing number of emerging diseases and reduced forest lands for economic importance. Given this context, the role of wildlife in human and domestic animal disease emergence has become widely recognized as a factor we can no longer afford to ignore. Thus, wildlife health monitoring and surveillance have become an integral aspect in the identification and management of potential threats to human and livestock animal health. This monitoring helps to detect early signs of diseases, identify potential threats, and assess the overall health status of wildlife populations.

Here are some key methods and considerations for health monitoring in free-range wild animals. 1. Non-Invasive Techniques, in free-range wildlife, invasive health monitoring methods are generally avoided to minimize animal stress and disturbance. Non-invasive techniques, such as remote sensing, camera traps, and collection of fecal or saliva samples, are commonly used toobtain valuable health-related information without direct contact with the animals. 2. Fecal and Saliva Sample Collection samples can be collected from free-ranging animals to analyze various health indicators, including hormone levels, pathogens, and genetic information. Non-invasive genetic sampling allows for individual identification and assessment of genetic diversity within populations. 3. Long-term Data Collection, Health monitoring in free-range wild animals often requires long-term data collection to identify trends and changes in health status over time. Longitudinal studies are essential for understanding the dynamics of diseasesand health patterns within populations.

Diseases in free-ranging wildlife, except for those of economic or public health importance, are rarely monitored. Tamil Nadu is home to several unique flora and fauna. The fauna involves various wild animals found living along the western ghats. The Western Ghats are home to four tropical and subtropical moist broadleaf forest ecoregions which provide shelter and food to very unique species. Many of these species are threatened in many ways, among which diseases caused by various factors including but not limited to the fragmentation of forests and an impact on immunology, genetic diversity, sexual selection and reproductive success etc., Species like Asian Elephant (*Elephas maximus*), Nilgiri tahr (*Nilgiritragus hylocrius*) and Lion-Tailed Macaque (*Macaca silenus*) are listed as

Endangered under criteria A2c, C2a(i), C2a(i) and A2abcd respectively (https://www.worldwildlife.org). A few studies have suggested that disease might have caused extinction or at least significantpopulation declines. Although there is no conclusive evidence to prove.

Despite advances in serologically testing the animals for any potential disease involving the collection of blood from the animal by tranquillizing it, it also poses a serious threat to the well-being. A relatively recent and growing body of literature indicates that parasites are important in almost every aspect of the evolution and survival of wild populations (Stumpf etal., 2016; Kumar et al., 2019). For example, we now know or strongly suspect that diseases and parasites have an effect on immunology, genetic diversity, behavior, predation, sexual selection and reproductive success, fecundity, ecology, animal community structure, speciesdiversity, and demography. A few studies have suggested that diseases might have caused extinction or at least significant population declines (Zachariah et al., 2013). The importanceof monitoring diseases in free-ranging wildlife has been greatly underestimated as far as endangered species and reintroduction projects are concerned. The study of diseases important to endangered species is often performed on captive animals, usually a surrogate species. Although valuable information about the disease and host may be learned, serious problems are associated with such an approach. Initially, differences among species in response to a particular disease are well documented. Second, an individual's response in an artificial environment is likely to differ greatly from the response of a free-ranging individual because protective measures that the host might mount cannot be achievedin captivity. Also, the collection of samples from free-ranging is also not easy due to the various complications involved in it. However, advancements in biotechnology, microbiology and bioinformatics can help in testing and experimenting from the sample obtained non-invasively and assess the health of the animal (Mokili et al., 2012; Wei et al., 2019).

Metagenomics and marker gene approaches, coupled with high-throughput sequencing technologies, have revolutionized the field of microbial ecology. Metagenomics is a culture-independent method that identifies and characterizes organisms from various samples. Whole-genome shotgun sequencing analyses the total DNA of a chosen sample to determine the presence of microorganisms from all domains of life and their genomic

content. Importantly, the whole-genome shotgun sequencing approach reveals the genomic diversity present, but can also give insights into the functional potential of the micro-organisms identified. The marker gene approach is based on sequencing a specific gene region. It allows one to describe the microbial composition based on the taxonomic groups present in the sample. It is frequently used to analyse the biodiversity of microbial ecosystems. The current study mainly focuses on the stool samples of free-ranging animals of Tamil Nadu. The habitats selected for the study are Anamalai tiger reserve and KMTR, Tamil Nadu. The methodologyadopted for the study is a recent trend called metagenomics. In metagenomics, whole genome sequencing means a total DNA of a chosen sample to determine the presence of micro-organisms from all domains of life and their genomic content. The present study targeted the presence of viruses and bacteria in the body of selected species. From this data, we can identify pathogenic or disease-causing organisms are present in a selected group of animals.

2. Objectives

2.1 Identification of vulnerable groups of animals (Elephant, Nilgiri tahr, lion-tailed macaque).

Based on the vulnerability, four species were chosen for the initial phase of the project Asian Elephant (Elephas maximus), Nilgiri tahr (Nilgiritragus hypocrisy) and Lion-Tailed and Macaque (*Macaca silenus*) are listed as Endangered under criteria A2c, C2a(i), C2a(i) and A2abcd, respectively.

2.2 Collection of non-invasive samples for disease diagnosis and assessment of prevalence of panel of communicable diseases by molecular analysis

Stool, Dung and pellet samples of the most vulnerable species, Lion-tailed macaque, Asian elephant and Nilgiri Tahr, were collected and subjected to molecular analysis using Whole Metagenomic High Throughput Screening/ Sequencing.

Interpreting metagenomics results is crucial in understanding the microbial communities in an environmental sample. This process involves analyzing and making sense of the vast amount of genomic data obtained from sequencing techniques. Here are some key points to consider when interpreting metagenomics results:

Taxonomic Composition

The first step is to determine the taxonomic composition of the microbial community. This involves identifying the organisms present in the sample, ranging from bacteria, archaea, viruses, fungi, and other microorganisms. Taxonomic profiling allows researchers to understand the diversity and relative abundance of taxa within the community.

Diversity and Richness

Assessing the diversity and richness of the microbial community provides insights into the complexity of the ecosystem. High diversity and richness indicate health and stability.

Ecosystem, with low diversity, may suggest disturbances or specific environmental conditions.

Comparative Analysis

Researchers often compare metagenomic data from different samples or habitats to identify ecosystem patterns and differences. This comparative analysis can reveal how environmental factors, such as temperature, pH, or nutrient availability, shape the microbial community structure.

Sample Contamination

Researchers must also consider the potential for sample contamination during the laboratory and sequencing processes. Careful controls and quality checks are necessary to ensure that the detected microbial DNA represents the community in the original sample.

Integration with Other Data

Metagenomic data should be integrated with other environmental data, such as physical and chemical parameters, and data from traditional biodiversity surveys to gain a comprehensive understanding. This integration allows for a more holistic interpretation of the relationships between microbial communities and their environment.

Interpreting metagenomics results is a multidisciplinary process that involves understanding complex genomic data, ecological principles, and environmental factors. By combining taxonomic and functional analyses, researchers can gain valuable insights into the structure and function of microbial communities, which is essential for advancing our understanding of ecosystems, supporting wildlife conservation efforts, and addressing broader environmental challenges.

3. Materials and methods

3.1. Sample collection

Fresh stool samples for wild animals (lion-tailed macaque – droppings, Asian elephants – dung, Nilgiri tahr – pellets) were collected within 2 hours of defecation from wild elephants picked from herds moving in Anamalai Tiger Reserve (ATR) and KMTR non – invasively using sterile wooden sticks/toothpick into sterile vials and preserved in absolute ethanol and GPS coordinates of the collected samples were noted. Samples were also collected from captive elephants and lion-tailed macaques from the zoo as a control.

A total of 10 samples was collected and pooled for the species of wild elephants (ATR & KMTR), Nilgiri tahr (ATR & KMTR); Lion-tailed macaque (ATR), and captive (AAZP), and2 dung samples of captive elephants from AAZP.

3.2. Whole metagenome shotgun sequencing

Nilgiri Tahr, Wild elephant and Lion-tailed macaque are the chosen species for the study. Study in tigers was omitted because of the rare chance of getting very fresh scat samples from the field.

All samples were sequenced on the Illumina NextSeq500 platform using 2 x 150 bp chemistry Ligation technology to construct NGS sequencing libraries. The process uses an enzyme to connect specialized adapters to both ends of DNA fragments. An 'A'- base is added to the blunt ends of each strand, preparing them for ligation to the sequencing adapters. Each adapter contains a 'T'-base overhang, providing a complementary overhang for ligating the adapter to the A-tailed fragmented DNA.

3.3. Bioinformatics Analysis

3.3.1. Data Statistics and Quality Control

Samples were sequenced on the Illumina NextSeq500 platform using 2 x 150 bp chemistry, and the sequenced raw data statistics are provided in the table below.

Whole Metagenome shotgun sequencing (WMS), is a relatively new and powerful sequencing approach that provides insight into broader community biodiversity and function. DNA samples were randomly broken ~150bp in length. The resulting fragments were subjected to end repair, A-tail, Sequencing adapter addition, purification and PCR amplification of library sequences. All libraries were then sequenced on Illumina Nextseq500 platform with 2 × 150 bp paired reads (Eurofins, Bangalore).

The sequenced raw data for 3 samples were processed to obtain high-quality clean reads using Trimmomatic v0.39 to remove adapter sequences, ambiguous reads (reads with unknown nucleotides "N" larger than 5%), and low-quality sequences (reads with more than 10% quality threshold (QV) < 25 phred scores). A minimum length of 100 nt (nucleotide) aftertrimming was applied. After removing the adapter and low-quality sequences from the raw data high quality reads were obtained. These high quality (QV>25), paired-end reads were used for de-novo assembly.

Parameters considered for filtration are as follows:

- Adapter trimming was performed.
- SLIDING WINDOW: Sliding window of 10 bp, cutting once the average qualitywithin the window falls below a threshold of 25.
- LEADING: Cut bases off the start of a read if below a threshold quality of 25.

- TRAILING: Cut bases off the end of a read, if below a threshold quality of 25.

- MINLENGTH: Drop the read if it is below 100 bp length.

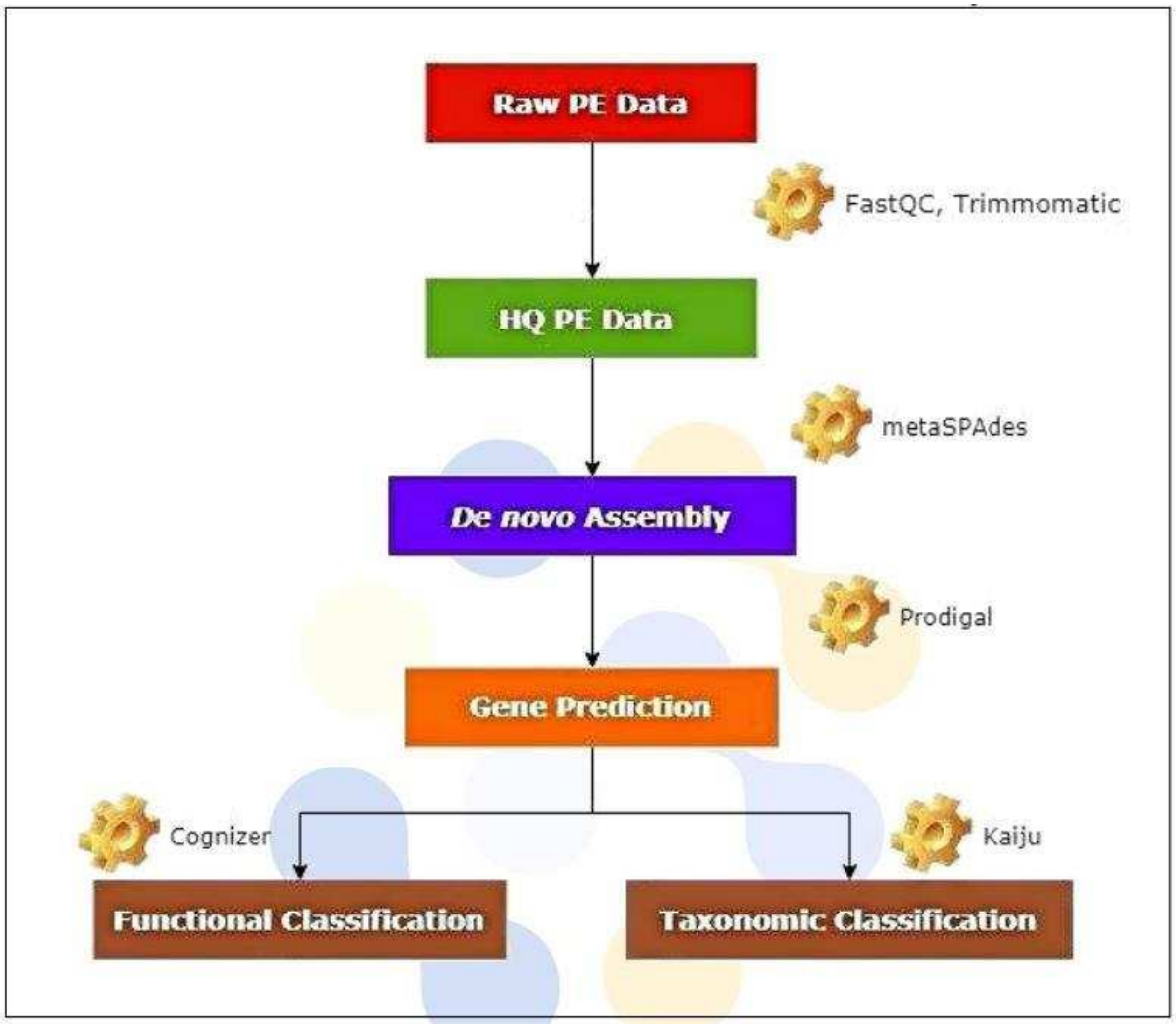


Figure 1: Bioinformatics Workflow

3.3.2. De-novo Metagenome Assembly

The filtered high-quality reads of 7 samples were assembled into scaffolds using SPAdes v3.12.0 [metaSPAdes mode]. Statistics of assembly are given in table 1 below.

Table 1: Asembly Statistics

Sample Name	Number of Scaffolds	Total length of the assembly (bp)	Average length of scaffolds (bp)	Scaffold N50 (bp)	Maximum length of scaffold (bp)
A1WC_NG1	3,31,753	37,36,28,122	1126	1,153	2,87,694
A1WC_NG2	2,19,388	28,92,37,148	1318	1,576	3,73,430
A1WC_NG3	1,21,881	13,41,21,214	1100	1,184	3,64,155
A1WC_4	352,226	338,478,396	961	933	338,392
A1WC_5	323,810	358,573,517	1,107	1,145	241,499
A1WC_6	272,682	368,771,944	1,352	1,642	258,941
A1WC_7	345,676	562,093,996	1,626	2,452	567,743

3.3.3. Gene Prediction

Genes were predicted from the assembled scaffolds of 7 samples using Prodigal-2.6.3 with default parameters. Statistics of genes are given in the following table 2:

Table 2: Gene Prediction Statistics

Sample Name	Number of genes	Average gene length (bp)	Maximum gene length (bp)	Minimum gene length (bp)
A1WC_NG1	6,51,402	512	20,778	60
A1WC_NG2	5,21,349	486	22,407	60
A1WC_NG3	2,39,390	493	19,188	60
A1WC_4	597,289	512	13,650	60
A1WC_5	674,731	467	23,145	60
A1WC_6	550,803	599	18,465	60
A1WC_7	738,651	698	29,595	60

3.3.4.Taxonomic Analysis

Taxonomic analysis of the predicted genes of 7 samples were carried out using Kaiju (kaiju.binf.ku.dk), which is a program for sensitive taxonomic classification of high-throughput metagenomic sequencing data. Kaiju uses either the set of available complete genomes from NCBI RefSeq or the microbial subset of the NCBI BLAST non-redundant protein database “nr”, optionally also including fungi and microbial eukaryotes. The results of the taxonomic analysis are described in the following result section.

4. Results

Taxonomic abundance at Kingdom level:

Nilgiri Tahr (ATR)

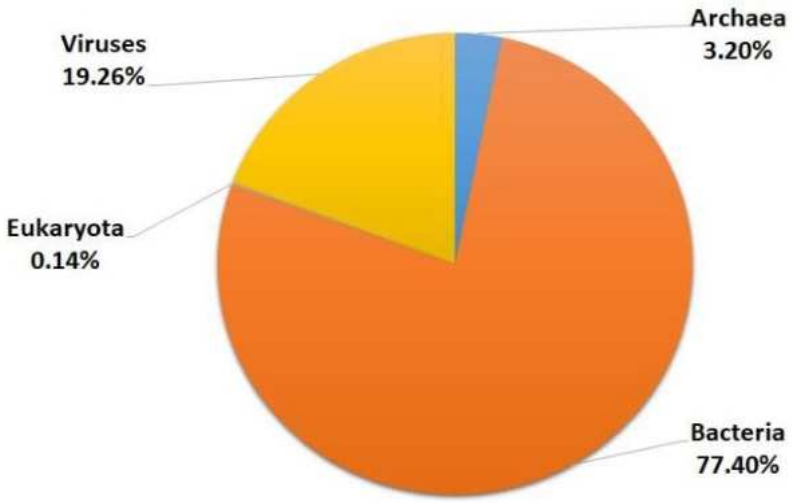


Figure 2: Kingdom level abundance for the sample of Nilgiri Tahr

Bacteria is more abundant in the stool of NilgiriTahr (77.40%) and followed by viruses (19.26%).

Wild elephant (ATR)

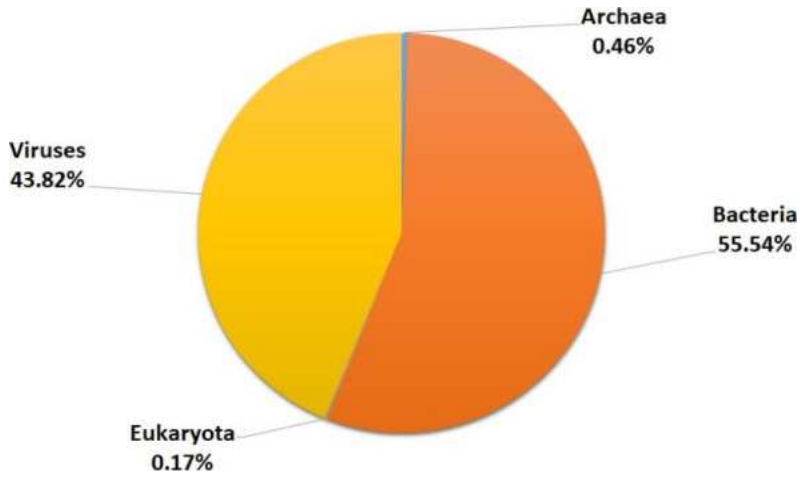


Figure 3: Kingdom-level abundance for the sample of Wild elephant (ATR)

Bacteria is the most abundant kingdom. In wild elephant stool sample, 55.54% of bacteria and 43.82% of virus abundance observed.

Lion tailed macaque (LTM) (ATR)

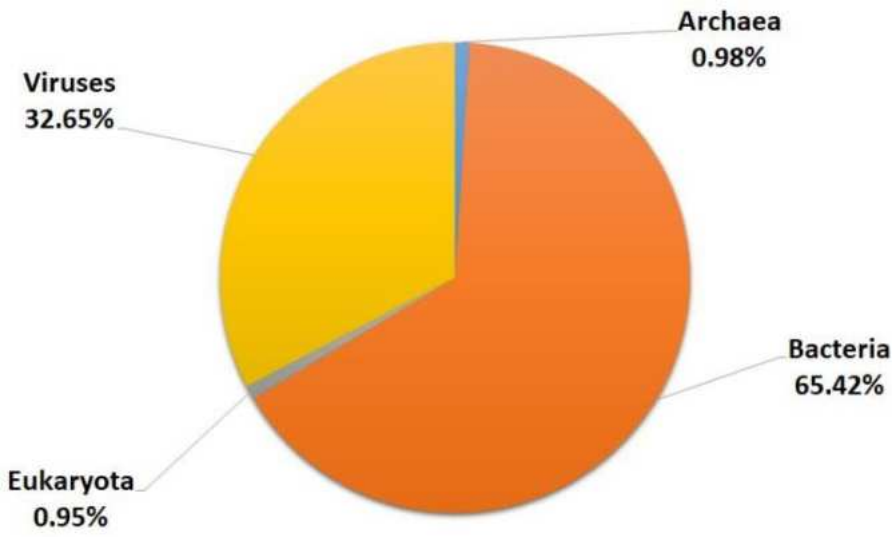


Figure 4: Kingdom-level abundance for the sample of LTM (ATR)

Stool samples of LTM from ATR contains, 65.42% bacteria and 32.65% of virus abundance observed.

Nilgiri Tahr (KMTR)

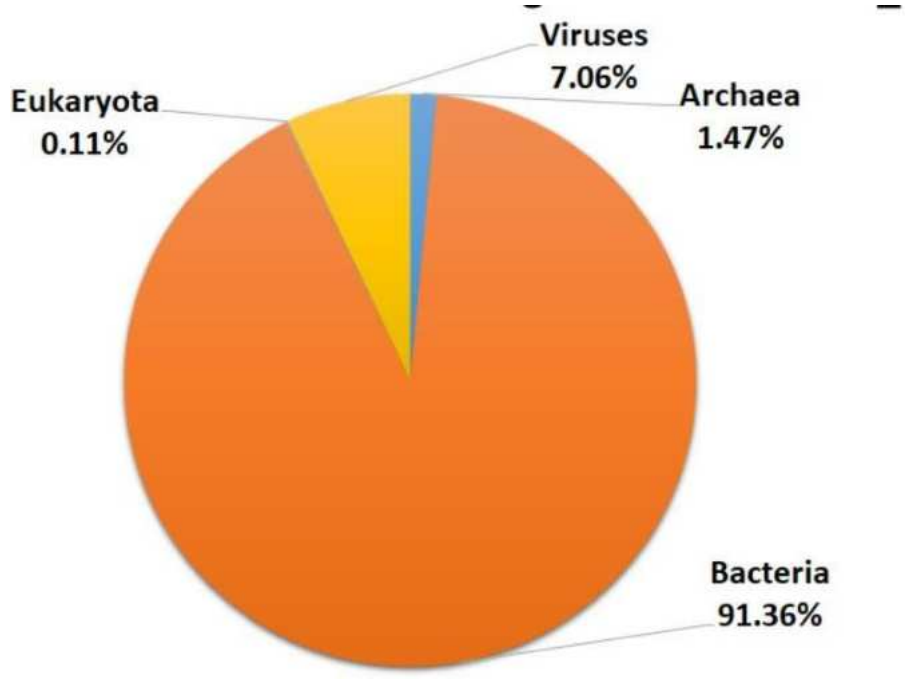


Figure 5: Kingdom level abundance for the sample of Nilgiri Tahr (KMTR)

Bacteria (91.36%) holds high abundance and virus with 7.06%.

Wild elephant (KMTR)

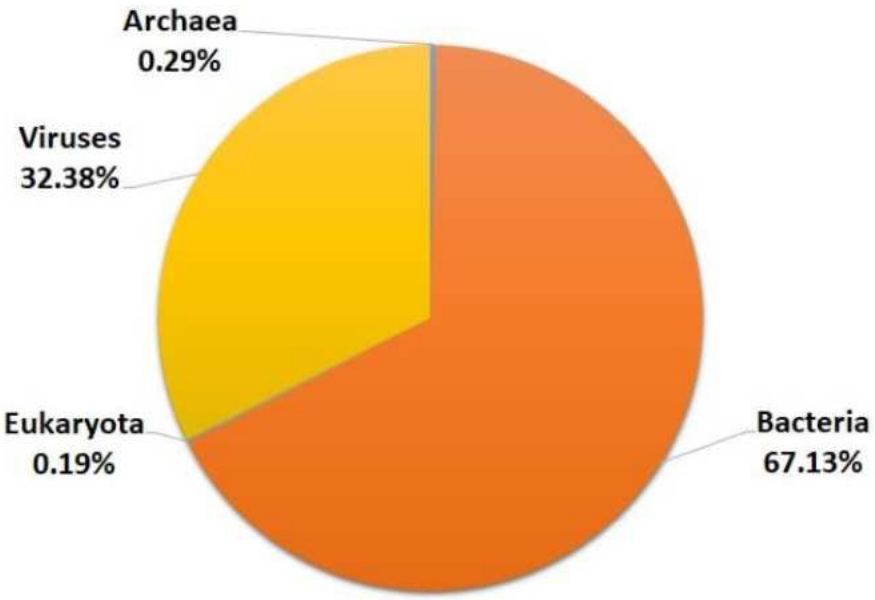


Figure 6: Kingdom-level abundance for the sample of Wild elephant (KMTR)

Dung samples of wild elephant of KMTR shows 67.13% bacterial abundance followed by 32.38% virus abundance.

Lion tailed macaque (Captive)

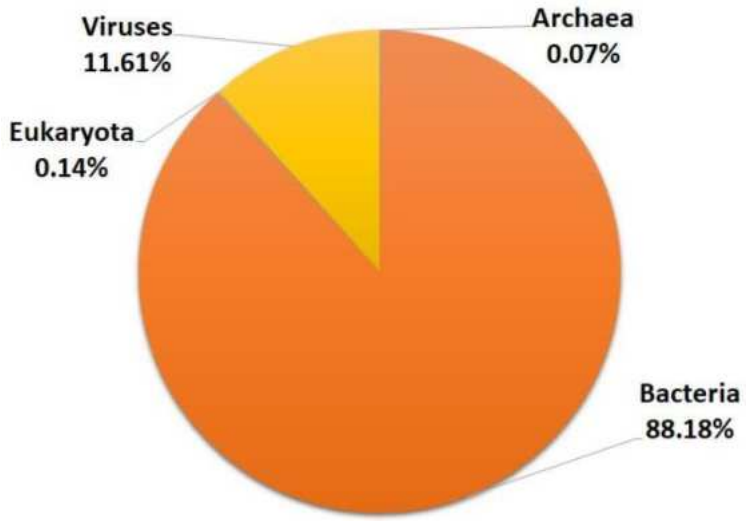


Figure 7: Kingdom-level abundance of Wild elephant (KMTR)

Bacteria (88.18%) is the most abundance kingdom in wild elephant of KMTR and 11.61% of virus.

Captive elephant

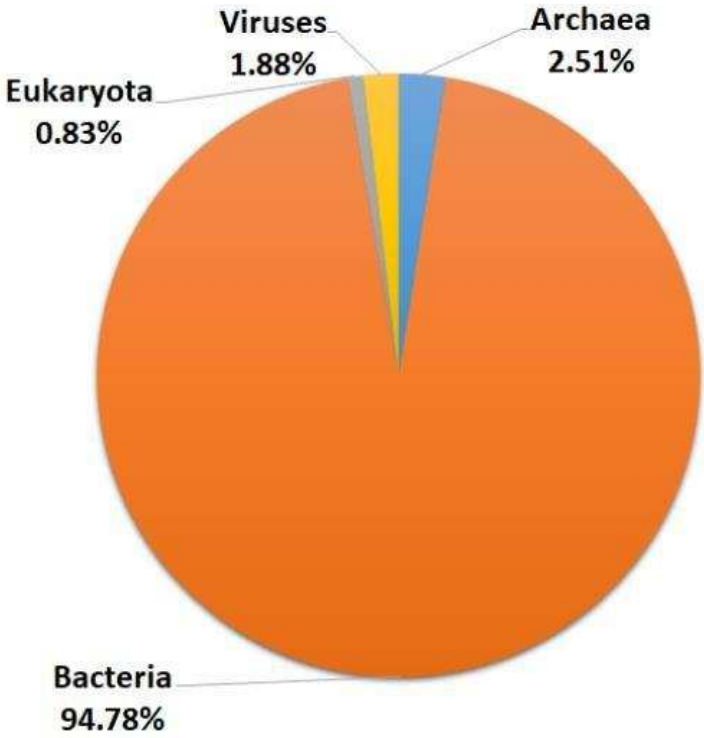


Figure 8: Kingdom-level abundance of captive elephant

94.78% of bacteria is more abundant in the dung sample of captive elephant and a very less amount of virus abundance (1.88%).

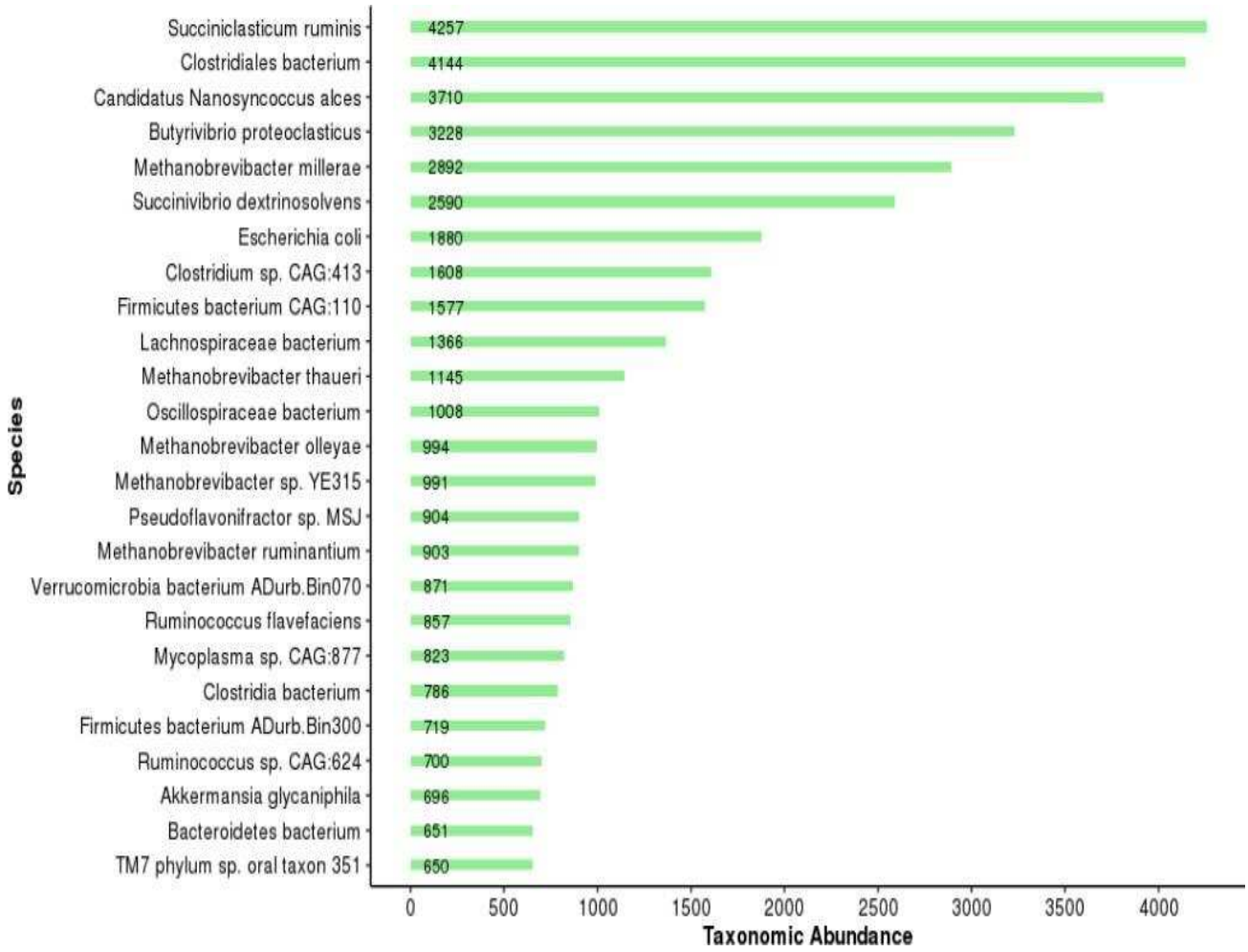
Table 3: Kingdom level abundance statistics of 7 samples

Sl.No.	Species	Archaea	Bacteria	Eukaryota	Viruses
1.	Nilgiri Tahr (ATR)	3.20	77.40	0.14	19.26
2.	Wild elephant (ATR)	0.46	55	0.17	43.82
3.	Lion tailed macaque (ATR)	0.98	54	0.95	32.65
4.	Nilgiri Tahr (KMTR)	1.47	65.42	0.11	7.06
5.	Wild elephant (KMTR)	0.29	91.36	0.19	32.38
6.	Lion tailed macaque (Captive) (AAZP)	0.07	67.13	0.14	11.61
7.	Captive elephant (AAZP)	2.51	88.18	0.83	1.88

Taxonomic classification at Species level (Bacteria)

NilgiriTahr (ATR)

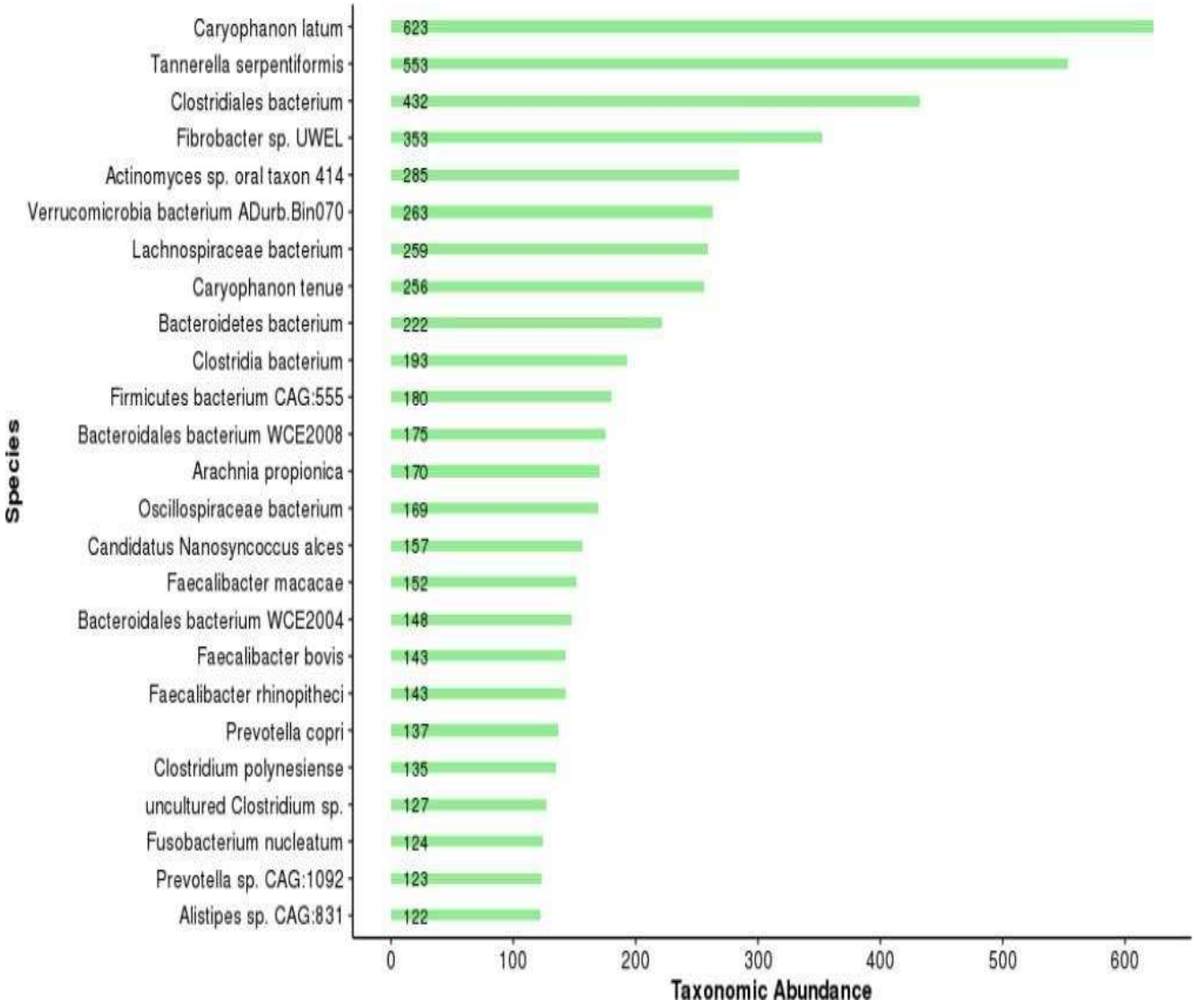
Figure 9: Taxonomic classification at Species level (top 25 most abundant) for the sample Nilgiri Tahr (ATR).



Bacterial species ***Succiniclasticumruminis*** is found to be rich in Nilgiri Tahr (ATR)

Wild elephant (ATR)

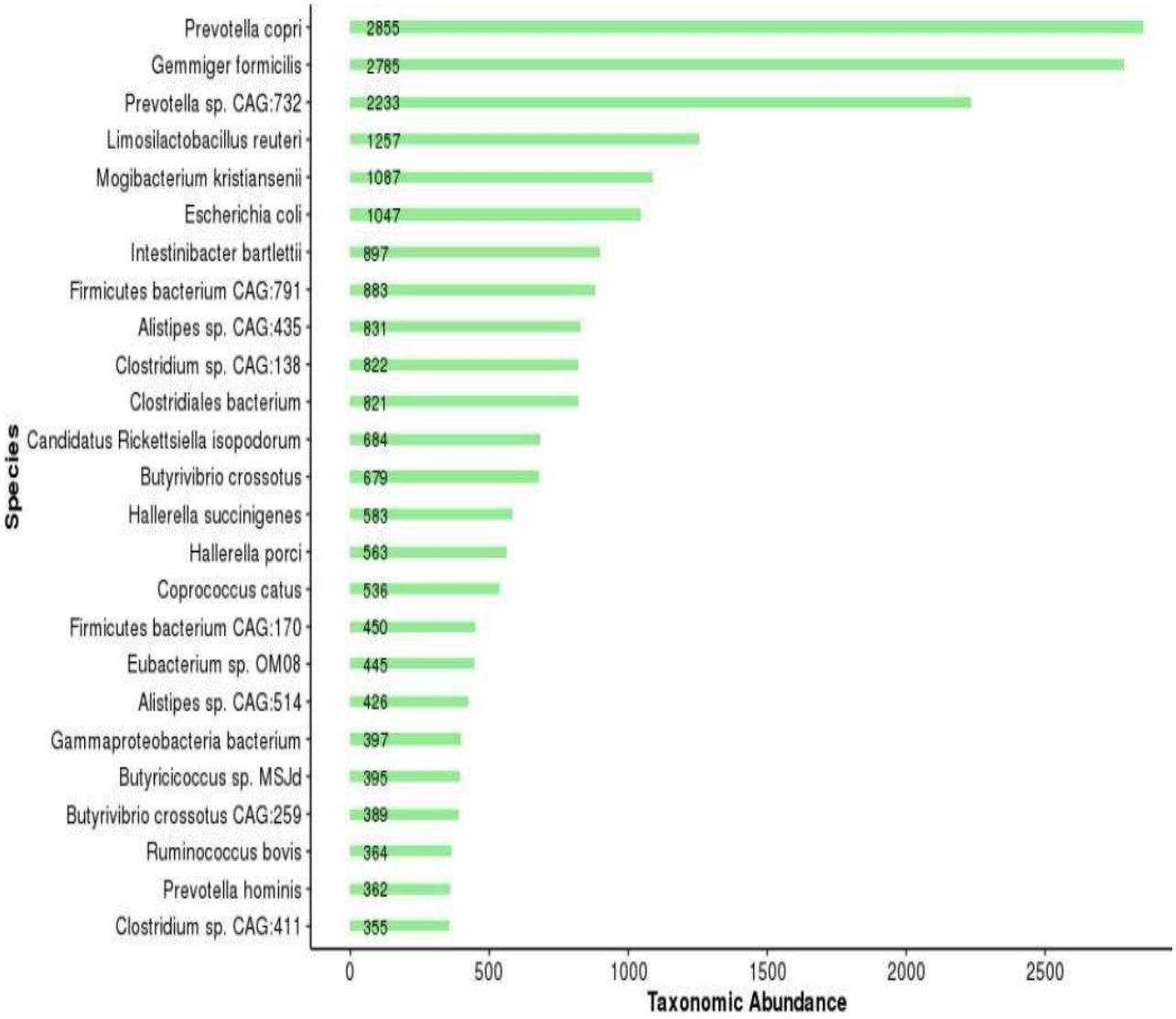
Figure 10: Taxonomic classification at Species level (top 25 most abundant) for the sample wild elephant (ATR)



Bacterial species ***Caryophanon latum*** is found to be rich in Wild Elephant.

Lion tailed macque (ATR)

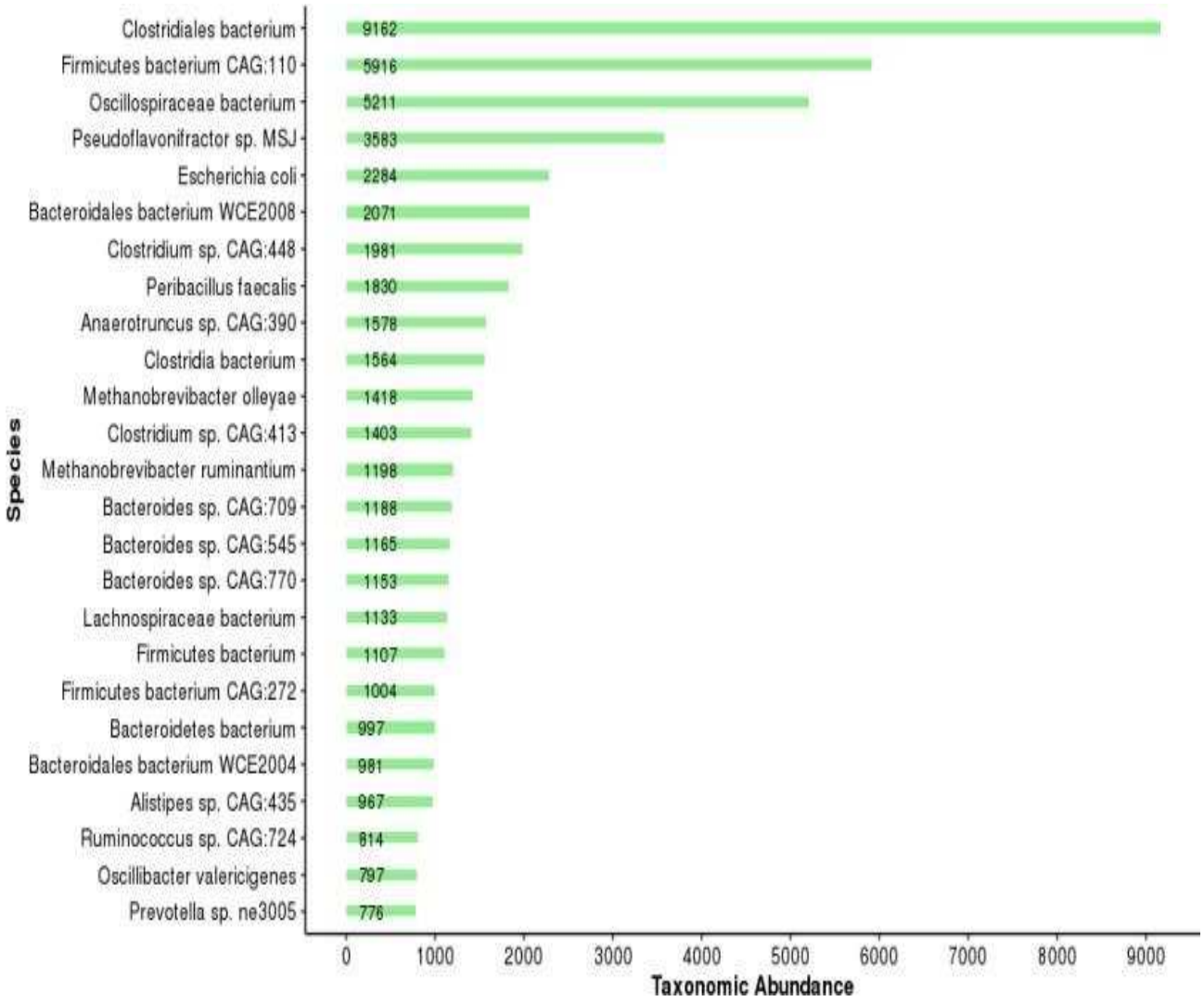
Figure 11: Taxonomic classification at Species level (top 25 most abundant)



Prevotella copri is the most abundant species.

Nilgiri Tahr (KMTR)

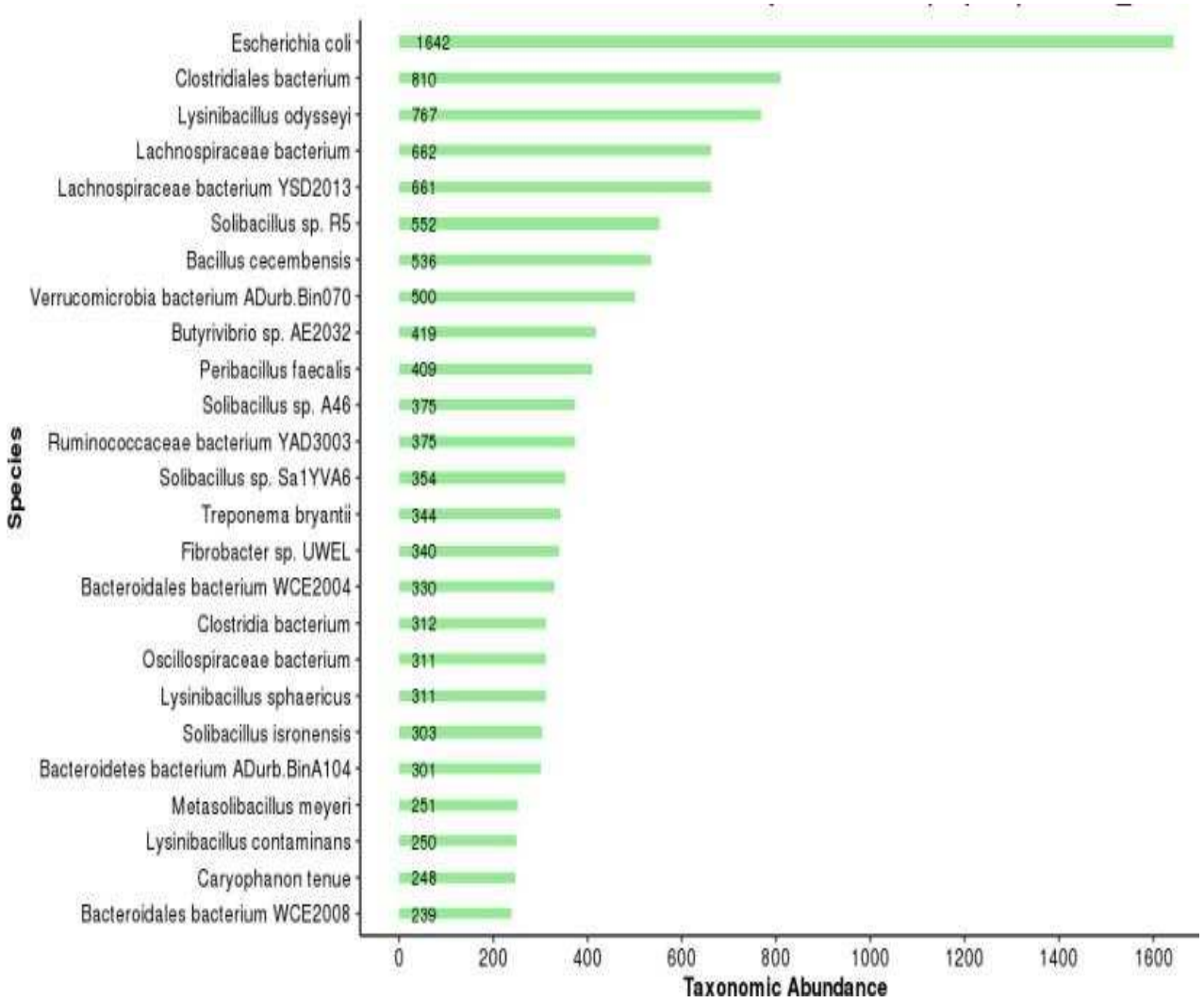
Figure 12: Taxonomic classification of bacteria at Species level (top 25 most abundant) of Nilgiri Tahr (KMTR)



Clostridiales bacterium is the most abundant species.

Wild elephant (KMTR)

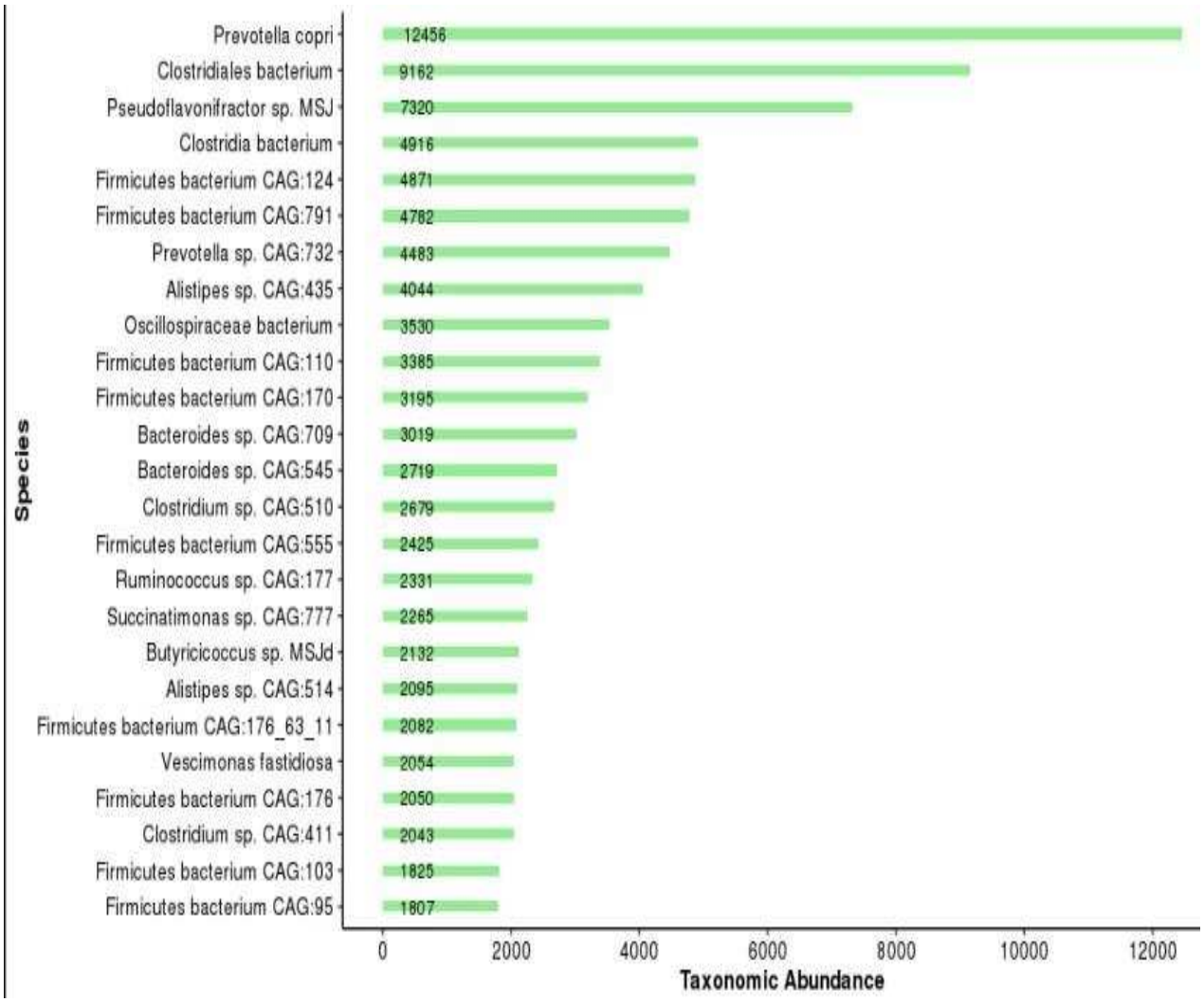
Figure 13: Taxonomic classification of bacteria at Species level (top 25 most abundant) of wild elephant (KMTR)



Escherichia coli is the most abundant species observed.

Captive LTM

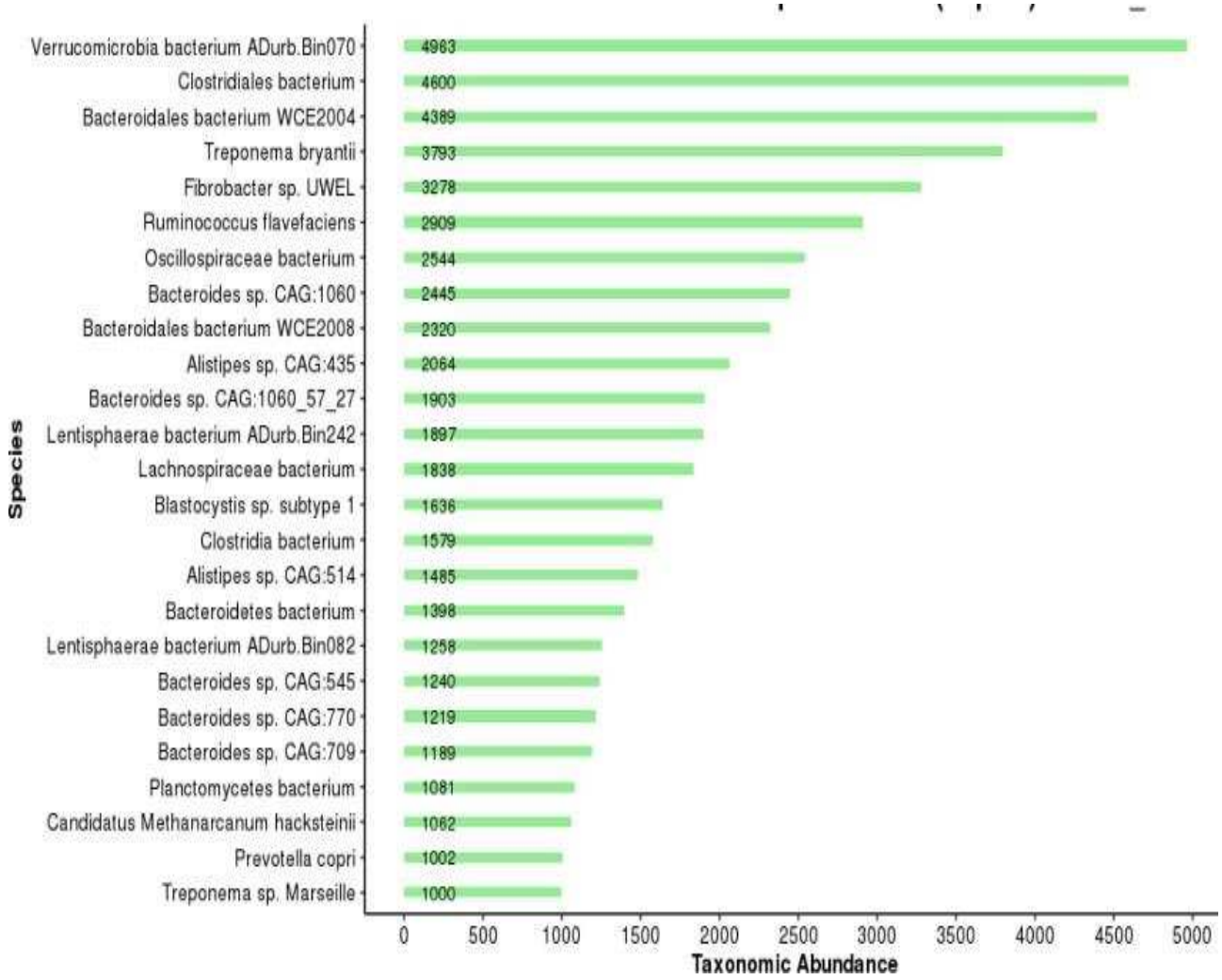
Figure 14: Taxonomic classification of bacteria at Species level (top 25 most abundant species in captive LTM)



Prevotella copri is the most abundant speices observed.

Captive elephant

Figure 15: Taxonomic classification of bacteria at Species level (top 25 most abundant species in captive elephant)



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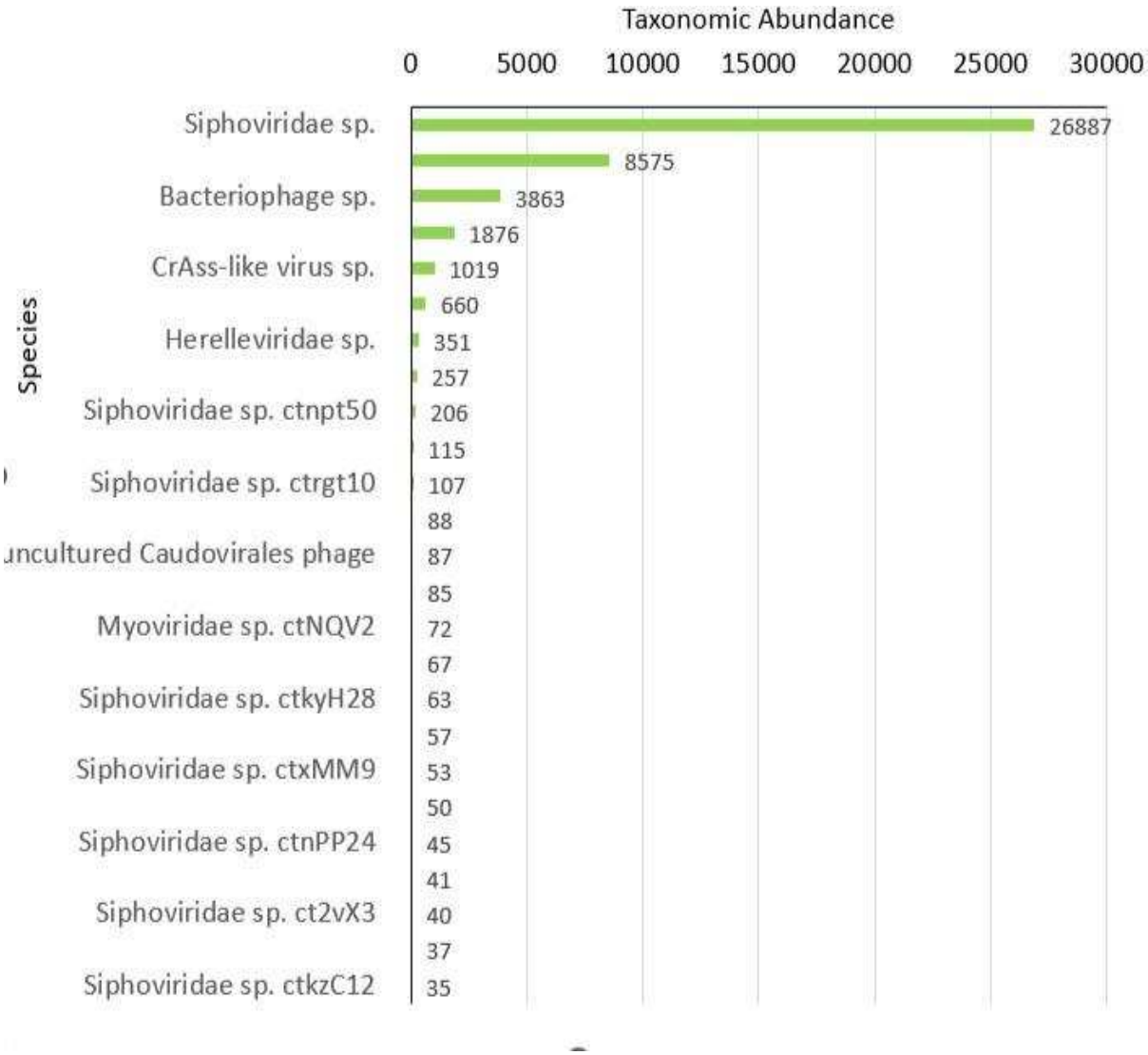
V*errucomicrobia bacterium ADurb.Bin070* is the most abundant species observed in captive elephant dung sample.

Taxonomic classification at Species level (Virus)

Nilgiri Tahr (ATR)

Figure 14: Taxonomic classification of virus at Species level (top 25 most abundant species in Nilgiri Tahr)



Siphoviridae spp. is found to be rich in Nilgiri Tahr.

Wild elephant (ATR)

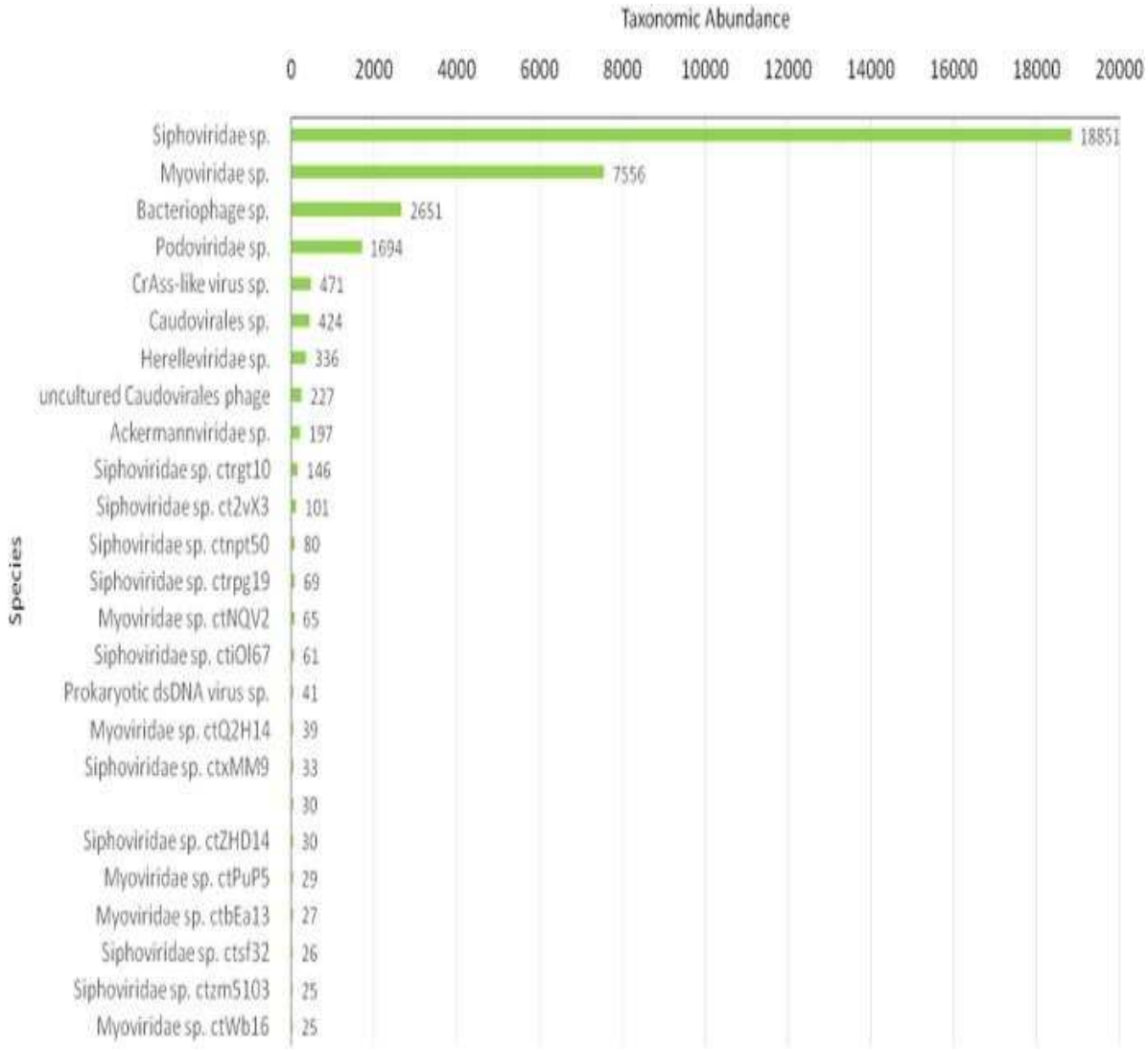


Figure 15: Taxonomic classification of virus at Species level (top 25 most abundant species in wild elephant). ***Siphoviridae spp.*** is found to be rich in Wild Elephant.

Lion-Tailed Macaque (ATR)

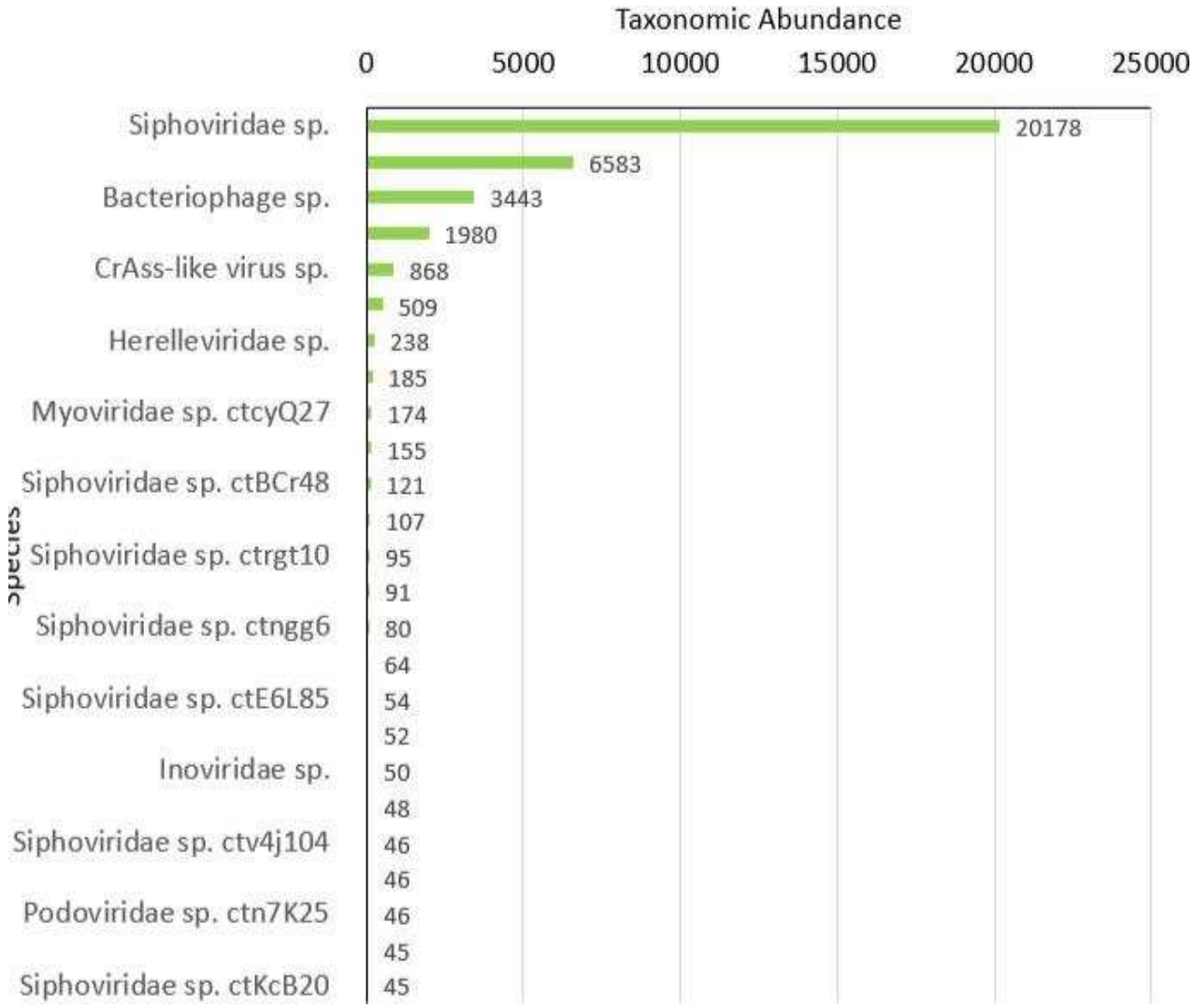


Figure 16: Taxonomic classification of virus at Species level (top 25 most abundant species in wild elephant). ***Siphoviridae spp.*** Is the most abundant species.

Nilgiri Tahr (KMTR)

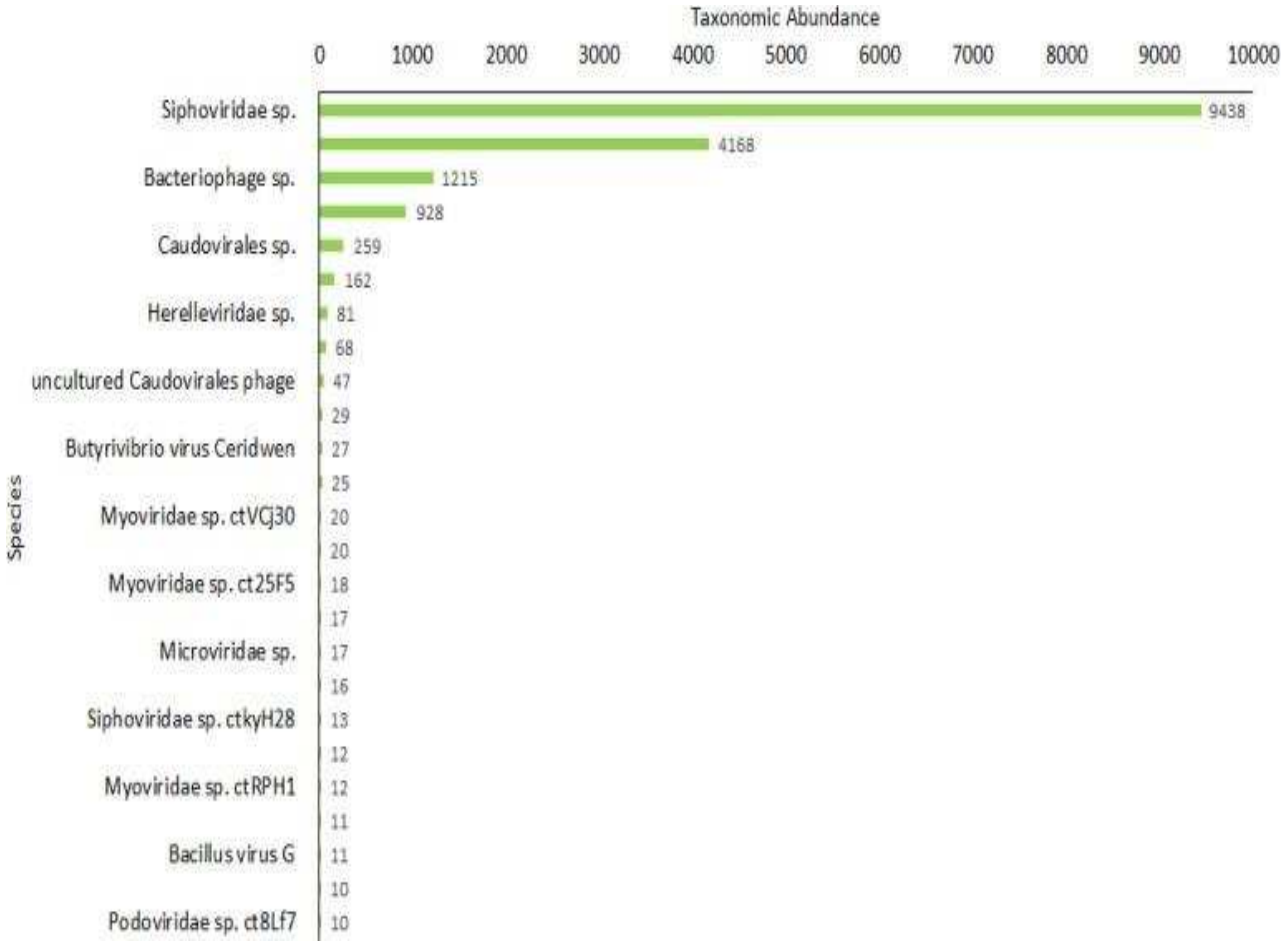


Figure 17: Taxonomic classification of virus at Species level (top 25 most abundant species in Nilgiri Tahr). ***Siphoviridae sp.*** is the most abundant species.

Wild elephant (KMTR)

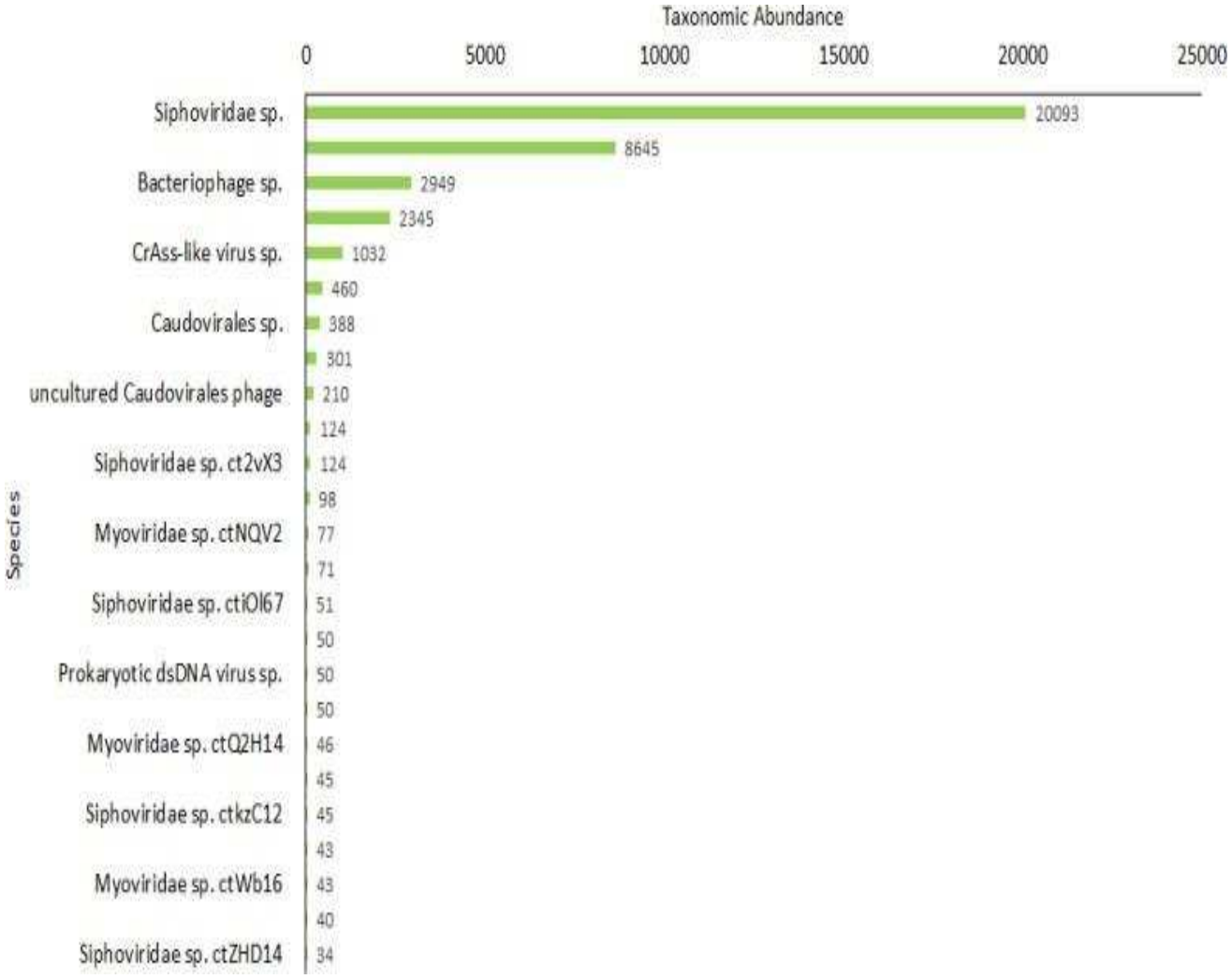


Figure 18: Taxonomic classification of virus at Species level (top 25 most abundant species in wild elephant). ***Siphoviridae sp.*** is the most abundant species.

Lion-Tailed Macaque (Captive)

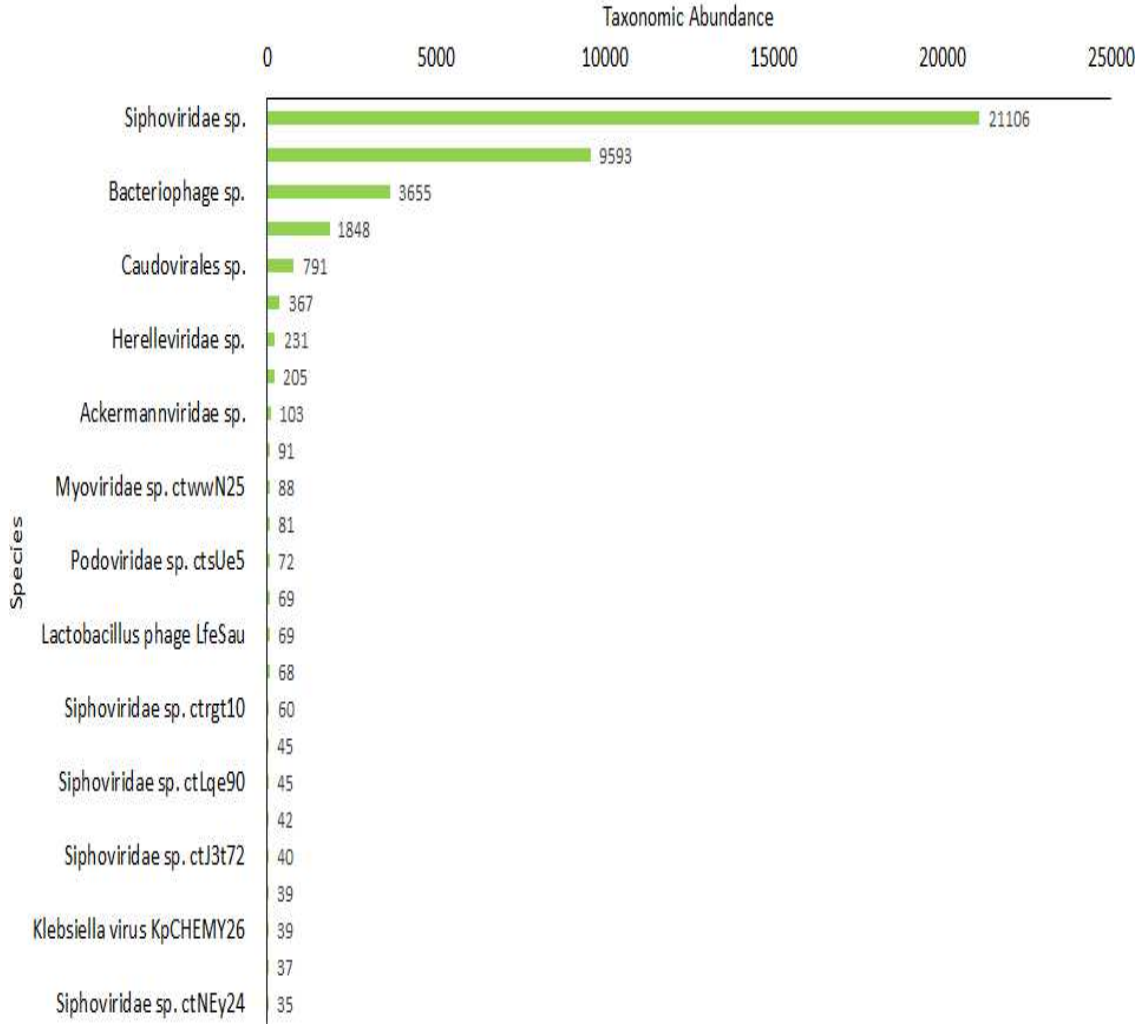


Figure 19: Taxonomic classification of virus at Species level (top 25 most abundant species in captive LTM). ***Siphoviridae sp.*** is the most abundant species.

Captive elephant

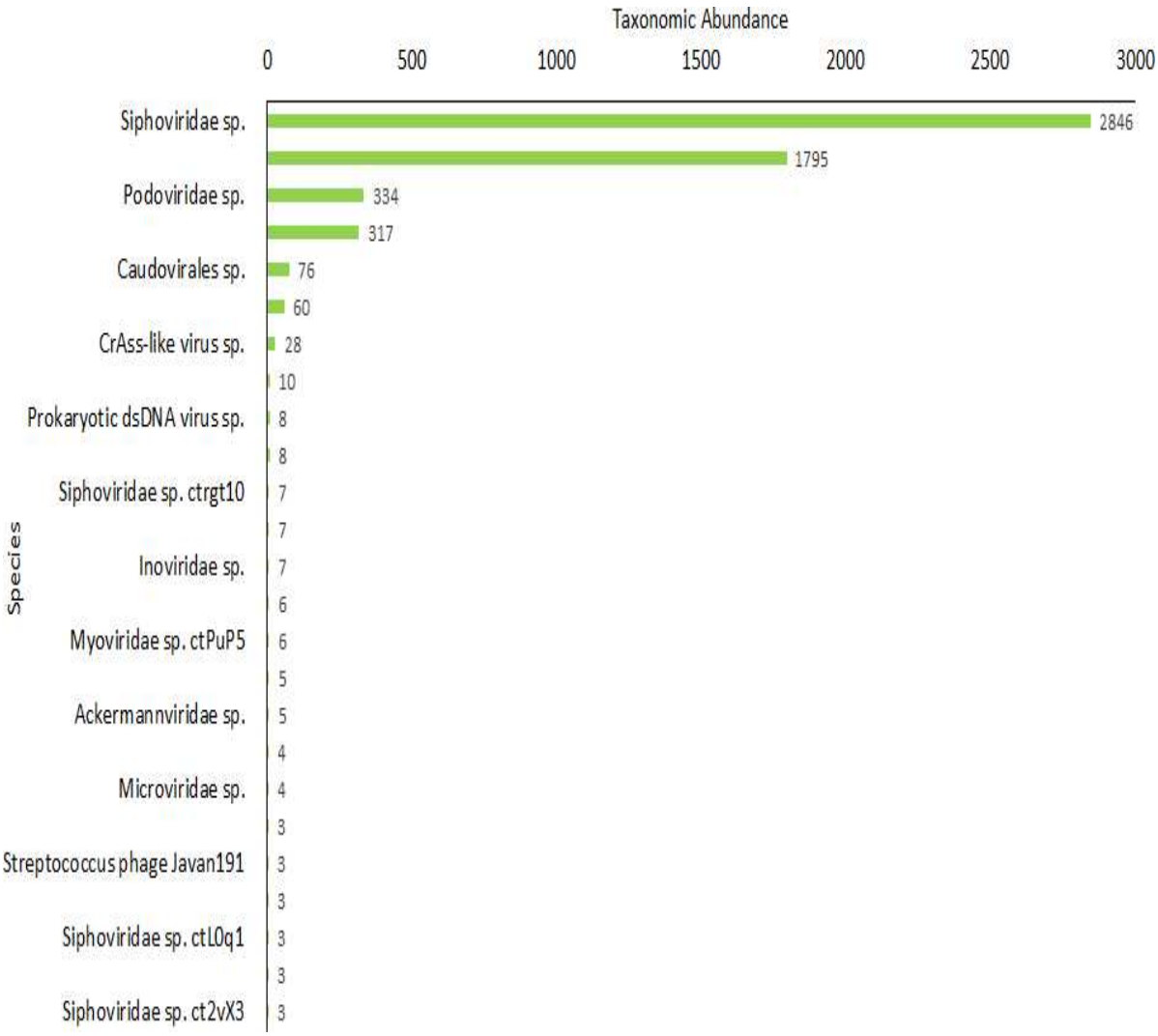


Figure 20: Taxonomic classification of virus at Species level (top 25 most abundant species in captive elephant). ***Siphoviridae sp.*** is the most abundant species.

Table 4: Most abundant sample at species level (Wild) Bacteria

Species	Most abundant organism at species level
Nilgiri Tahr (ATR)	*Succiniclasticum ruminis*
Wild elephant (ATR)	*Caryophanon latum*
Lion tailed macaque (ATR)	*Prevotella copri*
Nilgiri Tahr (KMTR)	*Escherichia coli*
Wild elephant (KMTR)	*Clostridiales bacterium*

Table 5: Most abundant sample at species level (captive) Bacteria

Species	Most abundant organism at species level
Lion tailed macaque (Captive) (AAZP)	*Prevotella copri*
Captive elephant (AAZP)	*Verrucomicrobia bacterium ADurb.Bin070*

Table 6: Most abundant sample at species level (Wild) Virus

Species	Most abundant organism at species level
Nilgiri Tahr (ATR)	*Siphoviridae sp.*
Wild elephant (ATR)	*Siphoviridae sp.*
Lion tailed macaque (ATR)	*Siphoviridae sp.*
Nilgiri Tahr (KMTR)	*Siphoviridae sp.*
Wild elephant (KMTR)	*Siphoviridae sp.*

Table 7: Most abundant sample at species level (captive) Virus

Species	Most abundant organism at species level
Lion tailed macaque (Captive) (AAZP)	*Siphoviridae sp.*
Captive elephant (AAZP)	*Siphoviridae sp.*

Table 8: Common bacterial disease in Nilgiri Tahr and its incidence in collected samples. (Harish B et al., (2019), Balakrishnan et al., (2017), Jones et al., (2018), Singh et al.,2014)

Species	Bacterial disease	Causative organism	Wild	
			ATR	KMTR
Nilgiri Tahr (ATR)	Brucellosis	*B. abortus*	-ve	-ve
	Campylobacteriosis	*Campylobacter fetus spp. fetus intestinalis, Campylobacter jejuni*	-ve	-ve
	Salmonellosis	*Salmonella spp*	-ve	-ve
	Listeriosis	*Listeria monocytogenes*	-ve	-ve
	Q Fever	*Coxiella burnetii*	-ve	-ve
	Tuberculosis	*Mycobacterium bovis or Mycobacterium tuberculosis*	-ve	-ve

Table 9: Common bacterial disease in elephant and its incidence in wild/captively collected samples. (Harish B et al., (2019), Balakrishnan et al., (2017), Jones et al., (2018), Singh et al.,2014)

Species	Bacterial disease	Causative organism	Captive	Wild	
				ATR	KMTR
Asian Elephant	Tuberculosis	*Mycobacterium tuberculosis*	-ve	-ve	-ve
	Salmonellosis	*Salmonella enterica*	-ve	-ve	-ve
	Tetanus	*Clostridium tetani*	-ve	-ve	-ve

Table 10: Common bacterial disease in LTM and its incidence in wild/captively collected samples. (Harish B et al., (2019), Balakrishnan et al., (2017), Jones et al., (2018), Singh et al.,2014)

Species	Bacterial disease	Causative organism	Wild	Captive
LTM	Shigellosis	*Shigella flexneri*	-ve	-ve
	Salmonellosis	*Salmonella spp.*	-ve	-ve
	Tetanus	*Mycobacterium tuberculosis*	-ve	-ve
	Compylobacteriosis	*Campylobacter*	-ve	-ve
	Helibacteriosis	*Helicobacter pylori*	-ve	-ve
	Listeria	*Listeria monocytogenes*	Prevotella spp. can act as a moderator	Prevotella spp. can act as a moderator
	Streptococcus pneumonia	*Streptococcus pneumoniae*	-ve	-ve

Table 11: Common viral disease in Nilglir Tahr, elephant, LTM and its incidence in wild/captively collected samples. (Harish B et al., (2019), Balakrishnan et al., (2017), Jones et al., (2018), Singh et al.,2014)

Species	Viral disease	Causative organism	Captive	Wild	
				ATR	KMTR
Nilgiri Tahr	Malignant catarrhal fever	*Alcelaphine gammaherpesvirus 1 (AlHV-1),* *Ovine gammaherpesvirus 2 (OvHV-2)*	--	-ve	-ve
Elephant	Elephant Endotheliotropic Herpesvirus (EEHV)	*Elephantid betaherpesvirus 1*	-ve	-ve	-ve
Lion-Tailed Macaque	Kyasanur Forest virus	*KFDV - Flaviviridae*	-ve	-ve	--
	Monkey B virus (MBV)	*Herpesviridae*	-ve	-ve	--
	Monkeypox (MPoX)	*Orthopoxvirus Poxviridae*	-ve	-ve	--

5. Discussion:

Health monitoring in free-ranging wild animals is a critical aspect of wildlife conservationand management. It involves the systematic assessment of the health and well-being of individual animals and populations in their natural habitats. Health monitoring in free-range wild animals plays a vital role in the conservation and management of wildlife populations. By employing non-invasive techniques, conducting disease surveillance, and establishing long-term monitoring programs, researchers and conservationists can gain valuable insights into the health status of wildlife populations and implement appropriate measures to safeguardtheir well-being and the integrity of their natural habitats. Metagneomic data provides insightsinto the ecosystem of the bacteria and virus that is present inside the gut of the organism andalso its multiple disease causing pathogenic bacterial ecosysytem. Gut bacteria have the ability to have a relationship and communication with the environment around them. They may not be directly beneficial but the abundance of the gut bacteria always has it impact on the metabolic and functional activity of the organism. Lot of human gut bacteria were studied with respect to the gut health of the organisms also talk about the the importance of their abundenc in maintaining the metabolic pathways correlates to the multiple organs linked to the gut.

The most abundant bacterial species observed in the stool samples of Nilgiri Tahr form Anamalai Tiger Reserve is *Succiniclasticum ruminis*. It is a gram-negative, anaerobic, non motile, non-spore-forming, rod-shaped bacterium that fermented succinate quantitatively. It is a common inhabitant of the rumina of cows that are fed production diets and of cows on pasture (Van, 1995). But, in the pellets of Nilgiri Tahr collected from KMTR, *Escherichia coli* is most abundant.

Caryophanon latum is the most abundant species of bacteria observed in the dung samples of Wild elephant from ATR. It is an extraordinary non-sporing, Gram-negative bacterium first described by Peshkoff from fresh cow dung (Pringsheim & Robinow,1947). Its pathogenic nature is not yet defined. But, *Clostridiales bacterium* is the most abundant bacteria observed in the dung samples of wild elephants from KMTR. *Verrucomicrobia bacteriumADurb.Bin070* is the bacteria found more in the dung samples of captive elephants from AAZP.

On the other hand, *Prevotella copri* were found more abundant in the stool sample of Lion tailed macaque of ATR and captive LTM sample collected from AAZP. Some studies reported that, P*revotella copri* act as modulator of infection caused by foodborne pathogen *Listeria monocytogenes* (Rolhion et al., 2019). Precolonization of germ-free mice with *Prevotella copri* strain resulted in a significantly thinner mucus layer and higher degree of intestinal inflammation in mice following *Listeria monocytogenes* inoculation compared to those preinoculated with other commensal bacteria (*Bacteroides thetaiotaomicron*), suggesting that *Prevotella copri* may impair intestinal mucus barrier function, and therefore making the intestinal epithelial cells and local inflammation system more vulnerable to pathogen invasion (Amat et al., 2020).

Siphoviridae sp. is the virus species observed most abundant in all 7 samples including wild and captive.

Antibiotic resistance genes (ARGs) profile Wild and Captive of Asian elephants

The emergence of antibiotic resistance is a growing threat to public health worldwide and other animals. Compared to the gram positive bacteria, gram negative bacteria acquire

resistance faster thus multidrug resistant negative bacteria pose the biggest threat to public health. Wild animals act as efficient antimicrobial resistance reservoirs and epidemiological links between human, livestock and the environment. Drug resistance in wildlife can develop on its own or by exposure to human waste or agricultural runoff with antibiotic residues. Compared to wild animals, zoo animal populations are more closely associated with human populations, thus it is highly likely that antimicrobial resistance (AMR) and ARGs in zoo animals are more similar to humans and livestock. Limited studies have been conducted to monitor antibiotic resistant bacteria in zoo animals (Feng Xin et al., 2023).

Based on the medical records from the zoo keeper, elephants did not receive antibiotic treatment for 6 months before sample collection. The two Asian elephants received antibiotic treatment in February and March which was about seven months prior to sample collection. Some antibiotic resistance genes do not vanish immediately after antibiotics end as some bacteria can pass on those genes to the next generation. Furthermore, animal food may also play a role in disseminating antibiotic resistance to zoo animals as antibiotic resistant bacteria and ARGs have been isolated from zoo animal foods. Heavy metals can drive the co-selection of antibiotic resistance in soil and water bodies.

Succiniclasticum ruminis is the most abundant species in Nilgiri tahr (ATR). *Caryophanon latum* is the most abundant species in Asian Elephant (ATR). Prevotella copri is the most abundant species, act as modulator of infection caused by foodborne pathogen Listeria monocytogenes (Rolhion et al., 2019) in Lion-Tailed Macaque.

Abundance of E.coli in Lion-Tailed Macaque could be a potential zoonotic disease. As the group of 100 primates move through the valparai town and enter the reserve forest area

near the pudhuthottam village. It is observed that these group frequently enter the residental and commercial buildings and also the shelters of the workers of the tea and coffee estate.

6. Conclusion:

Metagenomics, a cutting-edge field in genomics, has emerged as a powerful tool in wildlife conservation. Unlike traditional genetic analysis that focuses on individual organisms, metagenomics delves into the study of entire microbial communities present in environmental samples. By harnessing the potential of metagenomics, conservationists can gain valuable insights into the intricate relationships between wildlife, their habitat, and the microbial world. This discussion explores the role of metagenomics in wildlife conservation and its implications for biodiversity preservation.

1. Understanding Ecosystem Health

Metagenomics allows conservationists to obtain a holistic picture of ecosystem health by analyzing the collective DNA of all organisms present in a given environment. Microbial communities play a crucial role in maintaining ecological balance, affecting nutrient cycling, disease regulation, and overall ecosystem stability. By characterizing these microbial communities, conservationists can identify indicators of a healthy ecosystem and detect early signs of degradation or disturbance.

2. Monitoring Endangered Species

For many endangered species, acquiring direct genetic samples can be challenging and invasive. Metagenomics offers a non-invasive alternative, enabling researchers to study the presence and genetic diversity of endangered species indirectly through their shed DNA or microbiota left in their habitats. This technique provides valuable data for estimating

population sizes, monitoring individual health, and understanding the genetic diversity of endangered species critical for their survival.

3. Zoonotic Disease Surveillance

Zoonotic diseases, which can transfer between animals and humans, pose a significant threat to both wildlife and public health. Metagenomics can help in early detection and monitoring of zoonotic pathogens by identifying potential reservoirs in wildlife. By understanding the interactions between wildlife, their microbial communities, and zoonotic agents, we can implement targeted surveillance and preventive measures to minimize disease transmission.

4. Tracking Invasive Species

Invasive species can have devastating impacts on local biodiversity. Metagenomics allowsus to identify invasive species in their early stages before they become established and cause irreparable harm. By analyzing the environmental DNA in a region, conservationists can detect the presence of invasive organisms and respond promptly with appropriate managementstrategies.

5. Restoring Disturbed Habitats

When ecosystems face disturbances, such as pollution or habitat degradation, microbial communities are often among the first to respond. Metagenomics helps conservationists assess the resilience of habitats by observing shifts in microbial diversity and function. This knowledge is invaluable for devising targeted restoration plans that foster the recovery of bothflora and fauna.

6. Unraveling Symbiotic Relationships

Metagenomics enables the study of intricate symbiotic relationships between species, including those between animals and their gut microbiota. Understanding these interactions can shed light on the role of microbes in the health, nutrition, and behavior of wildlife. This knowledge can lead to innovative approaches in captive breeding and conservation programs to ensure the well-being of endangered species.

Metagenomics has revolutionized the field of wildlife conservation by offering a comprehensive and non-invasive approach to study the complex relationships between wildlife, their habitats, and the microbial world. From monitoring endangered species to understanding ecosystem health and detecting zoonotic diseases, metagenomics provides valuable data and tools to safeguard biodiversity for future generations. Embracing metagenomics in conservation efforts can empower us to make informed decisions and create sustainable strategies to preserve our planet's rich biological heritage.

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APPENDIX-I

Photographs of sample collection from the field.

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