Comprehensive surveillance of infectious diseases

Infectious diseases are caused by infectious pathogens like bacteria, virus or fungi and can also be transmitted through parasites from one host to another. Comprehensive surveillance of infectious diseases by different approaches can help in better analysis of the diseases and their prevalence in the society. In this project, we propose two different approaches to understand the epidemiology of infectious diseases- 1) Surveillance of infectious diseases by waste water-based epidemiology and 2) Surveillance for zoonotic vector borne diseases through analysis of tick-wildlife-human system.

1) Surveillance of infectious diseases by waste water-based epidemiology

Background: Supervision of the health of community can help in early detection of emerging infectious diseases in the individuals of the society. Waste water-based epidemiology can serve as a tool for early detection of the infectious diseases, can give a quantitative estimation of the number of individuals infected and can serve as a warning system for the governments to take appropriate measures to contain the diseases.

The ongoing COVID-19 (Coronavirus disease 2019) pandemic has affected the society in most unexpected ways. SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2), the causative virus is shed in the stools of the COVID-19 individuals regardless of whether the patient suffers from gastrointestinal upset or not (Wang et al., 2020a and Wang et al., 2020b). Even asymptomatic COVID-19 affected individuals shed the virus in stools and the virus can be detected in the stools for several days even after the patient tests negative in conventional nasopharyngeal tests. Analysis of waste water for the presence of SARS-CoV-2 genes can give a qualitative as well as quantitative idea of the spread of the infection in the corresponding society.

Capacity Building: We have standardized a protocol for the detection of the SARS-CoV-2 genes in sewage samples from STPs (sewage treatment plants) and drains from several cities in India. After quantitating the viral load in the waste water samples, the number of individuals infected in that community are estimated by mathematical calculations (Ahmed et al., 2020a and Hellmér et al., 2014). Based on the analysis of STP samples from Hyderabad city, we have estimated that approximately 1-7% of the population of Hyderabad was already infected with SARS-CoV-2 during 8th July 2020 to 6th August 2020 (Manupati et al, online 2021 Jan 6). We have also monitored the weekly infection trend for a period of two months in eight cities and weekly infection trend for a period of 1 month in 3 cities and 5 towns in India. The standard operating procedures (SOP) for detection of SARS-CoV-2 in waste water samples are already established by us (Fig. 1). The regular monitoring of SARS-CoV-2 or any such parasite or the biomarkers associated with candidate diseases in waste water samples from different communities worldwide can serve as a reliable and unbiased surveillance measure to comprehensively monitor the prevalence of the disease in the communities in real time.

Significance and impact: Along with SARS-CoV-2, several infectious agents and biomarkers associated with diseases can be detected in waste water surveillance. Sewage, being a centralized collection point for human wastes is a highly valuable and unbiased sample, can give an impartial idea of the disease epidemiology in a society or a community. Sewage surveillance would be relevant not only to understand the present epidemiology of the COVID-19 but would be an indispensable tool for early and easier detection of future COVID-19 or other such outbreaks. Correlation of sewage surveillance data with the timings of various steps taken to contain the disease, can provide essential quantitative data to ascertain the efficacy of the measures taken. Since sample collection from individual persons is not necessary for sewage surveillance, parasites which are not detected in regular detection methods can be picked up based on waste water surveillance.

The already established SOP at Centre for Cellular and Molecular Biology (CSIR-CCMB) can be coordinated in countries internationally and can be used for setting up waste water based human

health analysis centers worldwide. Samples collected worldwide can also be sent to CCMB for analysis wherever detection at local points is not possible. Waste water-based surveillance would definitely help in early detection of COVID or other such infectious diseases in future.

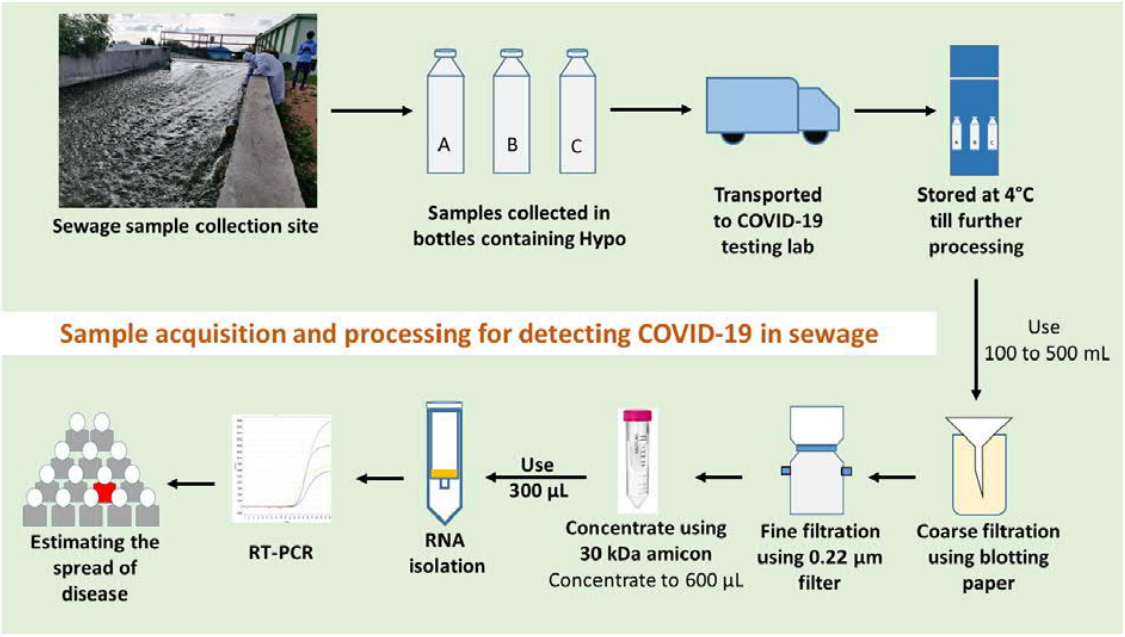

Figure 1: Sample acquisition and processing for detecting COVID-19 in sewage

(Manupati et al, Sci Total Environ. 2021 May 10; 768: 144704. Published online 2021 Jan 6. doi: 10.1016/j.scitotenv.2020.144704)

2) Surveillance for zoonotic vector borne diseases through analysis of tick-wildlife-human system

Background: Emerging Infectious Diseases (EIDs) are increasing these days due to factors like globalization and large densities of population, climate change, wildlife habitat destruction. Sixty percent of all known human pathogens and 70% of new infectious diseases are of zoonotic (wildlife) origin. Zika, H1N1, and Ebola, are all reminders of the devastation caused my emerging infectious diseases. Most EIDs are caused by pathogens being transferred to humans and associated domestic animals from wildlife. Ticks are a major vector facilitating the spread of EIDs (Table 1 and 2). After mosquitoes, ticks are likely to be the second most predominant vector for transmission of disease from animals to humans. They feed on hosts, and transfer pathogenic organisms from one host to another. It is imperative to understand the dynamics of the wildlife-domestic animals-human contacts through ticks, and their microbiome content. The composition of the tick biomes are dynamic and influenced by environmental inputs such as location, climatic conditions, habitat and prevalence of pathogens in the surrounding "biome" of the region. Tracking the composition of and the occurrence of known and novel pathogenic microbial species in tick microbiomes provides an opportunity to track emerging infectious diseases of zoonotic origins.

Scientific Relevance and capacity building: In this project, we shall collect ticks from various locations in India, in different breeding seasons, and from different hosts - humans, livestock and wildlife. We will use molecular biology-based methods to identify tick species and their hosts. Analysis of the microbiome content of these ticks would be done to understand pathogen prevalence, distribution and host specificity.

Currently, major limitations to monitoring EIDs are lack of epidemiological data, availability of biological material from afflicted animals, proper collection/ preservation/ processing of relevant biological samples and the development of modern molecular tools for detection. India, with its traditional farming practices, large rural economy and livestock populations, rich wildlife diversity and humid tropical climate, would be an ideal model to understand the occurrence of emerging infectious diseases of wildlife origin that can be transmitted through ticks to humans. There is a critical need to assess the status of dynamics of tick-borne disease prevalence in India's wildlife and the potential of their transmission to humans. There is a need to identify and categorize tick distribution, host specificity and microbiome analysis of ticks in India using state-of-the-art molecular biology approaches and microbiome analysis. LaCONES, at CSIR-CCMB, is uniquely situated to address the niche area, through extensive expertise in wildlife biology/forensics and its strong network with over 160 zoos and linkages with protected areas in the country. Once the procedures are standardised, using the model of India, this study can be extended to other countries as well.

Science/Technology Gap: Study of the tick microbiome is a fairly new and important strategy to understand the emergence and spread of infectious disease. There have been reports studying the tick microbiome, its composition, host specificity, and use in understanding the emergence and spread of tick-borne diseases (Greay et al 2014, Narasimhan and Fikrig 2015, Bonnet et al 2017, Swei and Kwan 2017). Molecular based assays for diagnosing tick borne diseases have exploded in the last decade with significant improvements in sensitivity and specificity (Ayden et al 2013; Mans et al 2015). These techniques have not only increased the accuracy of the detection of the pathogen, but have also expanded the capability of identifying new, previously unknown pathogens and distinguishing between species and strains of microorganisms (Baneth et al 2014). In Indian ecosystem, very few studies have been conducted to investigate the occurrence and distribution of ticks and tick borne diseases using molecular approaches. There is a critical need to carry out such analyses in India, which could be a hotspot for tick-borne diseases owing to its geo-sociology.

Objectives:

1. To identify various tick species and their host specificities. We shall use microscopy to create a repository of images to facilitate easy morphological identification of tick species.

2. To standardize molecular methods to identify tick species, to validate morphological methods, and create a repository which is absent in India and then validate it globally.

3. To reveal the microbiome of tick species, which is crucial in understanding the dynamics of emerging infectious diseases, so that it contributes to strategies on combat tick borne diseases of wildlife origin.

Project strategy: The project shall involve the following steps: 1. Collection of tick samples from wildlife, domesticated animals and humans; 2. Molecular identification of the tick species, as well as microscopy for morphological identification; 3. Sequencing of microbiome and bioinformatics analysis to identify composition and properties of the microbiome; 4. Analyse the collected data, and understand the dynamics behind the spread of infectious disease vectors and pathogens, and create a framework for further research.

NGS: Genomic DNA will be isolated from the stomach and salivary content of Ticks and will be processed for whole genome sequencing using NGS platform. The reads will be subjected to quality control to reduce noise in the analyses.

Bioinformatics Pipeline: Initially, host sequences will be screened out to the best extent possible using homology to available Tick genomes. The bioinformatics pipeline will have following tasks: 1. Taxonomic profiling using mapping against known genomes or markers; 2. De-novo assembly and genome annotation for strain identification; 3. Sequence based search of specific features such as

virulence islands. We will employ either clustering first or assignment first approaches depending on quality of sequence, computing resources etc.

In parallel, we will also perform 16S rRNA amplicon sequencing, using multiple primer sets for amplification. This will be used primarily to identify bacterial components of the biome, using resources such as Ribosomal Database Project Sequences. The WGS data will allow us to identify viruses, protozoa etc as well as bacterial species not covered by the RNA amplicon sequencing. The Bioinformatics pipeline will also use resources such as, Meta Velvet/ MetaVelvetSL for Metagenomic assembly, LikelyBin /MetaWatt for Binning, Glimmer-MG/ FragGenScan for gene predictions, InterProscan for Domain DB etc. We may also consider online portals such as MG-RAST, EBI-Metagenomics, IMG/M.

International partners: Research groups from Bangladesh- Jahangirnagar University Savar, Dhaka, Sri Lanka- University of Colombo and Nepal - National Trust for Nature Conservation, have agree to participate in the proposed surveillance program of infectious diseases.

Plan of action: We have already standardised the procedures for surveillance of SARS-CoV-2 in sewage water at CSIR-CCMB, Hyderabad, India. The standard operating procedures (SOP) for surveillance of zoonotic vector borne infectious diseases through analysis of tick-wildlife-human system would be established in LaCONES, CSIR-CCMB. CCMB would be a node and the SOPs would be shared with the international partners. The researchers would be trained at CSIR-CCMB. The surveillance SOPs would then be followed in the partner countries.

Project duration and funding requirements: Since the project extends from surveillance of SARS-CoV-2 to other infectious diseases, the duration of the project would be 3 years. In the first year, the surveillance of SARS-CoV-2 through sewage water analysis would be extended to more cities in India and abroad and the SOP for analysis of other infectious diseases through analysis of tick-wildlife-human systems would be established at LaCONES, CSIR-CCMB. The staff of other collaborating partners would also be trained. In the second year, the data for other infectious diseases would be collected from the collaborating countries at a larger scale. In the third year, all the data would be analysed at a broader scale and an information sharing platform would be generated.

Most of the infrastructure required for both the approaches of this project already exists in CSIR-CCMB. Funding is required for consumables and manpower. The total funding required for 3 years is 1,550,000£

Budget Head	First Year	Second Year	Third Year	Total
Equipment for node and other centres	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- Nil P2- Nil	P1- 100,000£ P2- 100,000£
Manpower	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 150,000£ P2- 150,000£
Consumables	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 150,000£ P2- 150,000£
Regional labs set up	P1- 25,000£ P2- 25,000£	P1- 25,000£ P2- 25,000£	P1- 25,000£ P2- 25,000£	P1- 75,000£ P2- 75,000£
Training and International facility set-up	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 150,000£ P2- 150,000£
Travel	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 50,000£ P2- 50,000£	P1- 150,000£ P2- 150,000£
Total	P1- 275,000£ P2- 275,000£ P1 + P2- 550,000£	P1-275,000£ P2-275,000£ P1 + P2- 550,000£	P1-225,000£ P2-225,000£ P1 + P2- 450,000£	P1- 775,000£ P2- 775,000£ P1+ P2- 1,550,000£

P1- Surveillance of infectious diseases by waste water-based epidemiology

Thus, the first approach, that is, surveillance of infectious diseases by waste water-based epidemiology would help in monitoring the status of infectious diseases in communities in real time whereas the second approach, of surveillance for zoonotic vector borne diseases through analysis of tick-wildlife-human system would throw light on the newly emerging infectious diseases which get transmitted from vectors like ticks from wildlife- livestock to humans. Taken together, this project would help in doing a comprehensive surveillance of infectious diseases which are newly emerging as well as of those which are already existing in humans.

References

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B. 2020a. First confirmed detection of SARS-CoV-2 in untreated waste water in Australia: a proof of concept for the waste water surveillance of COVID-19 in the community. Sci. Total Environ. 138764.

Andrea Swei, Jessica Y Kwan 2017. Tick Microbiome and pathogen acquisition altered by blood host mean. The ISME Journal Volume 11, pages 813-816

Aydin MF, Aktas M, Dumanli N 2013 Molecular identification of Theileria and Babesia in sheep and goats in the Black Sea Region in Turkey. Parasitol Res

Ghosh S, Bansal GC, Gupta SC, Ray D, Khan MQ, Irshad H, *et al.* 2007. Status of tick distribution in Bangladesh, India and Pakistan. Parasitol Res 2007;101 Suppl 2:S207-16.

Greay, Telleasha L et al 2018. Recent insights into the tick microbiome gained through next-generation sequencing. Parasites & vectors vol. 11,1 12. 4 Jan. 2018

Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T., Norder, H., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80 (21), 6771–6781.

Mans B-J, Pienaar R, Latif AA, A review of *Theileria* diagnostics and epidemiology, International Journal for Parasitology: Parasites and Wildlife (2015)

Manupati Hemalatha, Uday Kiran, Santosh Kumar Kuncha, Harishankar Kopperi, C.G. Gokulan, S. Venkata Mohan, Rakesh K. Mishra. Surveillance of SARS-CoV-2 spread using wastewater-based epidemiology: Comprehensive study. Sci Total Environ. 2021 May 10; 768: 144704. Published online 2021 Jan 6. doi: 10.1016/j.scitotenv.2020.144704

Minjauw B, McLeod A. Tick-borne diseases and poverty: The impact of ticks and tick-borne diseases on the livelihood of small scale and marginal livestock owners in India and eastern and southern Africa. *Research Report*, DFID-AHP. UK: Centre for Tropical Veterinary Medicine, University of Edinburgh 2003; p. 116.

Saminathan M, Rana R, Ramakrishnan MA, Karthik K, SinghY, Malik and Dhama K 2016. Prevalence, diagnosis, management and control of important diseases of ruminants with special reference to indian scenario. Journal of Experimental Biology and Agricultural Sciences, June - 2016; Volume – 4(3S)

Sarah I. Bonnet, Olivier Duron et al, 2017. The Tick Microbiome: Why Non-pathogenic Microorganisms Matter in Tick Biology and Pathogen Transmission. Front Cell Infect Microbiol, 7: 236.

Sukanya Narasimhan, Erol Fikrig 2015. Tick Microbiome: the force within. Review, Trends In Parasitology, Volume 31, Issue 7, Pages 315-323

Wang, J., Shen, J., Ye, D., Yan, X., Zhang, Y., Yang, W., Li, X., Wang, J., Zhang, L., Pan, L., 2020b. Disinfection technology of hospital wastes and wastewater: suggestions for disinfection strategy during coronavirus disease 2019 (COVID-19) pandemic in China. Environ. Pollut. 114665.

Wang, W. Xu, Y., Gao, R., Lu, R., Han, K.,Wu, G., Tan,W., 2020a. Detection of SARS-CoV-2 in different types of clinical specimens. Jama 323 (18), 1843–1844.

APPENDIX

Table 1. Important tick-borne Diseases of Human and Livestock (*Ghosh and Nagar, 2014)*

Name of the Disease	Causative Pathogen	Host	Name of the Tick
Viral diseases			
Kyasanur forest disease	Group B Toganvirus (Flavidiridae)	Human	Haemophysalis spinigera
Crimean-Congo haemorragic Fever (CCHF)	Nirovirus (Bunyaviridae)	Human	Hyalomma anatolicum
African horse sickness	Reoviridae (African horse sickness virus)	Horse	Hyalomma dromedarii
African swine fever	African swine fever virus	Swine	Ornithodorus mobuta
Nairobi sheep disease	Bunyaviridae	Sheep	Rhipicephalus appendiculatus
Rickettsial diseases			
Ehrlichiosis	Ehrlichia canis	Dog	Rhipicephalus sanguines
	Ehrlichia equi	Horse	
	Ehrlichia bovis	Cattle	Hyalomma spp.
Human monocytic ehrlichiosis	Ehrlichia senetsu Ehrlichia chaffeensis, Ehrlichia phagocytophilia	Human	Rhipicephalus sanguines
Cowdriosis	Cowdria ruminantium	Cattle	Amblyoma variegatum
Anaplasmosis	Anaplasma marginale	Cattle, Buffalo, Sheep	R. (B.) microplus
Indian tick typhus (ITT),	Rickettsia conorii	Human	R. sanguineus Dermacenter andersoni, Rhipicephalus (B.) decoloratus
Spirochete diseases			
Lyme disease	Borrelia burgdorferi		Ixodes ricinus
Bacterial diseases			
Tularemia	Francisella tularensis	Rabbits	Dermacentor spp
Protozoan			
Theileriosis	Theileria annulata, T. parva, T. hirci	Cattle	Hyalomma anatolicum, Rhipicephalus appenticulatus
Babesiosis	Babesia bigemina,	Cattle, Buffalo	Rhipicephalus (B.) microplus
	Babesia ovis	Sheep	Rhipicephalus spp.
	Babesia motasi	Goat	Haemaphysalis spp.
	Babesia equi	Horse	Hyalomma anatolicum
Human babesiosis	Babesia microti, Babesia divergens	Human	Ixodes spp.

Table 2. Important tick-borne diseases in India (*Ghosh and Nagar, 2014)*

Name of the Disease	Causative Pathogen	Host	Name of the Tick
Kyasanur forest disease (KFD)	Group B Toganvirus (Flavidiridae)	Man	Haemophysalis spinigera
Crimean-Congo haemorrhagic fever (CCHF)	*Nairovirus* group	Man	*Hyalomma anatolicum*
Indian tick typhus (ITT)	Rickettsia conorii	Man	R. sanguineus Dermacenter andersoni, R. (B.) decoloratus
Ehrlichiosis	Ehrlichia canis,	Dog	Rhipicephalus sanguines
	E. bovis	Cattle	Hyalomma spp.
Anaplasmosis	Anaplasma marginale	Cattle, Buffalo, Sheep	R. (B.) microplus
Theileriosis	Theileria annulata, T. parva, T. hirci	Cattle	H. anatolicum, R. appenticulatus
Babesiosis	Babesia bigemina,	Cattle, Buffalo	R. (B.) microplus
	Babesia ovis	Sheep	Rhipicephalus spp.
	Babesia motasi	Goat	Haemaphysalis spp.
	Babesia equi	Horse	H. anatolicum