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e ABSTRACTS

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Potential of vector control to reduce disease and deaths

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About two-thirds world's population is at the risk of at least one of the diseases transmitted by vectors such as mosquitoes (malaria, dengue, lymphatic filariasis, chikungunya, Zika virus disease, yellow fever, Japanese encephalitis, West Nile fever, sandflies (leishmaniasis), blackflies (onchocerciasis), tsetse flies (human African trypanosomiasis), triatomine bugs (Chagas disease), snails (schistosomiasis), ticks (Lyme disease, encephalitis) and mites (scrub typhus). These diseases cause enormous suffering, death and economic loss. In many settings, surveillance and control of vectors can prevent, control and help eliminate the diseases they transmit. For example, malaria was eliminated from Europe and USA in the 1960s due largely to DDT indoor residual spraying (IRS); and more recently between 2000 and 2015, two-third of the 37% reduction in global burden of malaria was achieved by use of insecticide treated nets and IRS.

Despite the progress made, there are several important challenges today in the fight against vector-borne diseases. The invasion of new territories by vector species, the emergence/re-emergence of vector-borne diseases and increased transmission risks are associated with environmental and ecological changes due to urbanization, industrialization, expansion in agriculture, and climate change. Apart from environmental degradation, vector control today faces several other challenges such as biological threats (insecticide resistance, outdoor transmission, changing vector biology), socio-political factors, dearth of new tools, insecticide quality control enforcement. The lack of predictable and sustained domestic and international financing has led to abysmally low core capacity for public health entomology and vector control in most disease endemic countries leading to loss of technical and professional guidance to the vector-borne disease control programmes.

Following the recent outbreak of Zika virus disease, WHO has initiated the Global Vector Control Response to focus the world's attention on addressing these issues. The core pillars are: strengthening inter- and intra-sectoral collaboration, engaging and mobilizing communities, enhancing vector surveillance and monitoring and evaluation of interventions, and scaling up and integrating tools and approaches. Enhancing vector control capacity and innovation and research are the foundation of this approach.

The research community has opportunity for impact in finding vector control solutions for rapidly changing environments such as those caused by urbanization, climate change, agriculture and water resources development. There is need for greater investment in implementation research to understand how to best use the tools we have, and in innovation research, to develop and deploy new tools, especially for vulnerable communities usually outside of the reach of public health services.

Finally, vector control has a development and economic angle. Greater investment in vector surveillance and control together with sustainable development and universal health coverage will accelerate elimination of vector-borne diseases and help to achieve many of the associated UN Sustainable Development Goals.

Malaria remains unshaken; and the mighty mosquito remains unbeaten.

Dr. P. K. Rajagopalan

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Fighting malaria has very little to do with the intricacies of science and biology. The key was learning to think like the men hired to go door-to-door and stream-to-stream, killing mosquitoes. Do you think we should think like a mosquito? No, said Fred Soper, who eliminated *Aedes aegypti* from Panama Canal Zone and *Anopheles gambiae* from Brazil. His method was to apply motivation, discipline, organization, and zeal, in understanding human nature to achieve the objectives. This was the crux of the malaria control programme. He was considered the General Patton of Entomology. If any Indian can be compared to Soper, it can be only T. Ramachandra Rao.

Many stalwarts from India and abroad had done excellent work on malaria control. But I would like to single out the contribution of Dr Paul F Russel and Dr T.Ramachandra Rao, in India, for strongly founding the revolutionary malaria-management tactic based on MOSQUITO ECOLOGY. Russel, from the International Health Division of the Rockefeller Foundation (IHDRF), along with TR Rao, who started his career as Medical Entomologist under Russel in 1936, did pioneering work for six long years from 1936 to 1942, in villages of Pattukkottai in Tanjavur Dt, Tamil Nadu, long before DDT came into use.. It is worth remembering that their work formed the basis for the anti-mosquito strategy in later years. Working from a rented house in Pattukkottai, they systematically surveyed populations of mosquitoes in differently managed agricultural fields; and examined the spleens of villagers in selected villages every year. As a part of their investigations, space spray with Pyrethrum extract mixed with Kerosene was done in all houses in one village with the aim of killing adult mosquitoes. Numbers of inflamed spleens, an indicator of malarial infection, dropped from 68 to 6% in the sprayed village over three years, while in the unsprayed villages, they remained steady at above 50%. Their finding was rated outstanding by the Rockefeller Foundation (RF) which had funded these studies, and the world at large. They also carried out a long term program of mosquito-larvae control by filling in pits, creating better drainage and water-control devices in rice fields, distributing mosquito larva eating fish (*Gambusia*), and spraying Paris Green (a potent rat and insect poison). A Sanitary engineer, Knipe, joined them and they focused on the design of appropriate spraying device to spray on adult mosquitoes in houses and on larvae in stagnant water bodies. What started as a routine mosquito control programme turned out to be a well-executed research event that bears immense impact on mosquito management in India even today? Most importantly they demonstrated the role played by stagnant water bodies in mosquito breeding and how irrigated agricultural fields act as reservoirs of mosquitoes. The people of Pattukkottai villages experienced a remarkable relief because of pyrethrum spraying, and which won an immediate and whole-hearted acceptance. Every householder welcomed the 'spray man'. People rejoiced the freedom from mosquito bites, from fevers, and from other plaguing insects such as bedbugs and cockroaches (and scorpions). Nearly 45 years later, sometime in 1980s, I again studied the malaria situation in Pattukkottai, where malaria was once controlled by Russel and Rao. I found that people still remember the good work done by the Rockefeller Foundation, and malaria had come back following the pattern in the rest of India, though on a lesser scale! The work of Russel and Rao actually laid the foundation for formulating later malaria control operations in India. Another landmark incident was that in 1942, at the time when the Rockefeller Foundation wound up the work in Pattukkottai, the then Governor of Bombay, and Sir Roger Lumley developed malaria after a tour in the District of North Kanara, then in Bombay

State. At Delhi, he told the Viceroy, Lord Linlithgow, his concern at the prevalence and intensity of malaria in that area. The Viceroy, who was also greatly interested in malaria control, sent for the then Director of Malaria Institute of India (Sir Gordon Covell), who put forward proposals for a permanent malaria organization for Bombay Province, which were immediately accepted by the Governor. Dr. D.K. Viswanathan, who was serving with the Army on the Burma front, was asked to take charge of the new organization. He was a awe inspiring personality known for his dynamism. Ramachandra Rao, after completing his stint with Paul Russel at Pattukkottai, had joined Bombay Government as Entomologist under Dr Viswanathan. The team of Viswanathan and Rao (like Russel and Rao before) did yeoman service to control malaria, as it was their work which led to starting a national programme. It was in the villages of Yellapur in North Kanara the efficacy of indoor residual spray with DDT was demonstrated for the first time in India, in 1944-45, resulting in drastic reduction of malaria. There was also this very interesting episode worth quoting here. When a large amount of money was demanded for spraying operations in North Kanara District, Sir John Lumley, the Governor, was hesitant saying that North Kanara District had not contributed much to the war effort. Viswanathan and Rao argued that the British Navy owed a lot of gratitude to the district, since all the valuable teakwood trees from the district (Yellapur, to be precise) have been cut and sent for building ships for the British Navy, denuding the teakwood forests completely. Immediate approval was then given for the field trial of indoor residual spray with DDT in Yellapur. (This incident was quoted by the late Dr T.R.Rao himself when I was working as his Research Assistant many decades ago in 1953).

From the outset control efforts were directed to the destruction of the adult mosquito. Spraying with pyrethrum insecticide, which had proved of value in preliminary experiments in southern India (and Delhi), met with only partial success, but the introduction of DDT in September 1945 resulted in a dramatic fall in malarial incidence. Operations were eventually extended so as to cover the whole of Bombay State, which had a population of over 30 million. In his book *Malaria and its Control in Bombay State* [Bulletin, 1951, v. 48, 305] Viswanathan described how a nation-wide malaria control programme for India would be a worthy objective to get American aid. All entomologists and malariologists should consider this as Bible. In December 1952 an agreement was signed between USA and INDIA. Viswanathan played a prominent part in these negotiations. Without this financial aid from the U.S.A. the National Malaria Control Programme, later to become one for total eradication in India, could not have been implemented. The striking success of the campaign led by Viswanathan and Rao in Bombay State not only resulted in the adoption of a nation-wide programme for India, but also gave impetus to similar schemes in many other countries. The rest is all history. Malaria was drastically controlled in 1960. I am quoting all these because the present day scientists and malariologists should know about Dr T.Ramachandra Rao, the great Malaria Entomologist who worked for malaria control, nonstop, from 1936 to 1952. (He left in 1952 to join the Rockefeller Foundation led Virus Research Centre in Pune, as Chief Entomologist, to establish studies on arthropod vectors of arboviruses. He returned to Bombay Govt. two years later to continue his work on malaria. His name is being immortalized here. I am proud to have started my career as Medical Entomologist in 1952 under this illustrious scientist and it was the initial training and guidance I got from Ramachandra Rao that had seen me through in later life and career).

I like to quote another very interesting episode. In 1958, while I was studying Public Health at the University of California, Berkeley, there were many stalwarts like Karl F. Meyer (Zoonosis), Fred Soper and Lewis Hackett (malariologists) as visting professors. India had started the malaria eradication

programme just then. In the open class, Hackett (who described spleen rates as an index of malaria measurement) laughingly talked about our India's malaria eradication programme, and wondered how anybody can ever think of eradicating malaria, where the parasite as well as the vector mosquito had evolved long before man! He was talking about the word Eradication. These were prophetic words. Dr Hackett was also malaria advisor to the Czar of Russia before the October Revolution, 1917, i.e. a Century ago.. At that time quinine was the only antimalarial drug. He had asked at a parade of soldiers where the Czar was also present, that all should take quinine mixture before going to bed.. Next morning they again met and the Czar was also there. Since quinine produces luminescence in the urine, and he asked for urine samples and the first sample from the Czar. The Czar himself had not taken quinine because of the bitter taste. He told the Czar, "Your majesty, you should have set an example for the soldiers for taking quinine, to control malaria". He narrated another story about the Spanish American War in Far East in 1899; A few battalions of soldiers were led through marshy territory breeding mosquitoes in the Philippines and all died due to malaria and the Americans lost the territory. This happened one year after the discovery by Ross, and how knowledge rarely reaches the field in time. He humorously ranked intelligence as Human, Animal and lastly military "in that order! This was in a class where there were quite a few Military officers sitting in the front! Those were the good old days when knowledgeable scientists were respected and obeyed.

What happened during the "DDT Era" and afterwards is all history. Malaria was once controlled drastically, and then in the later years of 1960s Malaria came back with a bang. And we are still grappling with the problem, even if it is of that intensity. There are many pockets in India where malaria cases and deaths are occurring, in spite of best efforts. In quantitative terms they are in thousands (World Malaria Report, 2018). A malaria-free India now appears to be a dream. Malaria control at the national level has always been an operational programme, with the motto, which during the initial days of indoor residual sprays with insecticides, SPRAY AND PRAY! Due to deficiencies in the control operations at that time, the mosquito developed resistance to DDT and later also to some other insecticides. This serious problem could have been corrected in time, if only there was constant entomological surveillance and research. A.P. Ray, the architect of India's successful malaria control programme, who could be compared to Fred Soper, due to the euphoria created during the early 1960s in controlling malaria, could not foresee vector adaptation to chemical pressure. Insect resistance to chemicals was not well known at the time. He thought that there would be no further need for entomologists in mosquito control work, and many were diverted to other operations or had their services terminated. This was the greatest tragedy. We are still paying the price for denigrating the role of Entomologists. Even now it is said that more than 50% of the sanctioned strength of Entomologists have not been filled. Qualified, trained, knowledgeable and field oriented Entomologists are a rarity in India now!

Another reason for malaria resurgence was that all other methods of malaria control, suggested by Russel, Rao, and many others, were totally neglected at one time as they did not seem to be necessary at all. Many naturalistic methods of environmental manipulation demonstrated by Hackett in Malaysia (Please see Hackett's "Naturalistic Methods of Malaria Control"), by Russel and Rao, and many others in India,, were not given much importance. Malaria research came practically to a standstill. There did not seem to be any more need for the Indian Journal of Malariology, one of the foremost journals on malariology in the world, and it stopped publication. The situation had become unmanageable with the number of malaria cases showing an upward trend. We plodded along, following WHO advice, and many innovations were suggested and implemented haphazardly. But even in recent years, our track record on

malaria control doesn't exactly inspire optimism. The disease has been a low political priority for many years, rendering the current malaria control programme ineffective and confusing to implement. No one even knows exactly how many Indians suffer from malaria, let alone die from it each year. A couple of years ago the government claimed only about 300 deaths from malaria, while the British health journal *The Lancet* reported 50,000 deaths taking place annually. Even Indian officials acknowledge that there are severe limitations to the official statistics that depicted steady progress on fighting the disease, but claiming that new malaria cases dropped by half between 2000 and 2014. There was large scale fudging of data on malaria incidence which stems from the early days of the National Malaria Eradication Program, in which officers would lose their jobs over poor health outcomes. There was a report in 2016 (*Economic Times*, or was it *Al Jazeera*) of a situation in which every worker in the program was living under constant pressure from his or her supervisor. The false data collected thus gets forwarded through the District and State level, to the national level where a rosy picture is usually painted –to the national Health Ministry, and these finally gets sanctified at the WHO level. The situation has not changed even now, assertions to the contrary notwithstanding!. While working on tribal Malaria in Odisha in 1980s I found that there were some villages which were never sprayed due to either remoteness or hostility of the tribal population and many hamlets were never even visited by the control teams, and therefore no malaria control worth the name ever took place. A research team of Vector Control Research Centre found a few cases of *P.malariae* and *P.ovale* (Identification confirmed at London School of Tropical Medicine) in addition to many cases of *P. falciparum* and *P. vivax*. These findings were so embarrassing to the District Collector, that one fine day the VCRC team was expelled from the area! Such problems were also highlighted by Dr V.P. Sharma, the founder- director of the National Institute of Malaria Research in an interview before his death in October 2015. His famous words: "You can never reform the present system because you're saying the problem isn't even there". There is also the problem of increasing occurrence of diseases like Japanese Encephalitis, Dengue and Chikungunya, obscures real malaria incidence and therefore which draws greater attention and support from the government. Consequently, malaria control no longer has any priority. At one time malaria overshadowed diseases like Dengue in wrong diagnosis and now it is the other way around, Dengue obscures malaria diagnosis.

Malaria Epidemiology abounds in instances where even a few gametocyte carriers present in a locality can become a focus (a micro focus perhaps) for transmission. It should be recognized that while one or two cases in a population of 10,000 may appear a low figure, even the one or two cases if they occur in a small circumscribed locality, say an isolated cluster of huts with 100 people (e.g. a tribal settlement), can be a serious matter. There is no more intense follow up surveillance any more. I know personally of several instances in a place (Borigumma, Odisha) where active transmission of malaria was going on in practically closed cycles in small outlying hamlets consisting of a few huts, while the main village itself, not more than a kilometer away, and was considered to be in a satisfactory position. These small insignificant-looking foci, sometimes undetected, sometimes detected but not fully liquidated, became centres for focal outbreaks, if the vectors were not under control. And there were just no competent entomologists or surveillance workers to monitor the problem in the field.

Malaria, therefore, can never be eliminated from India in the near future as there is no more infrastructure at present to handle such problems. Active surveillance, the key to success, is just not there. The situation cannot be compared with the achievements of several small countries in the neighborhood like Sri Lanka, Malaysia, etc. which have controlled malaria. The vastness of the country, with many different ecological zones, and varying cultural and political differences make it almost

impossible to have a uniform pattern of malaria control in India. Also health is a state subject and the National Control organization cannot enforce their writ strictly on the States. Area specific action plan could have probably achieved better results. But then, we in India depended more on advice from International Organizations, ignoring the examples set by Fred Soper (in Brazil), Lewis Hackett (in Czarist Russia), Paul Russel and our known our own T. Ramachandra Rao. Many years after his retirement, Dr Rao was very disillusioned. He was one of the few great malariologists alive in the 1980s and told the Union Health Minister what was wrong with the National Control Program, and asked for a National Malaria Commission! Malaria Control operations had also become a scam then!! He was called senile by the then administrators! Dr Rao opined that you take a donkey with a foreigner's label to the minister and when it brays, it becomes expert advice! The control set up even now does not function properly. As recently as March 2018, one scientist visited Kalahandi district, Odisha state, a hot spot of malaria, where he asked a ASHA worker about RDK (rapid diagnosis kit) and anti malarials. She replied that for the last six months there were no RDK and antimalarials. Then he met the female health worker and asked about supply and she also said there is no supply of RDK and anti malarials. He was shocked and thinking about malaria elimination returned to District HQ. He then met with the District Malaria Officer who agreed that there is a shortage of anti malarials and RDK in district. The supply was not given by the State. He tried to meet the state programme officer but she was away in Cuttack. " If we think about malaria elimination then we have to change our mindset and have qualified staff at each level, arrangements of logistics and funding and above all political will for malaria elimination"In another village he asked a householder about LLIN (Long Lasting Insecticide Impregnated Net), as the Government had distributed freely all LLINs. The lady of the house pulled out a new LLIN from a bag. LLIN distribution rate was 100 percent and user rate was Nil". There were also many other problems. In some places even microscopes are not functional or unavailable, nor there are technicians to do the job? The operational deficiency is glaring. Some of the technicians in areas where malaria is not rampant have even forgotten to identify the parasites! They should be given reorientation training. In most of the remote areas where the bivalent Rapid Diagnostic Kits (RDTs) are in use, quick diagnosis and treatment is never possible because of the time lag between blood slide collection (BSC) and their examination (BSE). So there is no treatment. Some of these bivalent kits do show false positives. Then there is non-availability of the drug to ensure prompt treatment. There is no coverage or proper spray of insecticides; also no trained staff to spray or supervisory staff to oversee the vector control operations anymore. The biggest tragedy is the absence of qualified and competent entomologists, to tackle the mosquito angle which is the most important aspect of Malaria Epidemiology. In 1980, VP Sharma started many Integrated Disease Vector Control (IDVC) units in several locations in India where malaria continued to be a problem. I think this was his greatest contribution to malaria control in India. There was special emphasis on environmental control. Many of IDVCs became Centres of excellence not only in controlling Malaria in those areas, but these also turned out several young malaria entomologists. Eliminating malaria is, and should be, a priority for the country, but grand pronouncements are meaningless as long as manipulated data distort our knowledge and bad governance impedes genuine attempts to fight the disease.

India's malaria efforts lag behind those of most Asian and African countries. Over 90 per cent of national spending on malaria control in 2014 went towards administrative costs, salaries, and expenses other than the nets, medicines and insecticide sprays that make a concrete difference. The average global spending on administrative costs and salaries, meanwhile, is just 35 percent. The new malaria elimination policy does include some promising measures, like a greater emphasis on community

participation in fighting malaria. The National Vector Borne Disease Control Programme (NVBDCP), which coordinates the malaria programme, said there is a study underway to better measure the number of malaria deaths. They also agree with the need to fill the empty slots on health staffs, and train and incentivize community health workers. India would need to invest a much larger chunk of its domestic budget in overall health care. And the interventions would have to step outside the realm of the government health model. As Health Ministry officials point out, some challenges are not in their hands. We lacked will power and administrative skills — the kind that allowed India to tackle polio. It is a tall order to implement. Malaria strikes the hardest in the Northeast, in places like Bastar in Chhattisgarh, or Koraput in Odisha that are already torn by internal disturbances. Ultimately it can only be eliminated once the people in these areas are included as participants in the country's development who can hold the state accountable to them. That would require greater transparency from the government, and a focus not on its global image but instead on the actual people dying across the country every single day. Malaria elimination is really doable by a good leader who needs the courage and political influence to make it happens overcoming political pressure. It is unfortunate that we in India have a “we know everything” and big brother attitude, and are hesitant to learn from examples set by smaller countries. Also, the National Vector Borne Disease Control Programme, as the name implies, has the responsibility for the control of other diseases like Japanese Encephalitis, Dengue, Chikungunya, etc and therefore they cannot devote much attention for malaria at present.

Swaziland, a very small country within South Africa, eliminated Malaria: “All cases were investigated within 48 hours. Trained personnel carried out vector control linked to case investigation and routine vector surveillance as well as established sentinel vector surveillance sites including insecticide resistance monitoring. Can such operations be carried out in many pockets in different states in India where malaria is a problem? Vector control guidelines should be revised in line with the requirements of elimination and the programme should implement and monitor Indoor Residual Spray where necessary after every spray round. The supply chain system especially the distribution of malaria commodities needs strengthening as well and a training programme started for malaria case management, microscopy as well as malaria diagnosis. We have to ensure community participation in public health programmes as this was identified as necessary for the elimination agenda. A much smaller country like Sri Lanka, which did all these, was declared by WHO as having eliminated malaria. The determined effort made by countries like Sri Lanka, Malaysia, etc to implement the various control measures is really admirable. None of these is happening or even possible in India, which is comparatively very large with varying ecological conditions and political set up. They have more problems at hand running the organization and which can be summed by what Sir Gordon Covell, the first director of Malaria Institute of India said “I spend only 10% of my time fighting malaria, but 90% of my time fighting people preventing me from fighting malaria!”

What about research, which should be on a continuing basis monitoring every aspect of malaria control? One reason is the “molecularisation and computerization” of malaria research since 1980s, in almost all institutions dealing with vector borne diseases. The importance shifted to white apron clad scientists working within the four walls of an air-conditioned laboratory fitted with computers! We followed the WHO example in the eighties when the famous Vector Biology and Control (VBC) division was renamed Molecular Entomology Division. What is the connection between molecules and vector control work in the field? It was market oriented research, funded by multinational companies, and foreign universities (seeking raw material for their research work), which are now the core funding

source of the Research Programme in our research bodies. Field work in India had long become out of fashion and meant occasional visits to the field, which Barcelato, a Scientist with the Tropical Diseases Research Programs, called Safari research. Apron clad laboratory "Scientists" replaced hardcore field workers. We really need 'boots on the ground' attitude of working in the field. We are looking for Easy solutions to complicated epidemiological and ecological situations in computers. What is actually required is Applied Operational Research in the field. Only papers with high impact factors are now produced, to improve the career prospects of the investigators, and none of them giving easy, practical and sure solutions for real malaria control. There are many experts (all retired and superannuated personnel and some with no knowledge of the subject) – a wit calls them ex-spurts, but no malaria workers. Efforts should have been made on how to apply known technologies to practical use in the field. How to make the personnel actually do the job they are supposed to do. First get malaria under control in the country by tackling operational problems faced by NVBDCP, before carrying out publication oriented theoretical academic research to advance your career. (Can we call it survival research – rather nit picking research of no importance?) . Most of our young scientists may not have heard the contributions of great malariologists like Fred Soper, Lewis Hackett, Paul Russel and others. I wonder whether they know even our own Ramachandra Rao's classical work.

It is sad that researchers in many research institutes have wrong priorities. Many are keen in by molecular biology, but then and as Prof A Raman of Australia puts it, today's molecular approaches are like "'recreating' organisms in the cyberspace and treating them as models to work on to solve real-life problems." We are informed of things that none can see, perceive, and visualize. Many of today's biologists speak in terms of outcomes that are unverifiable and untestable. I think all these need to be pitched on sound logic and sequenced, rational, convincing evidences --- the very foundation of science and scientific approach. It is also sad that such an approach is being prescribed as a substitute for every kind of problem in India for which solutions have to be found only through hard field work. Wilbur Downs said while molecular studies-for example vaccine development is fascinating. Some of the new scientific approach is also illusory. They deflect attention from the here and now. The "here and now" is that today there are millions of people in thousands of villages for whom the application of knowledge and means already at hand –an adequate supply of antimalarial drugs, simple control procedures carried out on the local problem, and differentiation of malaria from other diseases and efficient mosquito control – is still awaited. These are the "here and now" problems which had taken a back seat.

Many easy solutions are offered based on cage experiments, and without understanding anything about what happens actually in the field conditions, but have no buyers in many countries. On release of genetically modified mosquitoes as a magic solution to control malaria, Dr Ify Aniebo, a Nigerian molecular geneticist and malaria specialist, who is skeptical about the approach, wrote in November 2018 and quoted in Scientific American: "Africa doesn't need genetically modified mosquitoes. Dr Aniebo's first concern was the speed with which the technology is being deployed. More studies on GM mosquitoes need to be done, she says, "to ascertain safety and avoid unintended consequences before releasing them into the field. African countries do not have the infrastructure needed to regulate or solve any problem that may arise from this technology. It's worth noting that a careful analysis of the GM mosquito releases that have already taken place – in the Cayman Islands, Panama, Brazil and Malaysia, suggests that the claims of success made for these trials are not actually supported by the evidence." Just how top-down the genetic engineering approach is can be seen in the case of the research group behind the release of the GM mosquitoes in Burkina Faso. "Target Malaria is largely based in London – at

Imperial College – and has been funded to the tune of over \$75 million by the American billionaire Bill Gates, via his Foundation in Seattle. This follows a pattern seen in others of the Gates Foundation's activities – that is supposedly helping the world's poor it spends the bulk of its money on high-tech approaches in rich developed countries..... The deployment of such a highly experimental technology in Africa is justified by its backers on the grounds that there are no viable alternatives for adequately combating malaria. But, in reality, there are plenty of less drastic ways to fight malaria says Ify Aniebo. She points, for instance, to “sanitary engineering; getting rid of mosquito breeding sites; and swamp drainage” as some of “the interventions that have helped in the past and have proven to be sustainable solutions” (The same objectives Russel and Rao had in 1936). She asks: Why spend such vast sums “on developing genetically modified insects when the money could be directed towards environmental engineering projects that hinder the ability of mosquitoes to breed in the first place?” This kind of long-term approach should not be ignored if we are serious about combating malaria, she says. In fact, these “more conventional tools” can not only reduce malaria but even eliminate it – Sri Lanka, eliminated malaria- without resorting to genetically modified mosquitoes. Even in the age of drug resistance, some countries have still managed to attain [malaria] elimination status.” Several countries in Africa like Nigeria, Uganda, Swaziland and some others have done far more advanced work not only in Applied Research and their commonsensical, practical and economical approach to Disease Control. We have any number of low-technology ways to reduce mosquitoes and the diseases they spread. Personal protection measures, destruction of breeding sites in and around the home, fogging during outbreaks – all of these have an impact on disease incidence. The lessons of the Panama Canal (Gorgas) and Brazil (Soper), and many other smaller countries like Swaziland, Sri Lanka, and several others is that it does not take high technology to control mosquito vectors. Instead, political will, strict discipline helping sustained application, and constant surveillance, are what's needed to have real policy bite. For the foreseeable future, we have to manage mosquito-borne diseases through environmental, safe chemicals, rapid diagnostics and innovative drug therapies. People get lured by some exciting new discoveries, but before jumping on the band wagon they should ponder whether it is applicable in all cases. We have knocked our heads against this type of thinking long enough.

I must conclude by paying my tribute to the great Fred Soper, who spent all his life on *Aedes aegypti* control in Panama Canal Zone and *An. gambiae* control in Brazil and Egypt, etc. His name was synonymous with malaria control. Towards the end of his life, Fred Soper, met with an old colleague, M. A. Farid with whom he had fought successfully the malaria vector *An. gambiae* in Egypt years before. “How do things go?” Soper began. “Bad!” Farid replied, for this was in the years when everyone had gone against Soper's vision. “Who will be our ally?” Soper asked. And Farid said simply, “Malaria,” and Soper, he remembered, almost hugged him, because it was clear that malaria will remain unshaken. I as a medical entomologist and vector ecologist, have been writing and talking about problems faced in the control of mosquito borne diseases including malaria, at the fag end of my life (now 89) and feel very much disheartened. I wish we had with us people like Fred Soper, Lewis Hackett and Ramachandra Rao, among others.

So malaria will continue to be a problem in India for many years to come, though not in epidemic form, but widespread in many pockets in India. We have to live with it, along with many other vector borne diseases. And the mighty mosquito will also continue to remain unbeaten. There seems to be no other way forward in the near future.

Challenges & Perspectives for Dengue Control in Urban areas and way forward for Civic Bodies: A Case Study of Delhi

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Dengue fever is emerging as a major public health challenge in Delhi leading to high morbidity and mortality. Epidemiological data of Dengue in Delhi has revealed that patient's age group is predominantly adults i.e. those involved in outdoor activities and transition from urban to rural settings. This infers that vector control is not only required in residential area but at work place also. The data on incidence of dengue in Delhi shows loss of cyclic pattern and outbreaks have been reported in 2003, 2006, 2010, 2013 and 2015. Gap between outbreak years is showing decreasing trend. Despite large number of dengue cases being reported, the case fatality rate (CFR) due to dengue has declined due to better case management facilities and increased awareness leading to early health seeking behaviour. CFR during outbreak years has been recorded as 4.2% in 1996, 1.12% in 2006 and 0.37% in 2015. Delhi has literacy rate of approx 86% but prevention and control of vector still remains a major challenge thereby necessitating the use of tools having behaviour impact. In a KAP study carried out in Delhi, it showed 80.5% respondents felt it is duty of concerned government agency to control mosquito breeding and prevent dengue.

Under Urban Malaria Scheme, Civic bodies have been entrusted with responsibility of prevention and control of vector borne diseases. In 2012, following trifurcation of erstwhile Municipal Corporation of Delhi, now the National Vector Borne Disease Control Programme is implemented by all three civic bodies as per Octalogue of Midterm Action Plan of Directorate of NVBDCP with innovative strategies that are locally appropriate, acceptable and affordable to have greater impact on community. Delhi being Capital city of India with multiple agencies, seeking coordinated effort may be for surveillance or vector control remains a big challenge. The vector control strategy in Delhi now encompasses domestic as well as peridomestic areas, parks, offices campuses places of public agglomeration etc. Solid waste management, public and support of stake holders are required for sustained programme management with crucial role of Civic bodies.

This paper highlights challenges in vector control in an urban settings and the way forward to overcome these challenges. It is beyond purview of Health department alone to control Aedes breeding. These challenges can be overcome with multi-pronged approach in an integrated manner. Shift of focus from vector control to Integrated Vector Management needs to be understood and addressed by programme managers and implementers. Best practices of other Municipal bodies also need to be adopted. During peak transmission season, there is increased demand for fogging from higher echelon and community members as it gives them sense of action being taken by the Government agencies. This leads to collapse of source reduction programme.

In India with limited resources and to have sustained control programme, monitoring and evaluation should be made an integral component of the programme management. Manpower planning, Capacity building by filling up crucial vacant posts of entomologists and insect collectors and skill development of field staff and developing managerial skills of Programme managers is the need of day. In India, there is need for development and use of managerial skills for the sustained programme management. Optimal utilisation of available resources and strategies based on entomological & scientific evidence should be first priority later they can be supplemented by newer tools in the programme.

Key words: Municipal Corporation of Delhi, National Vector Borne Disease Control Programme, Strategy, Dengue Control, Aedes

Microbial control of mosquitoes – exploiting the nature for vector control

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The goal of our organization is to protect people from the mosquito-plagues and to preserve the unique biodiversity of the ecological sensitive floodplains of the Rhine River. Therefore, since 1980 we focus solely on the reduction of mosquito larvae by applying selectively acting and sterilized products based on *Bacillus thuringiensis israelensis* (Bti, AM65-52). Bti is a soil bacterium which was first isolated in 1976 by Yoel Margalith in the Negev desert. Not the bacillus kills the mosquito larvae, but protein pro-toxins. Once they are ingested by the mosquito larvae, they pro-toxins are activated in the gut which bind to specific glyco-protein receptors, the osmo-regulation of the midgut cells is disturbed, and the cells swell and burst. The larvae die within minutes to hours. The unique features of Bti are the enormous selectivity and high efficacy. It kills only mosquito larvae and larvae of some members of nematoceran flies such as blackfly larvae. Thus the selective control is guaranteed and the biodiversity is conserved.

Mapping: The basis for the successful and targeted use of biological larvicides, is a precise mapping of the breeding sites taking the quality and productivity of the mosquito breeding sites into account. GIS maps are a tool for quick correspondence during the control activities.

Control Strategy: The design of the control strategy is based on the productivity of the breeding sites of anthropophilic mosquitoes (threshold) and their bionomics (e.g. the migration behaviour). Furthermore, ecological data, such as the presence of rare or sensitive organisms, e.g. the mass breeding areas of chironomids are taken into account. Since recent years the sterile insect technique (SIT) is employed in an IVM programme against the Asian tiger mosquito *Aedes albopictus*.

Infrastructure: The most important key for the success of the control measures is a functioning infrastructure with clear areas of responsibility. Mainly biologists are responsible for the organization in the regions, districts and municipalities. The 300 people of ground staff are supported by two helicopters.

Modern techniques: The satellite-based assessment of breeding sites (Web-GIS) and the precise application of Bti-granules by helicopter guided by GPS increase the efficiency of the operation and reduce the costs. Improved application techniques and formulations (Bti-ice granules) ensure the targeted application of the control substances. The use of drones may decrease the costs when small areas have to be treated.

Research: About 5% of the overall budget of 3,4 million Euros/year is needed for research in order to consolidate the measures (e.g. Resistance Research). In the monitoring, the direct influence of the control measures on the mosquito populations as well as indirect effects on the food web and food resources for bats, birds and amphibians is to be assessed.

Vector Control in California

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This presentation will cover a short history and organization of vector control agencies. In California there are 64 agencies who spend about 180 million dollars annually. Part of the presentation will primarily focus on the funding of these agencies. Most of the vector control is carried out by a special branch of government known as "Special Districts" which are governed by their own Board of Trustees who are appointed by Cities and counties. Each agency controls mosquitoes but some of the agencies include other vectors such as flies, midges, wasps, Africanized honey bees, rodents, skunks and occasionally other vectors. As indicated above the governing body of these special Districts is Board of Trustees who hire District Manager (DM). The DM implements policies and supervises the entire program. Most of the control program is divided into four main areas namely Administration, Technical, Operations, and Public education. A detail of each of these areas will be elaborated.

Big data and drones in Mosquito Control

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The impact and the efficacy of mosquito control projects are often suboptimal, especially when dealing with Mosquito Borne Diseases. Mosquito control has to integrate in its every day practice the possibilities that the exponential development in technologies is currently offering. There is a lack in the culture and managerial capacity of organizations and corporations dealing with mosquito control for the imperative digital transformation. The involved mosquito control organizations have to identify the critical work packages for which this digital transformation can improve essentially performance. New technological possibilities will require new skills and contrary to the prevailing perception, these technologies can lead to an increase in the needed workforce, provided that appropriate training is performed.

To our experience, key domains for the technological upgrading of mosquito control are the following:

- New culture, managerial skills and digital capacities of the implementing organization
- Further development and fast adoption of new tools, such as
 - Cheap, early and easy diagnostics for epidemiological surveillance
 - Digital georeferenced field data (mosquito larvae and adults) and continuous GIS data analysis
 - Ground and space remote sensing for observation of potential mosquito breeding sites (water sensors / satellites / drones)
 - Ground and aerial spraying tools (drones and / or other)

Integrative exploitation of all available data for both, risk assessment for Mosquito Borne Diseases in the appropriate spatiotemporal resolution and decision making for control operations (translational epidemiology).

In an attempt to upgrade technologically the services that Ecodevelopment provides in the field of mosquito / vector control, representative actual examples from Greece for 2018 will be explored:

- Following the extensive use of the in-house developed electronic platform (e-bite©) for operational, strategic and communication purposes among field technicians, project management and contracting authorities, a first evaluation of its impact will be made. It's compulsory use has already led all involved stakeholders towards this new digital transformation of a wide area mosquito control project (50% of mosquito control in Greece).
- Examples for each of the four above mentioned categories of new tools will be presented with special emphasis on the use of spraying drones (legal framework, technical aspects, operational evaluation)
- Examples for the effective operational use of open source data (remote sensing, land uses, hydrometeorological data) together with raw field data (mosquito larvae, adults & spraying applications) for the control of the 2018 West Nile Virus epidemic in Greece will be presented.

The strategy of providing new technological standards for all mosquito control stakeholders to comply with, will improve the cost-effectiveness of the projects.

Debug Fresno: Implementing Wolbachia to control *Aedes aegypti* in Fresno County, California

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Since the introduction of *Aedes aegypti* into California during 2013, this invasive mosquito is now established with populations expanding into urban areas within twelve counties in central and southern California. In 2016, the Consolidated Mosquito Abatement (CMAD) partnered with MosquitoMate to evaluate the first release of Wolbachia infected male *Aedes aegypti* mosquitoes in the United States. This Sterile Insect Technique (SIT) involves the release of male mosquitoes into selected residential neighborhoods with established infestations of *Ae. aegypti*. Mating of released males with local females results in conditional sterility. Verily joined the partnership in 2017 with the launch of Debug Fresno. Daily male mosquito releases in a neighborhood (221 acres/89 hectares) in the City of Fresno resulted in a significant reduction in biting female *Ae. aegypti*, compared to non-release areas. In 2018, the Debug Fresno study was expanded to include an additional three neighborhoods within the City of Clovis (195 acres/79 hectares, 206 acres/83 hectares, 321 acres/130 hectares). All release sites are within cities in Fresno County, California. The presentation will discuss results and summarize the role of CMAD in establishing the study areas with ongoing monitoring of mosquito population trends in release and non-release areas. Community outreach efforts to increase public awareness of Debug Fresno will be presented, and resident perceptions, responses and interactions will be discussed.

Sounds: a novel way to identify mosquitoes

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Mosquitoes are important vectors of several viral, protozoan and nematode diseases responsible for the deaths of several millions of people every year and many more suffer from temporary or permanent disabilities worldwide. Many vector-borne diseases are untreatable such as Dengue, Chikungunya and Zika and their control mainly centres on mosquito management. In India, more than 400 species of mosquitoes are present that belong to subfamilies Anophelinae and Culicinae consisting of 11 tribes. Subfamily Anophelinae involves genus Anopheles consisting of 63 species. Genus Aedes and Culex from Subfamily Culicinae contribute the major proportion in diversity and contribute 175 and 85 species respectively. They are highly diverse in inhabitation of breeding places, host choice for blood feeding and biting rhythms. These species' can be recognized with morphological characters, however, it is tedious and cumbersome. We need a highly specialized person to collect the mosquitoes, process and identify them properly. These species can also be identified with molecular markers, however, it is a lengthy process. We have developed a novel method based on mosquito sounds to identify the mosquito vectors in the live stage which may provide a quick and cheap method. The present study evaluated the six species belong to genera Anopheles, Culex, Aedes, Armigeres and Mansonia to develop a database of sound frequencies. The results from the present study have shown a species-specific pattern in sound frequencies with significant variations among different genera and species. We also found that individual species generate a group of frequencies which are varied in sound intensity. This method is able to distinguish the male and female mosquitoes. The findings of the present study are significant to develop a novel method for species identification of mosquitoes and to develop an automated tool for species recognition. The similar method can be implemented to other insects for their identification.

Urban Mosquito Vectors And Challenges For Their Control In A Metropolitan City - Chennai, India

Eapen Alex

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The global urban population is expected to grow approximately 1.84% per year between 2015 and 2020, 1.63% per year between 2020 and 2025, and 1.44% per year between 2025 and 2030. It is estimated that 54% of world's population of 3.9 billion lives in urban centers. The process of urbanization involves increased movement and settling of people in urban surroundings. Also, landscape modification and transformation of environs through demands for resources and improved communications. Urbanization leads to many challenges for global health and epidemiology of infectious diseases mainly in third world countries. Different risk factors in an urban environment can lead to various issues such as the proliferation of insect vectors due to poor housing structure and design, making them easy targets for these infectious agents. The population explosion in urban settings is interlinked with inadequate water supply and increased storage practices, poor sanitation and hygiene besides, waste disposal and management practices. Poor housing and overcrowding can also contribute to vector mosquito proliferation. The density of inhabitants and the close contact between people in urban areas are potential threats for the rapid transmission of infectious diseases. The growing trend of urbanization around the world has shifted some infectious diseases, which has been traditionally considered as rural, to urban settings. The World Health Organization (WHO) has published a list of 17 neglected tropical diseases (NTDs), most of them have become a reality in the urban environment. Urban settings can act as a catalyst for the rapid spread of infectious diseases. If the different insect vectors can adapt to the urban situations, the potential implication of which can be of a great concern. The operational feasibility of implementation in fast-growing cities with slum tenements, high population density coupled with inadequate manpower and resources is a herculean task. A much better understanding of infections are extremely important in urban settings, which are hubs of population movement and migration. Hence, a thorough knowledge of the local conditions favouring transmission, the risk factors involved, intervention measures adopted and implemented, manpower availability and deployment, nature and type of breeding habitats, larval source management methods, parasite surveillance mechanism, implementation of legislative bye-laws, adult vector behaviour etc. are essential to know whether local or indigenous transmission of vector borne diseases is really happening or not. It is also pertinent to know the transmission risk factors in urban settings as socio-behavioural, other confounding variables, breeding habitats, pathogens and intervention measures are different from a rural setting. The recent upsurge in dengue and chikungunya cases in urban cities have further diluted the attention and focus on disease surveillance and vector control. Regular active surveillance is of utmost importance in the urban vector control programme. Furthermore, changes in vector behaviour due to urbanization and climate change in addition to other eco-socio-behavioural factors also pose a serious challenge for the control of urban vectors in Chennai.

Disease control using genetically modified mosquitoes

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Recent advances in genetic engineering provide researchers with unprecedented possibilities to address problems. Our projects aim at generating genetically modified mosquitoes by applying CRISPR-Cas9 and gene drive technologies. The potential release of modified organisms is a critical part of the projects and requires extensive study of the target populations. Here we present the combination of genetic engineering and population genomic approaches with the goal to alter natural mosquito populations for disease control.

WHO malaria technical updates and initiatives for malaria elimination in India

Dr Roop Kumari

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Malaria is preventable and curable and increased efforts have yielded positive results, reducing the malaria burden in several countries. World Health Organization (WHO) has developed the Global Technical Strategy (GTS) for Malaria Elimination 2016-2030. In May 2015, the World Health Assembly endorsed the Global Technical Strategy (GTS) for Malaria Elimination 2016-2030. This strategy lays down clear global goals, milestones and targets. GTS provides a technical framework for all malaria-endemic countries to support regional and country programmes to accelerate progress towards malaria elimination. The strategy sets the target of reducing global malaria incidence and mortality rates by at least 90% by 2030. The milestones for 2020 include reductions in malaria case incidence and death rates by at least 40% and the elimination of malaria in at least 10 countries.

As per World malaria Report 2018, an estimated 219 million malaria cases occurred worldwide in 2017. The largest declines occurred in the WHO regions of South-East Asia (54%), Africa (40%) and the Eastern Mediterranean (10%). Region-wide efforts over the past decade have made it a real possibility to eliminate malaria from the region. Several countries are moving forward towards elimination. In 2016, 44 countries reported fewer than 10 000 malaria cases. In SEARO, Maldives and Sri Lanka have already been certified by WHO as malaria-free in 2015 and 2016 respectively. Two other countries – Bhutan and Timor-Leste had fewer than 100 reported malaria cases in 2016 and zero malaria deaths. India is now fourth highest malaria burden country as per WMR 2018.

In accordance with the GTS for Malaria Elimination 2016–2030 and commitment made to APLMA, the National Vector Borne Disease Control Programme (NVBDCP), Ministry of Health & Family Welfare, Government of India has launched the National Framework for Malaria Elimination (NFME) on 11 February 2016 towards commitment to malaria elimination by 2030. Malaria elimination in India will be carried out in a phased manner. In sync with NFME and global framework for malaria elimination, the National Strategic Plan (NSP) for the period 2017-22 has been developed by NVBDCP and WHO country office in which focus is on district based planning, implementation and monitoring.

India is endemic for malaria and carries the burden of the two major parasites *Plasmodium falciparum* and *Plasmodium vivax*. India represents 4% of the global malaria burden. Progress made in reducing its disease burden include 24% reduction in cases and 68% reduction in malaria deaths recorded in 2017 compared to 2016. India has set a target of being malaria-free by 2027 and eliminating the disease by 2030. India is now developing an approach to eliminate malaria state by state.

In India, - Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh, West Bengal and NE states, which account for a major burden of malaria cases- reported a substantial decrease. Elimination in the eastern state of Odisha is a challenge, which has more than 40% of the country's malaria burden but has shown a drastic decline in 2017. WHO is providing support to malaria program for Policy and guidelines update for accelerated action towards elimination, capacity building, surveillance strengthening, program reviews and demonstration of tailored response in one high burden state (Chhattisgarh) and one low burden state (Punjab).

Lymphatic Filariasis Elimination in India: How to achieve Goal

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India was a signatory to the World Health Assembly (WHA) resolution, 1997 for Elimination of Lymphatic Filariasis (ELF) as a global public health problem by 2020 after the International Task Force for Disease Eradication identified lymphatic filariasis as one of the six infectious diseases to be "eradicable" or "potentially eradicable". Following the resolution, First meeting of Global Alliance to Eliminate Lymphatic Filariasis was convened at Santiago de Compostela, Spain on 4-5 May 2000 and Indian Health Secretary stated "it would be a long haul for India and that interim target dates would be necessary between now and the year 2020." Subsequently, National Health Policy - 2002, envisaged the elimination target to be achieved by year 2015 in India. Many challenges were tackled during 15-years journey and now the main challenge is how to achieve the elimination goal in set period. Knowing well about the global goal of elimination by 2020, the Indian programme policy set it for 2015, which was the first challenge but has now been resolved by aligning it with global time frame. The second challenge was the implementation of global strategy of two-drug combination which was delayed by three years after starting elimination campaign in 2004 and fully scaled up in 2009. Due priority for ELF was not accorded for various reasons in comparison to other vector borne diseases and other flagship health programmes which still remains a major challenge and everyone should be concerned about it. Resolving administrative issues and clearing financial backlogs along with availability of required financial resources need to be ensured. Technical clarity on ELF concept, supervised drug administration, its significance, adequate social mobilization with adequate focus on ELF while integrating with other health programmes and ensuring quality assessment for microfilaria or for antigenemia during transmission assessment survey, need to be regularly emphasized throughout year. Performance monitoring of each activity at grassroot level, if ensured with corrective measures instead of only impact monitoring, will certainly give a boost to the programme. To accelerate elimination efforts, alternate strategy of Ivermectin combination with DEC and Albendazole (IDA), has been successfully piloted and programme has considered to implement in identified hard core districts. The last mile challenges will, however, be very crucial in achieving the goal. These challenges are survey of non-endemic districts for current status of LF endemicity and liquidating foci in non-endemic districts with treatment and vector control under Integrated Vector Management (IVM). IVM towards ELF has somehow, been tagged as anti-larval programme in designated urban areas but a comprehensive guidelines is required which will facilitate district level health workers in intensifying a synchronized approach against vector mosquitoes. Indian programme on ELF has shown significant achievement duly acknowledged at international level but sustaining the achievements gained so far and preventing few failures in TAS are also crucial before programme steps in for verification. All these are possible with full commitment and sustained efforts by service providers and community partnership.

When to Stop IRS for Visceral Leishmaniasis

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Visceral Leishmaniasis also known as Kala-azar is endemic in India sub-continent. In India VL is at present endemic in 54 districts in the states of Bihar, Jharkhand, West Bengal and Uttar Pradesh. Cases are sporadically reported from Delhi, Assam, Gujarat, Sikkim, Himachal Pradesh, Uttarakhand and from Kerala also. An estimated 130 million population is exposed to the risk of VL.

The distribution and prevalence of kala-azar changed dramatically in the wake of regular insecticide spray instituted all over malarious areas in the country under NMCP/ NMEP for interruption of malaria transmission from 1953/1958-59 onwards has also reduced the sand fly (vector of Kala-azar) population to a very low level resulting in interruption of Kala-azar transmission. It has taken a serious dimension since late eighties, as a consequence of withdrawal of DDT spraying under NMEP from Kala-azar endemic areas as early as 1963-64.

Indoor residual spray (IRS) is the main tool for killing of Kala-azar vector. During 80s DDT 50% has given good results in terms of epidemiological impact. Over the years the refusal rate by House owner has increased due to no perceptible results of insecticide in killing sand fly and white spots on walls. In 2015, Programme Division (NVBDCP) has replaced DDT with Synthetic Pyrethroid on the basis of published vector tolerance towards DDT.

Indoor residual spray is a cumbersome process, requires large number of spray workers, their training and retention of skilled workers etc. Lack of monitoring, supervision and quality of spray still remains a challenge. To overcome this challenge, focal/reactive spray in and around new case may give good results in interruption of transmission as the team will do IRS with skilled workers throughout the year by following standard operating procedure. The quality of supervision and monitoring will also improve as spray operation will be few houses or few meters from positive KA case.

Challenges of Urban Malaria Elimination In India

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Malaria in urban areas was considered to be a marginal problem restricted to mega towns only and was considered that local bodies are capable of handling it. Therefore, while launching the National Malaria Eradication Programme in 1958 in India, Urban Malaria was not included. By 1970s, incidence of rural malaria came down drastically i.e. 0.1 to 0.15 million cases per year but the urban town reported rising trend. Madhok Committee in 1970 investigated the problem and assessed that 10 to 12% of total cases were contributed by urban areas. The committee recommended anti larval measures for containment of urban malaria, because it was feared that proliferation from urban to rural may spread and nullify the gains already made. The control of malaria in the urban areas was thought of an important strategy as a programme complimentary to the NVBDCP for rural areas. Modified Plan of Operation (MPO) was designed and submitted to the Union Cabinet to tackle the malaria situation in both urban and rural areas in the country simultaneously. Under MPO, it was decided to initiate anti-larval and anti-parasitic measures to abate the malaria transmission in urban areas. The proposal to control malaria in towns named as Urban Malaria Scheme was approved during 1971 and it was envisaged that 131 towns would be covered under the scheme in a phased manner. This scheme was sanctioned during November, 1971 and the expenditure on this scheme is treated as plan expenditure in centrally sponsored sector. The central assistance under this scheme was treated 100 per cent grant to the State Governments in kind.

Urban Malaria Scheme is protecting 130.3 million population from malaria as well as from other mosquito borne diseases in 131 towns in 19 States and Union Territory. The challenges for malaria elimination include the increasing population growth unable to match with the civic facilities leading to increased malaria vector breeding and invasion by *Aedes aegypti* the vector of dengue fever, Dengue Hemorrhagic Fever (DHF) & Chikungunya, population pressure is increasing in all towns & mega cities are expanding and new settlements in peri-urban areas have come up. These peri-urban situations have low infrastructure which lead to inclusion of *An. culicifacies* (a major malaria vector in rural areas of India) along with the vector of urban malaria *An. stephensi*. The mega towns are now expanding vertically creating new avenues of vector breeding. For firefighting exigencies require building of two storage tanks one at the ground and other at the top with high breeding potential of *A. stephensi*. Intermittent water supply developed storage practices in artificial containers which generated breeding sites for vector mosquitoes. Development project without Health Impact Assessment (HIA) resulted in malaria outbreaks in some Cities. Inadequate and trained health infrastructure in all the cities of the country will hamper the elimination activities. The immigration particularly population from disease endemic areas to urban cities/towns and poor disease surveillance are biggest challenges in urban areas of the country.

Piloting of an Entomological Surveillance Planning Tool to Improve Entomological Intelligence for Evidence-Based Vector Control Decision-Making towards Malaria Elimination

Neil Lobo

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To accelerate progress towards malaria elimination, the World Health Organization (WHO) Global Technical Strategy 2016-2030 calls for maximizing the impact of vector control by strengthening entomological surveillance and capacity and managing insecticide resistance and residual transmission. In response to these efforts, we have developed an Entomological Surveillance Planning Tool (ESPT) to distil WHO guidance into an operational, decision-support tool for national malaria programs to support cost-effective, locally tailored, evidence-based vector control. The ESPT aims to support countries in generating entomological intelligence that guides vector control intervention selection, deployment in time and space, and provides a platform to evaluate complementary strategies and tools. To this end, the ESPT consists of a series of decision trees to help guide countries in the collection of the priority entomological indicators needed to make decisions through: 1) baseline surveys, 2) routine sentinel surveys, 3) focus investigations, and 4) entomological surveys based on priority programmatic questions. These decision trees link to the priority minimum indicators, a trapping methodology matrix to guide collections, data collection forms, and guidance on selecting sites for entomological investigations. In collaboration with national malaria programs and local partners, pilots of the ESPT have begun in three countries: Mozambique, Namibia, and Panama. Parallel evaluation activities are underway and include: 1) qualitative assessments to measure the ESPT's feasibility, acceptability, utility, and impact on vector control program decision-making; 2) costing of entomological surveillance activities and the cost per indicator; and 3) tracking decision-making on vector control strategy as related to implementation of the ESPT. Preliminary data from the pilot evaluations will be presented.

Ecologic And Socio-Demographic Impact On Dengue And Its Management In India

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Dengue, the most important human arboviral infections is listed amongst top 10 global health threats by World Health Organization (WHO). WHO has estimated that 40% of the world population is at risk of Dengue with a 30 fold increase in global incidence over the last five decades. There are around 390 million infections occurring annually in more than 128 endemic countries. In India, currently all 36 States/UTs are reporting Dengue incidence with repeated outbreaks from many States. In 1996, the country witnessed a major outbreak of dengue with 16517 cases and 545 deaths. Transmission is continuing in subsequent years. During last three decades, many fold increase has been reported from India. Perennial transmission established in southern and western parts of the country with the expansion of transmission window. In 2017, a total of 1,88,401 cases and 325 deaths were reported.

Ecology is a great determinant for occurrence of Dengue. Changes in climate directly affect the environment and influence the dengue transmission. However, association between weather and Dengue varies across geographical locations and socio-environmental strata. Temperature and rainfall are important climatic factors in mosquito population and disease transmission dynamics. Rise in temperature is reported to expand the vector's geographic range, increase the mosquito's biting rate and shorten the extrinsic incubation period leading to faster virus replication and increased transmission intensity. Rainfall provides essential habitat for vector during the monsoon and post-monsoon periods. Both *Aedes aegypti* and *Ae. albopictus* are involved in transmission. Their breeding habitats differ from one part of the country to other due to diverse ecological conditions. There is a paradigm shift in dengue transmission from urban to rural ecosystem with rapidly increasing cases from the rural areas contributing 30-50% of the total Dengue cases. However, the shift is not uniform across the states due to ecological diversity.

Geographical alterations with socio-economic developments occurring over the past few decades including rapid urbanization, unplanned construction, industrialization, concurrent population growth, deficient potable water, improper solid waste management, ever increasing automobile industry, increased population movement, suboptimal entomological infrastructure etc. are few underlying causes for geographical expansion.

National Vector Borne Disease Control Programme (NVBDCP) is an umbrella programme under the Ministry of Health and Family Welfare, Govt. of India for prevention and control of six VBDs including Dengue. NVBDCP mandated for planning including financing, policy making, technical guidance, monitoring and evaluation, while States are responsible for implementation of the programme. For effective prevention, a laboratory based disease surveillance network through Sentinel surveillance Hospitals and Apex Referral laboratories which provides early warning of transmission in addition to free diagnosis across the country. In absence of any drug or vaccine against Dengue, entomological monitoring and vector control are the main components to eliminate/reduce the risk of transmission and outbreak containment. Entomological monitoring is carried out by States and Municipalities. Community participation is critical for successful programme implementation. Media mix behavioural change communication is emphasized for awareness of the community and stakeholders. The sustainable changes for dengue control need a holistic approach considering the eco-bio-social factors based on vector control and other current strategies with multi-sectoral approach.

Vector surveillance and control at Points of Entry in the context of expanding distribution of disease vectors and outbreaks of vector borne diseases in new areas

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In May 2005, the Fifty eighth World Health Assembly adopted new International Health Regulations (IHR) which came in to force in July 2007. One of the essential elements of the IHR was capacity building for vector surveillance and control at points of entry (PoE). This step was timely in recognizing increasing threats of invasion of vectors in newer areas and countries via aircrafts, ships, lorries, trains and other means of transport at the airports, ports and ground crossings. The IHR mandated that each member state would designate PoEs, prepare standard operative procedures, ensure sufficient monitoring and response capacities, create necessary infrastructure and initiate comprehensive programme for surveillance and control of vectors at the points of entry and at least in their 400 meter perimeter. In the context of global expansion of vectors (*Aedes aegypti*, *Aedes albopictus*, etc.), emergence and outbreaks of vector borne diseases (Zika, Dengue, Chikungunya, West Nile virus etc.), there is need for strict compliance and implementation of IHR provisions at the Points of Entry by all the UN member states. In 2016, Departments of IHR and Neglected Tropical Diseases (NTD) have prepared guidance in the form of handbook on "Vector surveillance and control at ports, airports and ground crossings" to assist countries in the implementation of provisions of IHR. This guidance is available online in 8 languages, English, Spanish, French, Russian, Chinese, Persian, Japanese and Turkish and can be freely downloaded (<https://www.who.int/ihr/publications/9789241549592/en/>).

How Kerala government is tackling Dengue, Malaria and other vector borne diseases, a policy analysis.

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Kerala, the southern state in the Western Ghats, is geographically and climatically prone to vector borne diseases. With heavy Pre-Monsoon, South-West Monsoon and North-East Monsoon in past two years vector borne disease like Dengue and Malaria were reported in large number from all over the state. The state government had taken notice of Dengue epidemic and high casualty during April-September 2017 and took various steps for vector control using various government machineries in its umbrella health program Aardram. State vector control officials had regular vector surveillance in panchayat and ward level. Aarogya Jagratha, a year-long health awareness project to prevent the spread of communicable diseases. 'Dry day', a container source reduction drive is being observed every Friday at schools, Saturday at offices and Sunday at homes. One person in 25 households is designated as Arogya Sena member who undergoes training in basic medical care. Primary health centres and ASHA workers do house visits to inspect breeding sites of mosquitoes. The general public is sensitised about the issue through print, audio and visual media. 30th Kerala Science Congress, held on January 2018, had the 'Viruses and infectious diseases' as the focal theme. Kerala sustainable development goals 2020 aims to have zero indigenous cases of Malaria and filaria control.

This paper will deal with the policy analysis of Aardram program with regard to vector control and review the implementation and effectiveness of various vector control initiatives and projects by the state government. The study hypothesises that the experience gained by the effective implementation of vector control measures helped immensely during post-flood vector control and epidemic prevention. The methodology includes epidemiological data analysis, policy analysis and interview with various stakeholders.

In 2017, 21993 cases and 165 deaths due to Dengue, and 1177 cases and 2 deaths due to Malaria were reported. With the effective implementation of projects like Aarogya Jagratha, Aarogya sena, 'Dry day' every week, sea shore cleaning, etc with the help of primary health centers, ASHA workers, students and officials resulted in a decrease in dengue, malaria and chikungunya cases and casualties in 2018, 3652 cases and 35 deaths due to Dengue, and 686 cases and zero death due to Malaria.

Conscientious implementation and well funding of vector control program round the year helped in sensitizing the public about vector borne diseases and its control. Productive participation from school children, Asha workers and government officials helped in garnering habit of vector control in the locality. The well-oiled state machinery worked efficiently during post-flood period resulting in an effective vector control and very low vector borne disease reports.

Characterization of Vector competence, Co-infection, Viral Interference and Transovarial Transmission of Zika and Dengue Virus by Brazilian *Aedes aegypti* and *Aedes albopictus* populations.

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Dengue and Zika are important arboviruses present in tropical and subtropical areas of the Americas whose main vector is *Aedes aegypti* and a potential vector or secondary vector is *Aedes albopictus*. Several factors may influence the epidemiological dynamics of these arboviral diseases, such as the immunological status of the host, the viral serotype, genotype or strain of the circulating virus, the vector density, the vector's competence, as well as environmental factors. The vector's competence is an intrinsic characteristic of the vector, which allows the entry of the pathogen, its multiplication, and subsequent transmission to vertebrates. This characteristic has been the object of several studies of vector-virus interaction that have been essential to further understand the dynamics of arboviruses. This study was carried out by conducting Dengue virus (DENV) and Zika virus (ZIKV) experimental vector infections followed by tests for positivity using molecular biology tools. Infections with different serotypes of DENV in different populations of *Aedes aegypti* were performed. *Aedes aegypti* mosquitoes were coinfecting with DENV and ZIKV and molecular biology techniques measured the result of this infection. Vector competence, vertical transmission and the influence of ZIKV on immature stages of the vector were tested. The susceptibility of *Aedes albopictus* to ZIKV was also tested using experimental infection. Variations were found in the susceptibility of *Aedes aegypti* to different serotypes of DENV. *Aedes aegypti* mosquitoes seem to be more susceptible and present greater vector competence for ZIKV in comparison with DENV in coinfection. Vertical transmission of DENV and ZIKV can occur at high rates of branch infection, and ZIKV can delay hatching and larval development. Finally, *Aedes albopictus* from the East coast of the United States is susceptible to ZIKV. In conclusion, the analysis of the vector's competence and other aspects of the virus-vector interaction allowed us to highlight some still non-elucidated questions, such as the variation in vector competence, occurrence and vertical transmission rates besides the susceptibility of the coinfecting mosquito vectors.

Molecular Tools For Mosquito-Borne Disease Diagnosis In The Context Of Zika Fever Infection

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Introduction: Zika virus infection, a severe mosquito-borne disease caused by a flavivirus (Flaviviridae) primarily affects peoples in Africa, Brazil and Asia. The ZIKV infection is usually characterized by arthralgia, myalgia, mild fever, headache together with cutaneous maculopapular rash in human beings. Zika virus infection has been misdiagnosed in viremic phase since it exhibits nonspecific influenza-like symptoms. The only arbitration affordable for Zika virus infection is the rapid diagnosis and Aedes spp mosquito control. The present scenario concerning the Zika virus outbreaks has generated an alarming emergency against public health of international concern necessitating diagnosis of life-threatening epidemiologic candidate Zika virus. Therefore, this article analyses the advanced molecular diagnostic tools and strategies regarding the reliable detection of ZIKV within short time together with a special emphasis on new hypotheses, current updates, that has been affordable for the medical professionals from developing countries and rural regions that urgently need to address this epidemiological challenge.

Methodology: Articles on molecular diagnostics of Zika fever infections were searched in online databases (PubMed®, Excerpta Medica data BASE Embase® and Science Direct) including NCBI using the appropriate keywords such as "Aedes aegypti", "zika", "ZIKV", "molecular diagnosis", "vector control", "PCR". A total of 70 articles were found that described approaches that have been employed for the rapid diagnosis of Zika virus infection. The articles opted for this study have been written in English and most of them were taken from WHO, CDC, and NIH. Titles, keywords, and abstracts of the studies have been screened and the articles those are ineligible were discarded.

Result: The rapid diagnosis of zika virus infection in the laboratory is generally based on the analysis of serum samples employing antibody-based and viral RNA detection assays. Molecular techniques are renowned as more consistent owing to the cross-reactivity prompted by IgM antibodies among the flaviviruses during serological estimation. Significant efforts have been made concerning the development of several molecular tools for virus detection. Lateral-flow immune chromatographic assays are also employed as a simple, inexpensive, rapid, and instrument-free approach for the detection of ZIKV with low specificity and sensitivity. Currently, the RT-PCR based diagnostic approaches offer high sensitivity and specificity.

Conclusion: This study summarizes the presently available molecular tools for the rapid diagnosis of ZIKA virus infection.

Keywords: Aedes aegypti; zika; ZIKV; molecular diagnosis; vector control; PCR.

Dengue Out Breaks In Tribal, Rural And Urban Settings Of Khammam District - A Case Study

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Man-made containers, extensive use of plastic and improper waste disposal is leading to extensive congenial atmosphere for Aedes breeding and spread of antiviral diseases outbreaks of dengue in different places in tribal and non-tribal district.

Out breaks have since been reported from different parts of the country. Epidemics have been documented during the period which usually corresponds with warm rainy season, when density of vector mosquito is very high. Undivided Khammam district is located mostly on the banks of the river Godavari bordering with Odisha, Chhattisgarh and Andhra Pradesh states of India and 27.4 per cent of the total population of the district comprises of tribal inhabiting the agency mandals. The district administration and public health personnel have taken meticulous precautions by providing health care measures due to which, the incidence of malaria has been declining, however, dengue has become a challenge and of late it's prevalence has increased from January 2016 onwards. First it was started in Gundalaallapalli and Mangapet, in the month of May 2016, in Patha Anjanapuram, there is particular period for occurring of dengue and transmission of virus, because of water storage practices in the community level. In the agency area of Aswapuram, dengue cases spurted in 2014 in April first week. In 2016, a Mandalvinaka and the surrounding areas within the radius of 30 kilometres have witnessed alarming levels of dengue incidence attracting the special attention of state govt.

The district administration and the health department have deployed more personnel and equipment to deal with the situation. In 2017, Mudigonda and Nagulvanca, old tractor tyres and in Dwajasthambum inauguration in Nagulvanca village, public kept many pots outside in which plenty of larvae were found. It was also one of the causes in 2018 in Khammam town as both dengue and Chickungunya outbreaks were reported. Lack of awareness, quacks, steroidal drugs prescriptions and in the corporate private hospitals, lack of isolation wards in some places. The district collector has instructed the private practitioners to collect 2 blood samples, and one sample to govt medical sector, increased surveillance, dry day activities particularly with 8th 9th high school children in the same village, and NSS junior college youth and local youth.

Temporary platelet centres were established in affected Primary Health Centers. District health administration arranged meetings with local practitioners of Medicine and gave counselling on protocol of treatment, arranged meeting between local govt., and private sector doctors, explained the protocol of dengue, facilities available at the govt hospitals. Health education meetings were conducted with govt health educators to sensitize the community. The facilities in the Govt. hospitals were revamped and improved to provide extra beds for patients and facilities to the public with positive impact. In urban areas particularly in big corporate hospitals, lot of antilarval and adult anti-mosquito measures were taken. Due to all these measures, morbidity and mortality drastically reduced in the study districts. These will be discussed in detail.

Ecological Undercurrents Of Aedes Albopictus Driven Dengue In Kerala, India

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Kerala, the south Indian state of India, experienced the first outbreak of Dengue in 1997 with 14 cases and 4 deaths in Kottayam district. By 2003, the entire state became endemic to the disease. Though both the established vectors (*Aedes aegypti* and *Aedes albopictus*) are prevalent in the state, *Aedes albopictus* has been found to be dominant in terms of abundance and geographical distribution. However, no systematic studies have been undertaken to quantify the role of this vector in the transmission of dengue in the state. Hence, a well designed study was under taken in the state with Kannur district as a representative area. The objectives of the study were 1. To test the hypothesis that *Aedes albopictus* is the major player in making the state endemic to dengue; 2. To compare the habitat diversity of *Aedes aegypti* and *Aedes albopictus*; 3. To understand the ecological conditions that promote the proliferation of *Aedes albopictus*; and 4. To suggest strategies to control *Aedes albopictus* driven dengue in the state. The study was conducted from January to December 2017. Vector surveys were conducted in all Panchayats and municipal areas of the district to study the prevalence of vectors. Epidemiological data were obtained from the district health authorities. The presence of *Aedes aegypti* and *Aedes albopictus* were observed in the coastal panchayats and municipal areas. *Aedes albopictus* was the only vector in the panchayat and municipal areas of sub-coastal and foothill areas. Out of 2772 dengue cases reported from the district during 2017, areas with *Aedes albopictus* alone contributed 2473 cases (89.2%) and the remaining 299 cases (10.8%) were contributed by the coastal areas where both the vectors were prevalent. In the coastal areas 16 different types of habitats were found to have *Aedes* breeding. Out of these 14 were artificial and the other 2 were natural habitats. Percentage of natural habitats in coastal areas was only 1.7 *Aedes aegypti* could be collected only from one natural habitat and 2 artificial habitats. In sub-coastal areas and foothill areas *Aedes albopictus* immature stages were collected from 22 different types of habitats. Out of this 8 were natural habitats. Percentage of natural habitats in these areas was 55.5. One of the major habitats was latex cups in rubber plantations. With about 3300 mm rainfall spanning six months and with a rich diversity of natural habitats and prevalence of rubber plantations, breeding potential of these areas was very high. The study revealed that the major player in dengue epidemiology in Kerala is *Aedes albopictus*. With its peculiar ecological and demographic conditions *Aedes albopictus* driven dengue in the state demands alternative control strategies, different from that driven by *Aedes aegypti*.

Threats of three medically important *Aedes* species in the context of changing environment: Time for a reality check

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There are considerable evidences for the resurgence and progressive increase of mosquito-borne viral diseases such as Dengue (DEN)/DHF, Chikungunya and Zika. All the above mentioned diseases are endemic to India except Zika. Some sporadic cases of Zika have been reported recently from the western part of India. However, further epidemiological studies are needed to ascertain the origin of its occurrence. Until now Yellow Fever has never been reported in India, believed to be a "Yellow Fever receptive area". Global warming, globalization, urbanization, changing land use pattern etc. are likely to reshape the ecology of many vector mosquitoes and vector-borne diseases as well. *Aedes aegypti* and *Ae. albopictus* are recognized potential vectors of the above mentioned diseases. *Ae. albopictus* has been included in the hundred most dangerous species in the global invasive species database. The global expansion of their distribution and introduction to new continents has been attributed by international trade routes through shipping and increased anthropogenic activities. All these viruses have been isolated from the above mentioned mosquito species along with *Ae. vittatus*, another important member of the genus *Aedes*. According to NVBDCP report, in India clinically suspected Chikungunya fever cases vary from 15977-64057 during the period 2010-2016 though, no casualty has been reported till date. With reference to Dengue, the incidence rate has gone up to 129166 in 2016 from 28292 cases reported in 2010. The number of deaths reported in this period is 52 ± 19.64 (mean \pm SE). The Case Fatality Rate (CFR) however, has decreased from 0.39 to 0.19 clearly depicting a better clinical management. *Ae. vittatus* is hitherto a neglected vector species, however, this species might have some role in the maintenance and transmission of these viruses in nature specially in the inter epidemic period. Experimental evidences also reinforcing this assumption. All the above said mosquito vectors are also prevalent in Kolkata and adjoining districts as observed in our studies. This area is endemic for Dengue and Chikungunya virus. Anti-Dengue virus antibody may enhance Zika virus replication. There has been a concomitant rise in the incidence of Dengue in West Bengal alongside India from 2010 to 2016 as clearly reflected by the mean \pm SE value of 7586.8 ± 2862.94 . Emerging arboviral infections generally stem from an animal reservoir but there is inadequate information on the natural history of the Indian arboviruses especially their methods of survival in the inter-epidemic period. This has been evident from the resurgence of Chikungunya virus in India in 2005 after a gap of 32 years. Therefore, a sustained active surveillance on the above three *Aedes* species and molecular sequencing studies of different isolates of Dengue virus, Chikungunya virus, Zika virus are required to understand the virulence, evolutionary trend of these viruses, to know the vector distribution pattern and vector competence in the context of changing environment. This has to be done at the national and international level in a coordinated and sustainable manner in order to combat and control these diseases.

Changing Climatic And Ecological Conditions Affecting Spatio-Temporal Distribution Of Vector-Borne Diseases In India

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There are six major vector borne diseases (VBD) namely malaria, dengue, chikungunya, Kala-azar, Lymphatic filariasis and Japanese encephalitis in India. Since 2005, several VBDs like chikungunya, scrub typhus, and Kyasanur Forest Disease and Crimean Congo Hemorrhagic fever have re-emerged, the reasons of which are mainly changes in climatic and ecological conditions. The role of climate is crucial in the epidemiological triangle of VBDs as the life cycle of vector species; their survival and development of pathogen in their body are affected by climatic conditions. The climatic conditions prevailing in a particular geographic area and socio-economic developments determine the ecology of the area, which ultimately provide receptivity for the vectors species to breed and thrive. In recent years, the issue of climate change has also emerged which has been affecting the spatiotemporal distribution of VBDs at global level. The impact of climate change affecting the future scenario of major VBDs in India by the year 2030s, 2050s and 2070s using climate models has been studied. In terms of malaria, the Himalayan region is projected to have opening of few foci of malaria transmission by 2030s and so on while increased intensity in northeastern states. Simultaneously, the ecological changes affecting the altered distribution of malaria vectors in Assam and link of canal-based irrigation with malaria endemicity in Karnataka will be presented. The efforts made by the Govt of India in addressing the issue of climate change and health will also be shared.

Impact Of Climate Change On Expansion Of Vectors And Vector-Borne Diseases In Baltic Countries

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In the past decade, vector-borne diseases have increased in their incidence and distribution in Europe, creating new challenges for public and animal health. The ecology and epidemiology of vector-borne diseases are complex and diverse, and affected by the relationship between the pathogen, the host (vector or vertebrate host) and the environment. Climate is a major driver that influences the distribution of vector-borne diseases, as climatic factors affect the survival of the vectors, their habitat suitability, geographical distribution and abundance, and the survival of pathogens within the vectors. The geographical and spatial distributions of some European tick vectors have been changing in the last few decades, and new viral, bacterial and protozoan tick-borne pathogens have been detected in former non-endemic areas in Baltic countries. During the past two decades *Dermacentor reticulatus* has expanded its range in the Baltic countries, and new localities with *D. reticulatus* occurrence have been found in Lithuania and Latvia. Canine babesiosis has emerged in Latvia and become widely distributed and quite frequent in Lithuania. Recently, a new tick species *Ixodes inopinatus*, which previously was found only in the Mediterranean regions, has been discovered in Baltic countries. Mosquitoes and mosquito-borne diseases have become widely established across Europe. Climatic changes, the significant increase of tourism and travel of dogs across Europe have caused an increase in the geographical range of *Dirofilaria* infections. In recent years Baltic countries have witnessed the introduction of previously unknown human and canine *dirofilariasis*. Fleas are among the most important ectoparasites of animals and humans and are the natural vectors of several important infectious diseases. In recent years the flea fauna and flea-borne pathogens in Lithuania have been investigated. The advances in molecular biology during the last two decades and using of molecular diagnostic techniques have allowed researchers in Baltic countries to better diagnose, trace and characterize pathogens, and have led to the discovery of new vector-borne pathogenic organisms.

Impact Of Ecological Changes On Culicidae Diversity In Punjab

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The ecology of agricultural state of Punjab in India has tremendously changed during the last five decades. The growing cultivation of paddy crop during summer and monsoon months has made the availability of water throughout the state in abundance. Mosquito transmitted diseases are flaring up in different parts of the state due to this reason. The rapid development, population explosion, an excessive use of insecticides and pesticides and other ecological changes have led to the increasing population of mosquito species in the state. Nobody has done collection surveys and studied Culicidae diversity for the last 70 years or so in a proper way. The knowledge on vector species is scanty.

Keeping in view the above mentioned situation, intensive and extensive collection surveys have been carried out to study the Culicidae diversity. As many as 40 species referable to 15 genera have been collected and studied in detail. An effort has been made to collect the data on temperature, humidity, rainfall, prevalence of species in order to study various kinds of variations among mosquito species. Our study concluded that many species have completely disappeared whereas many have made their way in this prosperous state of North India during the last 2-3 decades.

Role Of Climatological Factors On Dengue Transmission In India

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Climate change is occurring as a result of an imbalance between incoming and outgoing radiation in the atmosphere. These conditions help insect vectors to become more active at higher temperatures. High Temperature is an important determinant of biting rate, mosquito development, influence the development time of virus in the mosquito and survival at all stages of the mosquito life cycle. Dengue is a widespread vector borne disease globally and the interaction between vector, host and parasite are influenced by various climatic factors and this association was less explored in India. For the past ten years, the number of dengue cases has gradually increased in India. In the present study, we focused on the extrinsic incubation period and its variability in different climatic zones of India followed by assessment of the association of climatic factors with dengue through distribution of lag non-linear models. The study observed that dengue cases follow a strong seasonal trend as both temperature and rainfall were positively associated with dengue cases. The temperature is important in virus development in different climatic regions and may be useful in understanding spatio-temporal variations in dengue risk. The risk of dengue increases with increasing mean temperature above 24°C. The highest risk of dengue can be observed at 30°C with 0 to 3 weeks lag. Similarly, the risk increases more than two-fold when a minimum temperature reaches 26°C with two weeks lag period. The precipitation shows the high risk of dengue was observed between 8 to 15 weeks. The weekly cumulative rainfall of 60 mm with 12 weeks lag shows highest relative risk compared to 40 and 80 mm rainfall. The outcome of this study helps to develop a climate-based disease forecasting model to help the public health officials to reduce the disease burden.

Timing of Vector Emergence and Bird Movement on Malaria Transmission With Climate Change

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Global climate change is shifting the range of hosts and parasites at varying spatial and temporal scales-exposing host populations to longer transmission period, and increasing the risk of disease transmission to individuals without adaptive immunity. In the context of malaria, birds provide an ideal model system from a "One Health" perspective, to understand pathogen ecology and disease dynamics and help better model the links between climate change and health. Climate change is exacerbating the threat posed by avian malaria (*Plasmodium* spp.) by extending the area of suitable habitat for malaria-transmitting mosquitoes and causing declines in almost 7% of globally threatened bird species [e.g. Hawaii]. Therefore, identifying mechanisms that can mediate the spread of the disease could be crucial for both human health as well as wildlife conservation.

The degree to which vertebrate and invertebrate hosts in Himalayan region are exposed to avian blood parasites needs immediate attention. Birds harbour a huge diversity of haemosporidians (Apicomplexa: Haemosporida) that belongs to three main genera: *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. We conducted year-round sampling of *Culicoides* spp., vector for *Haemoproteus* parasites, across four sites across an elevational gradient in the western Himalaya. Using cytochrome oxidase gene, we barcoded *Culicoides* spp. and more than 20 species have been identified. The timing of *Culicoides* emergence and abundance (with negative binomial regression) varied significantly across sites. The emergence of *Culicoides* is driven by temperature and do not coincide with peak bird breeding season (April-May) suggesting a mismatch in phenology of vectors and hosts which determines the degree of interaction between host and vector species, thereby influencing the parasite transmission dynamics. We discuss the impact of climate-change on vector species which may undergo a geographic range shift and increasing the risk of exposure of naive avian hosts to novel parasites.

Recent Research Conducted At USDA On Passive Delivery Systems For Spatial Repellents.

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Increased emphasis has been placed on developing improved passive delivery systems for spatial repellents. Active ingredients being evaluated have focused on transfluthrin, metofluthrin, and various essential oils. Although mosquitoes are the major target species, efforts are also being directed on using spatial repellents against ceratopogonid biting midges and tabanids. Studies are being conducted under laboratory, semifield and field conditions.

Development Of Prolonged Action PMD (Lemon Eucalyptus Oil) Botanical Formulations To Provide 12 Hours Protection Against The Aedes Aegypti Mosquito Vector

Professor John E. Moses

La Trobe Institute For Molecular Science

Can modern pharma prolonged action technologies extend the longevity of repellency of botanical repellents to match or extend the protection offered by the "gold standard" DEET? The results of an ongoing 3-year bio-efficacy programme are presented including new dose response results for a novel Prolonged Action PMD rich botanical repellent versus increasing concentrations of DEET products.

Characterisation of Mosquito Repellents by Dose/Response Curve As Measured By Time to First Bite

Larry Goodyer

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The measure of 'Time to first Bite' is often used when conducting mosquito repellent cage tests to assess the longevity of an applied dose of repellent when compared to the same dosage as DEET. We describe a botanical repellent that shows different dose response characteristics to DEET and discuss the implications. Further characterization of the new compound with respect to its effective dose and persistence will also be presented.

Effective Pest Management In Agriculture And Vector Control Using Repellents

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An insect repellent is a chemical compound or blend of compounds that deters insect activity on or near otherwise attractive substrates. Although repellents have played an important role in personal protection against hematophagous arthropods—mostly contact repellents—and a large number of repellent semiochemicals, including some pheromones, have already been characterized for agricultural and forestry insect pests, repellents are not widely commercialized in agriculture or forestry. Also, there are virtually no spatial or areawide repellents available in the market, both in the health vector management and the agricultural sectors of the market. Repellents are labile semiochemicals that quickly vanish once applied, as conventional slow-release formulation technologies are often difficult to apply and/or inefficient in controlling the emission rate of the active ingredient. ISCA Technologies (Riverside, CA), together with collaborators from academic, government and private sectors, has been actively developing novel repellent formulations against several important vectors of disease and pest species. Here we will discuss some of parameters involved on the commercial development of safe and efficacious formulations using repellents to manage arthropods of importance.

The Effect Of Climatic Factors On The Mosquito Species Of Tribe Aediniin Chandigarh And Its Surrounding Areas

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Arbo-viral diseases are now considered to be the major contributors to global mortality and impermanence. Aedes borne diseases such as Dengue, Chikungunya, Yellow fever and Zika are an emerging health problem worldwide. The recent threats posed by these vector-borne diseases are highlighting the 21st-century problem across the globe. These are the most important arboviruses that are present in tropical and subtropical regions of India. Dengue has recently spread to many states including union territories. In addition due to the increased number of cases and severity of the vector-borne diseases, there has been a major shift in the geographical range of the disease. Out of all the mosquito species of tribe Aedini, *Ae. Aegypti* and *Ae. albopictus* are the most important vectors of some fatal diseases, such as Dengue, Yellow fever, Chikungunya and Zika. In the recent year (2017-18), these mosquito-borne diseases particularly Dengue and Chikungunya have become emerging and dreaded health problems in Chandigarh. Maximum number of cases of Dengue were observed during the peak months of mosquito prevalence. Several ecological, biological and social factors influence larval and adult population and transmission of these mosquito-borne diseases. The climatic factors mainly temperature, humidity and rainfall alter the distribution of vector species. Climate has been the official determinant of the epidemiology of vector-borne diseases. Climate variability and breeding activity of mosquitoes are important environmental contributors to transmission of Aedes borne diseases. Warm temperatures (21- 39°C) and high humidity favour increased longevity of the adult mosquitoes thus leading to faster and increased virus transmission intensity. Temperature is the key factor for the expansion of the mosquito population in the regions as it governs the reproduction, maturation rate and mortality rate of the long-legged creatures but rainfall also has a strong impact on the colonization of these mosquito species in regions as it generates breeding grounds for larvae and pupae of the deadly vector species.

Hence, keeping in view the influence of environmental variables on the distribution and prevalence of these vector species, an entomological survey was carried out in Chandigarh and its surrounding areas. Then mosquito identification was done with the help of recognized keys and catalogues. Two new records have also been found which were not reported by previous workers. The study will highlight the effect of climatic factors on the distribution of vectorial species and disease transmission.

Studies On Seasonal Impact Of JE Vectors With Respect To Various Meteorological Parameters In Different Ecological Habitats Of Chandigarh

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The mosquitoes are the most familiar insects under family Culicidae in order Diptera. They include 110 genera in subfamily Culicinae, out of which genus Culex is the most dominant one. It is further divided into different groups, subgroups and complexes by various workers. Several species of Culex are potentially competent in transmitting various deadly diseases like Filariasis, and Japanese encephalitis (JE), West Nile virus, etc. The Japanese encephalitis (JE) is an important cause of viral encephalitis which is prevalent in almost all states of India like Bihar, Uttar Pradesh, Assam, Manipur, Maharashtra, Goa, Tamil Nadu, Kerala, Haryana, West Bengal and Punjab. Various JE virus isolations have been made from 9 species of genus Culex in different regions of India.

Keeping in view, the medical importance of JE vectors of genus Culex, detailed entomological surveys have been conducted during premonsoon, monsoon and postmonsoon seasons spanning two-years to explore the mosquito fauna of Chandigarh. During present investigations, a total of 6112 mosquitoes of genus Culex belonging to 15 species have been found with four new records of JE vectors in this area. It has been observed that the impact of weather variables like temperature, relative humidity and rainfall directly or indirectly influence the abundance of mosquitoes distribution in urban and rural areas of the region. These meteorological parameters are further co-related with the seasonal prevalence and relative abundance of JE vectors in various ecologically divided habitats of Chandigarh. The results of the present study will provide information on the occurrence of vector species of Japanese encephalitis in Chandigarh and its adjoining areas which will be beneficial for the authorities so that appropriate measures could be taken in time for the control of these vectors.

Molecular ecology of *Aedes aegypti* in India: Elucidating genetic structure, dispersal potential and connectivity among populations

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Ae. aegypti is the mosquito species that transmits several arboviruses including dengue, Chikungunya, Zika, etc. In India, dengue is an important public health problem which has expanded both spatially and numerically over the past decade. Since mosquito control is the only option to contain the spread of dengue infections, the Indian authorities have started looking for novel area-wide mosquito control technologies. Genetically modified *Ae. aegypti* developed by Oxitec, UK and Wolbachia-infected *Ae. aegypti* mosquitoes developed in University of Monash, Australia, have already reached Indian laboratories. However, before releasing them in the field to test their efficacy under natural conditions, a good understanding of *Ae. aegypti* populations naturally existing in India is required. With this in mind, this study has been designed with two major objectives. First objective is to determine whether *Ae. aegypti* populations taken from different geo-climatic zones of India belong to homogenous gene pool or is a group of conspecifics (locally adapted and genetically diverged). Since India is a large country with a vast ecological and geographical variations, genetic isolation within *Ae. aegypti* populations due to local adaptation and eco-geographical barriers is expected. This information is important as the same mosquito control strategy may not be effective in distinct target populations and thus can interfere with the nationwide vector control programs. To test this, *Ae. aegypti* populations from different geo-climatic zones of India will be screened using a panel of genetic markers and the data will be analyzed to determine the pattern of genetic differentiation between populations and the factors responsible (geographic distance, eco-geographical barriers, demographic history) for it. The second objective is to determine the dispersal potential of *Ae. aegypti* mosquitoes within an urban setting. Hypothesis is, though *Aedes* mosquitoes can fly up to only few hundred meters human-aided dispersal is enough to homogenize geographically distant populations within a well-connected urban area. Using this information it may be possible to deduce i) how far the introduced/modified mosquitoes can move beyond the release site and ii) what are the landscape/anthropogenic (road, bridge, human density, water body) factors that affect the mosquito dispersal within a human-populated area. *Ae. aegypti* collection from different regions has been started and the collected mosquitoes are being processed in the lab for genotyping. The genetic data generated from this study will be analyzed to explore genetic diversity, population structure and extent of gene flow among *Ae. aegypti* population in India at varied geographical scales.

This study is important not only to predict the potential of novel genetic methods, it will also help in tracking the emergence and spread of insecticide resistance and other important biological characters like vectorial capacity, symbiotic bacteria, etc. Because if mosquito can disperse to long distances it can quickly spread deleterious as well as important mutations from one region to another.

Antimalarial activity of biosynthesized silver nanoparticles using *Jussiaea repens* leaf extract against malarial parasite, *Plasmodium falciparum*

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BACKGROUND: The utilization of various plant resources for the biosynthesis of metallic nanoparticles is called green nanotechnology, and it does not utilize any harmful chemical protocols.

RESEARCH HYPOTHESIS: The present study reports the plant mediated synthesis of silver nanoparticles using the plant leaf extract of *Jussiaea repens*, which acts as a reducing and capping agent. The aim of the present study was to assess the anti-plasmodial activity of synthesized AgNPs against the malarial parasite, *Plasmodium falciparum*.

METHODS: The obtained nanoparticles were characterized using UV-visible spectroscopy; EDX (energy-dispersive X-ray), SEM (Scanning electron microscope), XRD (X-ray diffraction) and Fourier transform infrared (FTIR) analysis. The efficacy of green synthesized AgNPs at different concentrations (25, 50, 75 and 100 µg/ml) were tested on *P. falciparum*.

RESULTS: Synthesized AgNPs particles were confirmed by analysing the excitation of surface plasmon resonance (SPR) using UV-vis spectrophotometer at 422 nm. The scanning electron micrograph showed structures of spherical, cubic shape, and the size range was found to be 40–60 nm. The EDX spectra showed the purity of the material and the complete chemical composition of the synthesized AgNPs. XRD study shows that the particles are crystalline in nature with face centered cubic geometry. The FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. The parasitic inhibition was dose-dependent. The synthesized AgNPs showed significant anti-plasmodial activity when compared to aqueous leaf extract of *J. repens*. The maximum efficacy (100%) was observed in synthesized AgNPs against *P. falciparum* at IC₅₀=100 µg/ml.

CONCLUSIONS: This study provides first report on the anti-plasmodial activity of synthesized AgNPs using *J. repens* against *P. falciparum*.

The toxic effects of *Persicaria hydropiper* prompted free radicals against the dengue fever vector *Aedes aegypti*(Diptera, Culicidae)

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Background: Mosquito borne diseases including dengue, yellow fever, zika fever infection remains a main source of sickness and death. The aforementioned diseases produce morbidity and mortality in human beings around the world. Personal protection is recognized as one of the affordable and easiest approach concerning the prevention of mosquito bites. Apart from the synthetic repellent, the mosquito nets and several other approaches have gained much more attention against the mosquito-borne disease epidemics since they can be used anytime and anywhere. However, frequent use of synthetic insecticides against *Aedes aegypti* vector mosquitoes has disturbed the biological control systems. This has also induced the undesirable effects on non-targets organism together with insecticide resistance in the mosquito population. Several botanicals offer great promise as sources of phytochemicals for the control of mosquitoes. Several studies have reported the applicability of natural vector control approaches, but very few studies have pursued free radicals induced larval death in *Aedes aegypti* larvae.

Methods: We examined *Persicaria hydropiper* induced free radical formation against the *Aedes aegypti* fourth instar larvae using ammonium molybdate method with slight changes. About 50g of *Persicaria hydropiper* fine powdered leaves were used for obtaining aqueous extract in a Soxhlet apparatus with water. 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml of *Persicaria hydropiper* water extract was tested against the fourth instar larvae of *Aedes aegypti*. Triplicates and control groups were maintained for all executed experiments. UV–Vis spectrophotometer was used for determining the optical density of the sample solutions.

Results: The *Persicaria hydropiper* water extract made significant modifications in the naturally produced reactive oxygen species against the *Aedes aegypti* fourth instar larvae. *Persicaria hydropiper* water extract generates excessive free radicals within the body of *Aedes aegypti* fourth instar larvae. Only few radicals were found to be present in the control group of *Aedes aegypti* mosquito larvae homogenate and distilled water. The significant result obtained from the present investigation provides novel insights in to natural mosquito vector control approaches. The *Persicaria hydropiper* water extract generated free radicals within the body of larvae responsible for the death of mosquito larvae.

Conclusions: The extensive use of medicinal plants with potent ability to generate free radicals in *Aedes aegypti* vector mosquitoes can be an important new tool for the mosquito-borne disease eradication programs.

Keywords: Vector control, *Aedes aegypti*, *Persicaria hydropiper*, Oxidative stress, Mosquitoes.

Analyzing the Diversity of Mosquito Vectors of Medical Importance Using Mitochondrial COI Sequence

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Background: The global trade and increase of travel together with the environmental and climatic change have upsurged the risk of the adaptations and introductions of mosquito vectors to new environments. The precise identification and analysis of genetic diversity of the *Aedes* spp., *Anopheles* spp., *Culex* spp. is essential to design approaches for managing mosquito-borne diseases. The current epidemics of mosquito-borne diseases in India, especially in Kerala have exposed the need for a detailed understanding of the genetic diversity of mosquito vectors in this region.

Methodology: The diversity and molecular identification of *Aedes*, *Anopheles*, *Culex* vector mosquitoes have been carried out using the mitochondrial COI sequence. The MEGA7 software was used for phylogenetic analysis and the genetic divergence was evaluated using the NJ-K2P method. SPSS was used for estimating the Shannon index.

Results: The phylogenetic analysis of mitochondrial COI sequence has revealed a distinct clustering of individual mosquito species within every genus along with strong bootstrap support. In total, our investigation effectively identified three species of *Aedes*, three species of *Anopheles* and three species of *Culex* vector mosquitoes. *Aedes albopictus*, *Aedes aegypti*, and *Culex quinquefasciatus* were distinguished as common and observed at all the three study regions.

Conclusion: This study used for the first time mitochondrial COI sequence to determine the genetic diversity and molecular taxonomy of vector mosquitoes collected from Nelliampathy, Kerala, India. The mitochondrial COI sequence-based phylogenetic analysis and genetic diversity determination offer reliability, and accuracy than conventional methods to reduce the harmful effects triggered by the awful diseases of mosquito vector origin.

Keywords: COI, DNA barcoding, Mosquitoes, Taxonomy, Phylogeny, Diversity.

Zinc oxide nanoparticles an ecofriendly arsenal to prevent dengue vector proliferation

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Mosquitoes are a key threat for millions of people worldwide, since they act as vectors for devastating diseases. Mosquito young instars are usually targeted with organophosphates, insect growth regulators and microbial control agents. Indoor residual spraying and insecticide-treated bed nets are also employed. However, these chemicals have strong negative effects on human health and the environment. Newer and safer tools have been recently implemented to enhance control of mosquitoes. The present study describes the cost-effective eco-friendly synthesis of Zinc oxide nanoparticles from *Eryngium foetidum* aqueous extract. The SEM analysis shows the synthesized ZnONPs are spherical and hexagonal wurtzite in structure. The FTIR spectra revealed the role of Chemical moieties in the formation and stabilization of ZnONPs. The *Eryngium foetidum* ZnONPs shows promising larvicidal efficacy and it shed light upon the role of bioactive compounds transferred from the plant during synthesis. The larvicidal efficacy of ZnONPs was very limited in the literature. The *Eryngium foetidum* ZnONPs may expect to serve as an alternative ecofriendly tool to control the spread of mosquito borne diseases.

To assess the efficacy of commercially available different mosquito repellents against *Aedes aegypti*

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Repellents are commonly used personal protection measures to avoid mosquito bites. Chemically based insect repellents can be natural or of synthetic origin. The most commonly used mosquito repellent DEET (N, N, and diethyl-3methylbenzamide) was discovered over 60 years ago and has been in use since the 1950's. In the present work, bioassays were conducted in the laboratory as per the standard WHO protocol to assess the repellency of commercially available mosquito repellants, viz. Advanced Odomos, Advanced Odomos Natural, Odomos Gel and Good knight Natural against *Aedes aegypti*. Their efficacy was tested on human volunteers applied with two different concentration (2.5 and 5mg/cm²). Advanced Odomos exhibit significant activity and further tested with 7.5 and 10mg/cm². Percent protection against *Aedes aegypti* exposed to human volunteers applied with four different cream formulations in cage bioassays in laboratory conditions were calculated.

A Smart Internet of Things Trap for Mosquito Vector Control

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Mosquito-borne diseases like malaria, dengue, chikungunya, encephalitis, and Zika have serious public health impact, and are being aggravated by Global Warming. India has an estimated 34% of global Dengue cases, and 11% of malaria cases. Mosquito traps are used for trapping mosquitos in the field and analyzing in the lab for control and surveillance purposes, and are increasingly used in homes also. The technology of the trap has largely been unchanged for several decades, with issues of energy efficiency and portability. The analysis, classification and counting of trapped mosquitoes is expensive and time- and labor-intensive.

In recent years, research on traps for automated identification has focused on use of optics and lasers, sensors for wingbeat frequency identification, etc. In this work, an inexpensive smart mosquito trap is designed and developed using Internet of Things (IOT) approach with computer vision and machine learning. The trapped mosquitoes are classified in situ, and the count data are automatically reported to public health authorities or research laboratory using GSM wireless communication. The data tagged with geospatial and date/time coordinates can be stored in a database in a central server for vector control purposes using statistical and analytics methods. Access to server trap data is provided using a mobile phone app.

The smart mosquito trap developed is meant for deployment in the field and for standalone operation in public spaces (schools, hospitals, etc.). The trapping mechanism is similar to a conventional trap: UV or LED lights are used as mosquito attractants, with a TiO₂ coating used for CO₂ generation, and a small fan is used to suck in and dehydrate the mosquitoes. The mosquitoes are captured on video with one or two low-cost USB webcam-microscope-light systems. The mosquitoes in the video/still images are classified and counted using computer vision software with template matching of standard mosquito images/keys. The OpenCV open source computer vision software is installed on on-board low-cost, open source Raspberry Pi micro computer. The mosquito data (numbers and types of mosquitoes, along with date/time, and location coordinates) are transmitted using a GSM module. The mosquito data from traps in different locations can be stored in a central server with public health authorities. The server data can be mined for possible infection spread, and trends in mosquito prevalence

For improved classification, mosquito image classifier is trained using Inception v3 network with transfer learning, a type of machine learning. The Inception v3 model is a huge image classification model with millions of parameters that can differentiate a large number of kinds of images. It is made up of many layers stacked on top of each other which are pre-trained. A new final layer is added to train the layer using the trapped mosquito images given by the user. A Python script with 4000 training steps is written to run the classification software.

The smart IOT trap was designed, developed, and successfully tested using images of trapped mosquitoes taken with USB uScope (200x magnification, 30 frames per second, 640 x 480 pixels). Multiangular views of different template species - from ICMR Centre for Research in Medical Entomology, Madurai - were used as training data.

The mosquitoes in flight can be analyzed in real-time, but the image processing and machine learning tasks are more challenging and are open problems for future study.

Dengue epidemics attaining Himalayan foothill district Bilaspur of Himachal Pradesh.

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Background:

Until the early 2000s, dengue in India had been mostly confined to the hot and humid regions of the country, but now due to climate change and unplanned urbanization, the disease has geographically expanded to cooler hill states such as Assam, Arunachal Pradesh and Mizoram. Sporadic cases of dengue are being reported from Uttarakhand, Jammu and some districts of Himachal Pradesh viz. Solan and Bilaspur etc. since 2015 and 2016 respectively. In July 2018 dengue epidemic was witnessed in the district Bilaspur. The preliminary visits to the epidemic area revealed that unplanned urbanization and drought has led to paucity of water which in turn compelled the residents to use water storage containers. In view of this an epidemiological investigation was carried out to recommend an appropriate control strategy and answer the question that "why dengue epidemic has occurred in the district Bilaspur".

Methods:

Climatic data of the district for last 30 years has been obtained from Krisivigyan Kendra and climatic factors responsible for epidemics were investigated. Entomological survey for *Aedes* was undertaken in three wards of PHC Diara and surrounding villages where dengue cases were clustered. A total of 120 households and their peri-domestic areas were surveyed. Indoor resting adult vectors were collected using hand catch method; immature stages of vectors were collected from containers by help of a ladle. Man-hour density and other entomological indices were accordingly calculated and vector species were morphologically identified.

Results:

In villages Sheola and Muhana, under PHC Diara, majority of the households were found to store water due to inadequate piped-water supply. Our visits during dengue epidemics revealed that most of the households have stored water in syntax tank and plastic containers. Of which, majority of such containers were found positive for immature stages of *Aedes*. The Breteau Indices (BI) ranged from 80 to 175 in the surveyed areas (BI >50 is indicative of a dengue outbreak). Other indices such as House Index (HI), Container Index (CI) and Pupal Index (PI) were also as high as 90, 40 and 170 respectively. Both the vectors of dengue i.e. *Ae. aegypti* and *Ae. albopictus* were field collected from houses in affected areas with high Man Hour Density.

Interpretation & conclusions:

The high BI and PI indicate that stored water in the containers is a forcing factor for breeding of mosquitoes. The unplanned urbanization and drought have led to paucity of water. The possibly due to climate change, the changes in the intensity and shifting of the rainfall pattern has added to existing woes of water shortage. This has led to a compelling situation where the residents start storing the water. The reported high entomological indices are the proof of successful establishment of vector population in the district. Introduction of dengue virus through infected person served as an index case, and the disease was picked up by the established vector species to cause sudden epidemic of the disease where there were no earlier indication of sustained dengue cases in the preceding years. The possible entry of the dengue virus into the transmission cycle that caused an outbreak has also become an enduring threat for following years as the virus has a transovarian path of transmission. In view of above findings it was highly recommended that control of both *Ae. Aegypti* and *Ae. albopictus* should be taken up by the responsible municipalities and the National Vector Borne Disease Control Programme (NVBDCP) in such areas. We are also interpreting the possible role of the climate change in the establishment of vector population in the Bilaspur region including the surrounding areas.

Vulnerability assessment of dengue outbreak and transmission suitability in Kangra district, Himachal Pradesh, India.

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Background: In recent past dengue has been spreading in newer and higher grounds every year including colder and sparsely populated hilly regions due to climate change. It has now spread to peri-urban and rural areas as well as in addition to primary vector *Aedes aegypti*, *Ae. albopictus* is also playing a major role as vector. All the state and union territory of the India are now infected by dengue and to prevent outbreaks of dengue infection in hilly areas where dengue spells are not felt, there was a need of field survey for assessment of risk of outbreak and to find out transmission window. District Kangra in Himachal Pradesh state of India is an important tourist destination and vulnerable to dengue outbreak.

Methods: Both biotic and abiotic factors viz. entomological survey for *Aedes* and other environmental parameters viz. rainfall, humidity, indoor, outdoor and water body's temperature and household construction, and population density were studied. Surveys were undertaken in 10 PHCs of Kangra district. Localities of sporadic dengue cases without travel histories within two weeks were also surveyed. A total of 194 households and their premises were surveyed. Indoor resting adult vectors were collected using hand held aspiration tube and a torch light, immature stage of vectors were collected from household containers and peri-domestic water collections in manmade and natural collections including tree-holes with the help of a ladle/dropper, man hour density calculation of adult vector collection, other entomological indices and identification of vector species were done.

Results: Observations in Kangra district showed that *Aedes albopictus* was found to be the most common species, followed by *Aedes pseudotaeniatus* followed by *Ae. vittaus*. Average household construction was good and most of the cemented houses were protected by wire netting in the entrances and windows. The MHD was low inside the bedrooms protected by netting. Peoples were using netting in the doors and windows to prevent entrance of mosquito and other insects to avoid nuisance but not exclusively for preventing any vector borne disease. The overall HI (House Index), CI (container index), BI (Breteau Index) and PI (Pupal Index) were 39.2, 21.9, 42.3 and 70.6 respectively. *Aedes albopictus* was more prevalent in peri-domestic water collections in manmade and natural collections including tree-holes and was the predominant species found in all localities.

Interpretation & Conclusion: Due to ample peri-domestic breeding habitat of tree-hole breeder *Ae. albopictus* and people's carelessness to lay scraps that can contain rainwater, profuse breeding of *Ae. albopictus* was observed during monsoons. It was observed that plastic containers have the highest container positivity followed by grinding stones while fridge tray, OHT and UGT were negative for *Aedes* breeding. Though people's knowledge towards prevention and control of dengue was low, majority of the household could afford to prevent mosquito entrance to their house to avoid nuisance which indirectly provided some protection against vector bites. High entomological indices during monsoon indicated increased potential risk of dengue outbreak and feasibility of transmission during the period. The risk of introduction of dengue virus was found to be high as tourist from all over the country and the world come to this tourist destination.

Insecticidal activity of Nanoemulsion formulation from essential oil against *Culex quinquefasciatus* Say (Diptera:Culicidae)

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The aim of present study was to prepare a novel essential oil based nanoemulsion formulation (NEF) pesticide in various ratios incorporate with essential oils, emulsifier, Stabilizer and water. The nanoemulsion composed of essential oils and emulsifier was formulated in different concentrations of the oil and emulsifier based nanoemulsion formulations (NEF). The efficacy of larvicidal and pupicidal effect of the formulated essential oil nanoemulsion formulation was evaluated against *Culex quinquefasciatus*. The O/W emulsion was prepared using with essential oils, emulsifier, Stabilizer and water. The nanoemulsion formulation was characterized by dynamic light scattering (DLS), UV-vis spectrum, and scanning electron microscopy (SEM). The lethal concentrations (LC₅₀ and LC₉₀) of the nanoemulsion against *Culex quinquefasciatus* was recorded after 12 h and 24 h for larvicidal and pupicidal at 12h. The nanoemulsion formulation (NEF) was found to be an effective larvicidal and pupicidal agent. It could be a good alternative insecticide as a potent larvicide and pupicidal for management of *Culex quinquefasciatus*.

Key words: Essential oils, *Culex quinquefasciatus*, nanoemulsion formulation, larvicidal, pupicidal

Field efficacy of VectoMax FG as a mosquito larvicide in Goa, India

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Bacillus thuringiensis israelensis (Bti) has been extensively used in the field for the control of Anophelines and Culicines in a variety of habitats of mosquitoes under National programmes worldwide.

In the present study, a new formulation i.e., VectoMax FG, a fine granule formulation based on combination of Bti, strain AM65-52 and *B. sphaericus*, strain ABTS-1743 was evaluated in the phases II and III field trials in natural habitats with *Anopheles stephensi* alone or its mixed breeding with *Culex quinquefasciatus* and/or *Aedes aegypti* in clean water habitats.

In clean water habitats (Curing Waters) against *An. stephensi*, the residual efficacy (>80% kill) was observed for 19 days, whereas in the rain waters on the terraces of new build under construction where both *An. stephensi* and *Aedes aegypti* were breeding the effectiveness was observed for xx days against these two species of mosquitoes.

These results shall be presented in detail.

Study of Larvicidal Activity of Brahmi

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Phytomedicine is the scientific medicinal investigation and application of plants for curing a disease or for supplementing a diet. Bacopa monnieri also known as Brahmi is a Sanskrit word derived from "Lord Brahma" or "Brahman" the creator God of the Hindu divinity. Brahmi literally means the power ("Shakti") of the lord. Bacopa monnieri is a well known and highly reputed Indian medicinal plant belonging to the family Scrophulariaceae. Bacopa monnieri (BM) is being extensively studied through research and literature and is used for the various ailments. Natural products containing Brahmi are much preferred as the suitable remedy and used as a neuropsychotropic drug. The plant is clinically being used for treating various disorders associated especially with CNS such as depression, anxiety, Alzheimer's, sleep disorders, cognition along with other disorders like gastric problems, and various other diseases. Bacopa monnieri is the well known plant and various studies are being carried out on it but, some aspects related to it are not completely discerned and need some clarity. The main aim of the study is to isolate and characterize the phyto-constituents from the aerial parts of Bacopa monnieri with the help of techniques like column chromatography, HPLC, LC-MS, NMR and IR. The isolated and characterized constituents were then subjected to docking studies so as to identify any activity against one of the most prevalent maladies of the mankind – vector borne diseases. A preliminary testing was done against the larvae of two different species mosquitoes - Aedes aegypti and Culex quinquefasciatus. The larvicidal activity of the extract gave promising results and high mortality rate of 84% and 88% respectively. Jujubogenin isomer of Bacopasaponin C (Bacoposide X) was identified as the major constituent possessing the activity from docking studies which is further corroborated from the analysis.

Integrated approach in malaria vector control

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Each year, an estimated 250 million people become ill with malaria, and nearly 1 million die. Every 60 seconds, an African child dies of malaria. More than 90 percent of the world's malaria deaths occur in sub-Saharan Africa. Malaria is a leading cause of death of young children in Africa. Malaria is a preventable and treatable disease. Integrated vector management (IVM) is a decision-making process for malaria vector control. The aim of the IVM approach is an efficient vector control process for achieving the global targets for vector-borne disease control in a cost effective & sustainable manner. Key elements of an integrated vector management (IVM) are advocacy, social mobilization and legislation, inter sector collaboration, integrated approach & capacity-building. Integrated approach element is one major element which addresses chemicals & non-chemical vector control tools. Vector control remains the most generally effective measure to prevent malaria transmission. The current malaria control strategy calls for the selection of those control measures which are most appropriate to local circumstances and capabilities and malaria risk. Vector control methods vary considerably depending on situation. Choice of vector control will depend on the magnitude of the malaria burden, the feasibility of timely and correct application of the required interventions and, most important of all, the possibility of sustaining the resulting modified epidemiological situation. Basic objective of integrated approach is reduction of human-mosquito contact by using Insecticide-treated nets, repellents, protective clothing, screening of houses; destruction of adult mosquitoes by Insecticide-treated nets, indoor residual spraying, space spraying, ultra low-volume sprays; destruction of mosquito larvae in peri-domestic sanitation by Larviciding of water surfaces, intermittent irrigation, sluicing, biological control; source reduction by environmental sanitation, water management, drainage etc. Any strategy should be responsive to changes in local ecological and epidemiological conditions. The issues to be taken into account in planning vector control are: the target vectors, the timing of implementation, the areas of implementation, the entities involved in implementation and the entities responsible for implementation and external monitoring and evaluation.

Use Of Information, Education, Communication (IEC) And Community Perception Study In Vector-Borne Disease Management

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The major vector-borne diseases together account for around 17% of the estimated global burden of communicable diseases (WHO, 2017). Amongst them, mosquito-borne diseases (malaria, dengue, chikungunya, Japanese encephalitis, yellow fever, Zika etc.) are responsible for 90% of the communicable diseases. Every year more than 700000 people are dying due to these communicable diseases (WHO, 2017) although these diseases are preventable, manageable and curable. 21st century is the age of information and communication technology and is playing a pivotal role in the development of knowledge economy and environmental health. Application of Information, Education and Communication (IEC) has thus gained importance and priority in the policy of inclusive management of vector-borne diseases. Keeping in view the above perspectives, applicability and suitability of various tools and techniques of IEC in order to generate scientific temper and awareness about vectors and diseases amongst the community will be presented. Communities play a major role in the success and sustainability of the vector control. A preliminary survey through a structured open-end questionnaire amongst the college students of humanities background of age group of 18-20 has also been conducted in 2017 in Kolkata, in order to perceive their Knowledge, Attitude and Practice (KAP) about Dengue and Malaria that are endemic to this region. Though respondents were aware that these two diseases are transmitted by mosquitoes yet only 22.6 % of them use mosquito nets at night. 54.8 % respondents were aware that dengue is a viral disease but only 18.2 % were aware that malaria is caused by parasites. This study reveals that emphasis should be laid by putting knowledge into practice by more aggressive health education campaign. This study would be helpful to formulate community based strategies for the prevention and management of vector-borne diseases.

A Review on Larvicidal Activity of Phytoextracts against the Major Dengue Fever Vector, *Aedes aegypti*.

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Since *Aedes aegypti* is considered as the major vector of dengue fever with which millions of people are infected per year, development of strategies to accomplish improved vector control without much interference in the environment composition are most desirable. As phytochemicals are now in the run for achieving this goal due to the destructive effects that are witnessed by the chemical insecticides around the globe, this review is an attempt to recognise the plant species and their larvicidal efficacy with their inhibitory action on the life cycle of the species of interest, that have been documented through various studies conducted till date. Here we also discuss the synergistic impact of a number of phytoextracts which will provide more efficient control measures for mosquito vectors. All of these studies are a search for a risk-free vector control tactic, which would also lead to the exploration of the splendid flora that we are blessed with to replace the current chemical insecticide application for the benefit of our nature.

Larvicidal efficacy of *Catharanthus roseus* and characterization of possible bioactive compounds against *Aedes aegypti*, a potential Dengue fever vector

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Background: Mosquito borne diseases remain as a main threat for the existence of mankind all over the world. Majority of the world population are in the clutches of these mosquito borne diseases like Dengue, Zika, Yellow fever, Chikugunya, etc. The emergence of resistance among these vectors against synthetic insecticide paves the way for the rapid progression of mosquito borne diseases. This urges the need for an innovative approach for vector control. Larvicidal efficacies of phytoextracts could be used as a biocontrol tool against conventional use of synthetic insecticide.

Hypothesis: Overuse of insecticides had a negative effect on both environment and mankind. This necessitated the need for a more eco-friendly and target specific control strategies. Larvicidal efficacy of phytoextract could be tested as an effective biocide against conventional synthetic insecticide.

Method: *Catharanthus roseus* were collected from the nearby areas of Thrissur district, Kerala, India. Crude leaf extracts of *Catharanthus roseus* was prepared. The phytoextract of *Catharanthus roseus* was then tested against laboratory reared fourth instar larvae of *Aedes aegypti* to detect larvicidal efficacy. In the later step, the extract was used to isolate alkaloids. The qualitative tests were conducted to reveal the presence of alkaloids in the test sample. Using Gas Chromatography the phytochemical constituents in the alkaloid were analyzed.

Result: The crude extract of *Catharanthus roseus* showed 100% larvicidal effect against *Aedes aegypti*. The alkaloid was extracted from this aromatic plant sample using petroleum ether, methanol and ethyl acetate. The extracted alkaloid from *Catharanthus roseus* is subjected to Gas Chromatography (GC). The GC result confirms the presence of single compound catharanthine alkaloid from the test sample.

Conclusion: The present study investigate and identifies the potential larvicidal activity of catharanthine a terpene indole alkaloid from the leaves of an aromatic plant *Catharanthus roseus* against dengue fever vector *Aedes aegypti*. The larvicidal property of this plant can be well utilized as an effective vector control measure. Due to the wide availability of *Catharanthus roseus* all over the Indian subcontinent, this plant extract can be used as an alternative botanical insecticide against synthetic ones.

Keywords: *Aedes Aegypti*, *Catharanthus roseus*, catharanthine

Knowledge, Attitude and Practice of Lymphatic Filariasis Disease among ASHAs in Warangal District (Erstwhile) of Telangana state.

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(This study was carried out under the guidance of Dr. Anuradha Medoju, Senior Regional Director, ROHFW, Govt. of India, Hyderabad)

Background

Lymphatic Filariasis (LF) is an endemic neglected disease in tropical and subtropical countries caused by (*Wuchereria bancrofti*). It is transmitted to humans by mosquitoes of the genus *Culex* (in urban and semi-urban areas), *Anopheles* (in rural areas of Africa and elsewhere) and *Aedes* (in islands of the Pacific). It was estimated that 120 million people world-wide are affected with Lymphatic Filariasis and 1 billion are at risk to acquire during their lifetime. Since the human beings are the only reservoir of *Wuchereria bancrofti* infection that causes 95% of pathology, mass drug administration (MDA) has been launched for the effective elimination of infection/ interruption of transmission by administering antifilarial drugs like diethylcarbamazine (DE and albendazole. However, lack of knowledge with regard to the disease and prevailing attitudes and perceptions towards this programme is resulting in lower MDA compliance. Hence, the present study was aimed to assess the Asha's perceptions and knowledge with regard to causation and transmission of chronic manifestations of LF in erstwhile Warangal District.

Methodology

A total of 68 participants were interviewed in Warangal District (erstwhile) of Telangana State where the MDA programme is still in persistence. Social-economic status of the individuals such as education, area of living, Symptoms of LF, transmission of Disease, problems faced by Lymphedema patients, knowledge about MDA, Morbidity management, mosquito breeding sites, protection from mosquitoes, control of mosquitoes and other related information were collected using specific pro forma questionnaire duly cross checked by public health specialist. Statistical analysis was performed using SPSS software (20.0 version)

Results

Out of 68 participants, 54 (82%) of the persons were aware of LF and 12 (33%) of the persons were aware as LF as a communicable Disease, 64 (97%) of the persons known that LF is transmitted by mosquito, 55 (83%) of the persons aware the source of mosquito, 36 (55%) of the people were fully aware of the effected parts of LF, 34 (51%) of the people were fully aware of the symptoms of LF, 64(%) of the people were aware of the problems faced by patient suffering from elephantiasis, 64 (97%) of the persons were aware of Drug schedule of MDA, 26 (39%) of the people were aware 12 days treatment by DEC and Nil knowledge is the outcome in the study with regard to on Morbidity management like types of Lymphedema management involves components.

Conclusion

The basic knowledge of the disease needs to be improved for the successful elimination of the programme. Effective training on treatment, Morbidity management activities are essential to ASHAs. The Morbidity management which happens to be the 2nd pillar as per the ELF guidelines are required to

Assessment of knowledge about malaria among community

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Introduction: Malaria is an ancient disease in the world with 0.6 million deaths annually and 40% of world's population is at risk of malaria. Decrease in malarial mortality and morbidity is important to meet the overall objectives of reducing poverty and to achieve these target it is essential to have active community participation in the programme activities. Lot quality assurance survey (LOAS) methodology has been used extensively for monitoring success of health programmes at the health centers and at the community levels.

Method: Using Lot quality assurance method survey was done in six zones of Surat city. From each zone, we selected 19 different areas which had high malaria incidence in 2017.

Results: At community level, 94.74 % peoples were aware that malaria is transmitted by mosquito bite, 72.93 % peoples knew that mosquitoes lay eggs in water, 96.99 % peoples knew the symptoms of malaria and 95.49 % peoples knew how to prevent malaria.

Conclusion: Knowledge about where mosquitoes lay eggs is relatively poorly understood even in urban area. The current global malaria elimination campaigns lays emphasis on the strengthening of health systems in developing countries. In order to realize the benefits of this approach, it is also vital to invest in the 'people' component of health systems and the community awareness and understand the multi-level factors that influence their participation in the programmatic activities. The success of the programme depends on local health priorities and conditions, and the development of effective information, education and communication package to support behavioural interventions which require good community based data and understanding of the local socio-cultural context about malaria. This information was used to develop a BCC strategy for implementation in Surat city of Gujarat.

Molecular characterization of Glutathione –S-Transferases epsilon 4 gene of *Anopheles stephensi*

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Background: The emergence and progression of insecticide resistance in mosquitoes is creating major challenges for the control of vector borne diseases. Amongst various mechanisms for insecticide resistance; metabolic resistance is the main mechanism in which quicker detoxification of insecticide in resistant mosquitoes occur compared to sensitive one. Glutathione – S – Transferase (GST) class of enzymes are crucial component for metabolic resistance. Over expression of epsilon 2 and 4 class of GSTs was reported earlier in insecticide resistant population of few Anopheline species. However molecular mechanism behind over expression of GSTe4 gene in case of metabolic resistance in mosquitoes is yet to be explored.

Methods: Complete cDNA sequence of DDT and pyrethroid resistant *A. stephensi* mosquitoes was synthesized from total RNA. Further GSTe4 gene was amplified with gene specific primers and cloning was done. Afterwards Sanger sequencing was performed to analyze the sequences of these recombinant products.

Results: Full length coding sequences of GSTe4 gene was found to be 675bp which encodes 224 – amino acid protein. Further Molecular characterization revealed point mutations at 3 different positions which leads to the emergence of various isoforms of GSTe4 gene. These isoforms arise due to change in amino acid at three positions i. e. A89S, K180N and R213L.

Conclusion: Overexpression coupled with appearance of various isoforms of GSTe4 gene in insecticide resistant population of *A. stephensi* mosquitoes would play crucial role in metabolization of insecticides. Ultimately these alterations may confer the elevated level of insecticide resistance and role of various isoforms needs to be explored.

Molecular characterization and identification of *Anopheles sundaicus* from Myanmar

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Anopheles sundaicus s.l. is a major malaria vector, primarily in coastal areas of mainland and islands of Southeast Asia. Reportedly, *An. sundaicus* s.l. is a complex of four sibling species *An. sundaicus* s.s., *An. epiroticus*, *An. sundaicus* D and *An. sundaicus* E. However, specific status of sibling species of *An. sundaicus* found in Myanmar is not known. Dead mosquitoes were obtained from Myanmar which was morphologically identified as *An. sundaicus* were subjected to DNA sequencing for ribosomal internal transcribed spacer-2(ITS-2). Alignment of ITS-2 sequences of Myanmar revealed 100% homology with the *An. epiroticus* s.s. of the Sundaicus Complex. This is the first report of *An. epiroticus* from Myanmar.

Spatial distribution of Haemaphysalis species ticks and human Kyasanur Forest Disease cases along the Western Ghats of India, 2017-2018

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Background: Kyasanur forest disease (KFD) is a viral haemorrhagic fever; transmitted to humans and other hosts by a tick vector of genus Haemaphysalis. It affects 400-500 people annually in the Western Ghats region of India through spring to summer season with a case fatality rate of 3-5%. In recent times the disease incidence has expanded geographically across different regions of the Western Ghats in India (KFD is now endemic in five states). The seasonal outbreaks are in relation to the vector tick activities. Therefore, an entomological surveillance for ticks was conducted to understand the relationship between species composition, distribution, and abundance of Haemaphysalis ticks with KFD occurrence among humans.

Methodology: The study was conducted between October 2017 and January 2018 in endemic taluks (Sub-districts) of India. A total of 105 sites were selected based on grid sampling from five taluks representing five KFD endemic states in south India. One epidemiologically suitable site in each grid was surveyed for host-seeking ticks by using standard flagging method. The ticks collected were species identified and pooled for nucleic-acid extraction. The extracts were screened for NS-5 gene of KFD virus using Real-time RT-PCR technique. Further, the *H. spinigera* abundance was categorised and compared with the incidence of human KFD cases during the same season.

Results: A sum of 8373 ticks were collected during the study period. The study showed a wide distribution of host seeking tick species among the selected taluks, wherein *H. spinigera* was predominant in 3/5 taluks, *H. bispinosa* at 1/5 taluks, and both the species at 1/5 taluks. Since the number of *H. spinigera* in all of the first quartile were 0, the grids identified with 0 *H. spinigera*/man-hour were considered as less abundant. The second quartile was considered as moderately abundant (i.e., 1-11), the third quartile as high (i.e., 12-41), and the fourth quartile as very high (i.e., >41).

Discussion and Conclusion: In three out of five sites studied in the Western Ghats region of India, we identified *H. spinigera* (Primary vector of KFD) as the dominant host-seeking tick species. All the three sites had Lab confirmed human KFD cases in the season. During the study period only 23 out of 105 grids studied had reported human KFD cases. In which the grids with very high and high *H. spinigera* abundance had 69.75% of the human KFD cases reported. Hence, this method of tick surveillance could be efficiently used as a standard model for KFD transmission risk assessment and prediction of impending outbreaks.

Malaria Control in Kozhikode District of Kerala

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Malaria was a rural problem in Kerala in the past but the state is now facing the problem of 'urban malaria'. *Anopheles stephensi* is the major vector responsible for the urban malaria problem in Kerala. There has been phenomenal increase in the incidence of malaria in the last three years in Kozhikode. A total of 168 cases and 1 death have been reported from the district during the period 2016. The malaria problem was mainly confined to the coastal belt on the western side of the district. The first malaria case of that year in the coastal belt was reported in a migrant labourer which spread to the other areas as high density of vector was recorded. An entomological survey conducted in different parts of the district revealed the presence of a large number of breeding places of *Anopheles stephensi* on the coastal areas of the district. The breeding places were comprised of over-head-tanks, cisterns, ground-level-tanks, cement-ringed shallow wells, discarded fishing boats, ice trays, plastic fish boxes etc. In order to tackle this problem, a full-fledged Integrated Vector Management (IVM) strategy was developed and implemented on a war-footing during 2016. This IVM strategy included IRS, anti-larval operations, introduction of guppy fishes in wells, mosquito-proofing of over-head-tanks and wells, space sprays etc. Regular entomological surveys were carried out to assess the adult vector density as well as larval density. The insecticide resistance monitoring was also done during the period. The results of the entomological surveys showed significant reduction in per-man-hour-density (PMHD) after the implementation of the IVM strategy. The insecticide resistance studies showed that *Anopheles stephensi* is susceptible to cyfluthrin, deltamethrin and lambda cyhalothrin. As a result of the implementation of the IVM strategy during 2016 to 2018, the indigenous malaria cases could be brought down from 22.02% in 2016 to 4.39% in 2018

Key words: Urban malaria, *Anopheles stephensi*, IVM, insecticide resistance.

Epidemiological trends in Malaria occurrence- A Retrospective study through Kerala Sectors

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Background: Malaria is one of the most serious parasitic diseases in most of the developing countries. It continues to be the major health concern in India because of its massive progress, reemergence and drug resistance. Even though Kerala is a low incidence zone, the reintroduction of parasites makes the state vulnerable to disease outbreaks.

Research Problem: The present retrospective study was designed to evaluate the trends in malaria prevalence during 2014 to 2018 in three districts, Kasargod (North), Thrissur (Central) and Thiruvananthapuram (Southern) of Kerala. The northern and southern sectors have the similar geographic and cultural conditions as that of the neighboring Karnataka and Tamil Nadu states. The malaria occurrence of these sectors was compared with that of the neighboring states.

Methods: The annual malaria surveillance data of Thrissur and Thiruvananthapuram sectors were collected from the National Vector Borne Disease Control Programme of respective districts. The surveillance data of the Kasargod district was obtained from the Integrated Disease Surveillance Programme. The entire data were collected in de-identified manner, during regular intervals. The data were analyzed using MS-Excel 2008 (Microsoft, Redmond, WA, USA) and SPSS 20 (IBMCorp, Armonk, NY, USA).

Results: During the five- year study period, the predominant occupancy of malaria was observed in the Kasargod district, the northern sector, which was directly influenced by the high prevalence of malaria condition in the Karnataka state. There was a decreasing pattern observed in the recent malaria occurrence in all the three study sites. Compared to the other sectors, Thrissur district showed a consistent downward trend in the incidence from 0.0471/1000 population in 2014 to 0.0261 in 2018. Between the assessment times, a total of 569 cases were reported in the central sector, of which 394 were Plasmodium vivax cases, 99 were dreadful Plasmodium falciparum cases. The annual blood examination rate of the three study sites remained almost unchanged during 2014- 2018. The northern site showed reemergence of malaria condition from 0.185/1000 population in 2015 to 0.226 in 2016 and southern site incidence exhibited a substantial uphill from 82 confirmed cases in 2014 to 135 in 2015.

Conclusions: Our analysis revealed annual decline in recent malaria cases. There were evident variations in the recorded diagnosis and prevalence of malaria conditions between different study regions of the state. It showed the reemergence of dreadful P. falciparum cases in various declined regions. The study also flashed light on the influence of neighboring state in the existence of predominant malaria incidence in the northern study sector. A sharp surveillance system, effective screenings, more governmental initiatives and other multiple approaches are vital for the complete eradication of the parasitic condition.

Upregulation of carboxylesterase gene (COE) in Deltamethrin resistant *Anopheles stephensi*

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Pyrethroid group of insecticides are currently choice of insecticides and only class of insecticide recommended for Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) for the control of malaria vectors due to rapid killing action, low mammalian toxicity and degradability in nature. *Anopheles stephensi* is one of the major malaria vector found throughout India, Pakistan and Iran. In India, it is regarded as urban malaria vector. Although there is no report of resistance in *An. stephensi* so far against pyrethroids, we attempted to understand the molecular basis of insecticide resistance in laboratory colonies of *An. stephensi* which were selected for deltamethrin resistance by exposing them low dose of insecticide over generations. We report evidence of upregulation of carboxyl esterase gene in deltamethrin resistant strain of *An. stephensi*. Carboxyl esterase (COE) are important class of detoxification enzymes known to play a major role in resistance against organophosphate and carbamate group of insecticide, but in few recent studies, few esterase enzymes such as mixed function oxidases and beta esterase are found upregulated in *An. arabiensis* against permethrin by metabolizing the insecticide at a higher rate in mosquitoes. To understand the role of carboxyl esterase gene against pyrethroid, overexpression of this gene was monitored by synthesis of single stranded cDNA (using oligo-dT primer) from total RNA of susceptible and laboratory selected deltamethrin-resistant strain of *An. stephensi* mosquito and a quantitative real-time PCR was performed by using gene specific primers. Quantitative real-time data shows that the relative amount of COE transcript was significantly higher in the deltamethrin-resistant strain of *An. stephensi* as compared to the susceptible strain. Further study to characterize this gene in deltamethrin resistant and susceptible strain is under investigation as this gene is considered a potential target to understand the mechanism of pyrethroid resistance that may help in effective integrated vector management.

Development of multilocus neutral DNA marker in an Indian *Anopheles culicifacies* sibling Species

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Plasmodium is one of the most successful parasites ever known to mankind. After thousands of years, malaria remains the world's most pervasive infection, affecting at least 91 different countries and some 300 million people. In India the major vector of malaria is *Anopheles culicifacies*. *Anopheles culicifacies* contributes to about 60–65% of all malaria cases of India. It is species complex of five sibling species A, B, C, D and E. Vector control, an essential component of malaria control, has become less effective in recent years. *An. culicifacies* has developed resistance to DDT in 286 districts and to DDT and malathion in 182 districts of India. Resistance to SPs has also reported from some areas. Due to development of resistance in *An. culicifacies* the vector control program is less effective. Considering malaria can be a fatal vector-borne infectious disease, inference on population genetic structure and demography could be of help in the long run for malaria vector management and control. Using the published genome sequence information of *Anopheles gambiae* we designed EPIC primers to amplify DNA fragments in *An. culicifacies* species A and B nuclear genome. For this, we scanned the whole *An. gambiae* X-chromosome and selected introns of length 300–700 bp, present in orthologous genes. Thirty such primer pairs were tested for amplification in *An. culicifacies* genome. Apart from these, 20 primer pairs earlier developed in *An. gambiae* (Stump et al., 2005) and one developed in *An. minimus* (Sharpe et al., 2000) were also tested for PCR amplification in Indian *An. culicifacies*. Out of 51, four markers were successfully amplified and sequenced in both sibling species. All the four DNA fragments were found to be polymorphic for single nucleotide polymorphisms (SNPs) in a population sample of *An. culicifacies* species A and B from India. Several tests of neutrality have been done. The four multilocus nuclear DNA fragments thus could be considered as 'putatively neutral' and be used to infer population structure and demographic history of *An. culicifacies* species A and B.

Biocontrol of *Aedes aegypti* larvae by the fungus *Penicillium citrinum*Deeparani K. Prabhu¹, Ashwani Kumar¹ & D. Jayarama Bhat²

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The resistance of vectors to chemical insecticides and the damage to the environment make biological control of mosquito populations an attractive alternative. *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Diptera: Culicidae), the principal vector of dengue and yellow fever (Flaviviridae) as well as Chikunguniya (Togaviridae) viruses was susceptible to the conidia of *Penicillium citrinum* Thom, an anamorphic fungus. The fungus was isolated from 2nd instar larva of *Anopheles* sp. collected from curing water at a construction site in Panaji, Goa, India. The fungus was studied in culture. The larvicidal efficacy of fungal conidia was evaluated by performing in vitro bioassays against the 3rd instar larvae. The LD50 value obtained against *Ae. aegypti* larvae was 80.13 x 10⁶ spores/ml at 24 h and 66.03 x 10⁶ spores/ml at 48 h respectively.

The difference in mortalities in *Ae. aegypti* larvae was not significant between the 3 replicates/dose ($F = 1.571$ $p = 0.230$), while it was highly significant between the 4 doses ($F = 55.755$, $p = 0.00$).

Targeting vector salivary proteins for combating disease: A Computational Biology Approach

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Conventional approaches currently being used for vaccine development are cumbersome that can take decades for locating a region which can initiate a significant immune response. Therefore, developing vaccines against vector borne diseases to prevent their outbreak has not been possible. "Vaccinomics" is an in-silico approach to design epitope based peptide vaccines which has become possible in the recent years due to sequencing of genomes of various organisms and development of protein sequence databases. Novel immuno-informatics based approaches can be used to design vaccines not only against disease causing pathogens but also the vectors carrying these pathogens. Lyme disease caused by bacteria *Borrelia burgdorferi* is a tick borne disease spread by *Ixodes persulcatus*, *Ixodes ricinus*, *Ixodes scapularis* and has a world-wide frequency of 365,000 per year. In the present study an in-silico informatics-based approach was used to design an epitope based vaccine against the salivary proteins of the tick, which acts as a vector for a number of bacteria and viruses, so as to elicit an immune response to prevent the entry of the parasite into the human host. Salivary gland of vectors such as ticks and mosquitoes carry out various activities not only for the survival of the vector but also for effective blood feeding which results in transfer of the pathogens to human hosts. The antigenicity and immunogenicity of the salivary proteins affects the infectivity of parasite. Salivary proteins of tick were retrieved from NCBI protein database and analysed using various in-silico tools. VaxiJen was used to identify and score antigenicity of the sequences and T-cell epitopes were analyzed using NetCTL server followed by predication of the structure of the vaccine construct. Finally protein-protein docking of the peptide vaccine and the human host protein TLR having affinity for parasite ligands was carried out to evaluate the binding efficacy of the vaccine. The validation of the prediction is expected to be done by the wet lab researcher for translation of the research from bench to bedside. Vaccinomics based in-silico approaches hold much promise in the timely development of new vaccines that is critical in recent times in view of an ever rising global burden of disease.

Proteogenomic Approaches To Investigate Mosquitoes

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Proteogenomics involves the use of proteomic information to curate the genomes. Peptide data derived from high-resolution mass spectrometry is invaluable for increasing the accuracy of genome annotations. The successful outcome of such analyses includes identification of novel genes, novel exons, novel initiation codons, exon extensions, intronic genes, and cSNPs. We carried out a systematic effort to apply proteogenomic concepts to annotate the proteomes of *Anopheles gambiae*; *An. stephensi* and *Aedes aegypti* on a high-resolution Fourier transform LTQ-Orbitrap Velos ETD mass spectrometer. As with most genomes, the exact number of protein-coding genes in any given species will remain as a matter of debate until the computationally predicted genes are validated by any method. We carried out transcriptome sequencing and deep proteome profiling of multiple organs of these mosquitoes. In addition to validating a large number of computationally predicted genes in these mosquito genomes, we added a large number of novel protein-coding genes and corrected some predicted protein sequences. Evidence at proteomic level enabled us to revise the status of several predicted 'non-coding RNAs' to conventional mRNAs coded by protein-coding genes. We also extended novel genomic events identified in these genomes to improve the assembly and annotation of other publicly available Anopheline genomes.

Key words: malaria, dengue, vector mosquito, bioinformatics

Tissue-specific splicing events in Indian malaria vector, *An. Stephensi*

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Genome sequencing of *Anopheles stephensi*, a major malaria vector in Asia, has accelerated research to understand its vectorial ability at the molecular level. The diversity and complexity of the cells and tissues comprising an organism although dictated by the same set of genes, it is regulated in part by their expression. The diversity is further enhanced at the transcript level considering the post transcriptional modifications, including alternative splicing. Tissue specific profiles of these transcript variations – at the level of spliced forms and expression helps in understanding the molecular differences in these tissues and probable functional implications. In this study, we summarize the transcriptomic profile of four important organs of an adult female *An. stephensi* mosquito – midgut, Malpighian tubules, fat body and ovary. In all, we identified over 21,000 transcripts corresponding to more than 12,000 gene loci from these four tissues and provide the tissue-based expression profiles of majority of annotated transcripts in *An. stephensi* genome and dynamics of alternative splicing in these tissues. Understanding the transcript expression and gene function at the tissue level would immensely help in enhancing our knowledge of this important vector and decipher the putative role of these tissues. This knowledge would in turn provide the basis of selection of candidates for future studies on vectorial ability.

Proteins associated with host response in *Anopheles stephensi* during infection by *Plasmodium berghei*

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Introduction: *Anopheles stephensi* is a primary vector of malaria in India. Mosquito midgut is the first site of vector-parasite interaction. It is now known that several midgut proteins are involved in recognition and subsequent development of malaria parasite in the vector mosquito. However, a systematic quantitative proteomic analysis has not been carried out to identify mosquito midgut proteins involved in vector immunity and parasite-vector interaction.

Methodology: In this study, 3-plex quantitative iTRAQ labeling approach was used to study differential expression of proteins in the midgut of female *Anopheles stephensi* after feeding 3 separate batches on sugar, uninfected blood and *Plasmodium berghei* infected blood.

Results and Discussion: Mass spectrometry analysis of iTRAQ labeled peptides led to identification of 2,327 proteins out of which 55 and 204 proteins were over-expressed in *Plasmodium berghei* infected mosquito midguts as compared to blood fed and sugar fed mosquito-midguts, respectively. Another 176 and 290 proteins were down regulated in *Plasmodium berghei* infected midguts as compared to the blood fed and sugar fed midguts, respectively. These differentially expressed proteins were found involved in blood meal digestion, sporogonic development or inhibition of malaria parasite development. These proteins are attractive candidates that can be pursued for their role in parasite invasion, inhibition or survival.

Conclusions: The proteins identified in this study may serve as an important starting point for further targeted studies in the context of their potential role in malaria transmission.

Midgut and Salivary Gland Proteomics of *Aedes aegypti* Linn, Protein-Protein Interactions & Involvement in Various Pathways

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Aedes aegypti (Linnaeus, 1762) is a vector mosquito species belonging to Phylum Arthropoda, class Insecta, order Diptera and family Culicidae. It is medically one of the most important mosquito, being the primary vector of Dengue, Chikungunya, Yellow fever and Zika. Salivary glands and midgut are two largest and most important organs in *Aedes aegypti* from the point of view of transmission of disease agents.

In this study, using high-resolution mass spectrometer, analysis of 18 fractions of *Aedes aegypti* proteome resulted in acquisition of 83,836 peptide spectra from salivary glands sample and 1,16,773 peptide spectra from midgut samples. 1208 proteins from the salivary gland and 2277 proteins from midgut were identified. Thus a total of 3485 proteins were catalogued from *Aedes aegypti*. To the best of our knowledge, this is the largest catalogue of proteins from *Aedes aegypti* so far.

Out of the total 1208 salivary proteins identified in this study, 402 (33.27%) proteins were found to be involved in one or more metabolic pathways as described for *Aedes aegypti* in KEGG Pathway. There is a representation of 59 metabolic pathways in the dataset of salivary glands while in midgut proteins there is a representation of 110 KEGG metabolic pathways with involvement of 709 unique proteins in multiple pathways. 29 proteins from the salivary gland dataset and 41 proteins from the midgut dataset of this study were found listed in ImmunoDB dataset. This implies that not only they play role in the immune responses of *Aedes aegypti* but also suggests that such responses perhaps originate from or are localized in these organs. A total of 70 immunogenic proteins have been identified in this study.

The results of our study have been able to not only characterise the proteome of these organs but also delineate certain important roles that these proteins play in immune response, metabolism, protein-protein interactions and transmission of *Aedes aegypti* borne diseases. They provide novel targets for devising new interventions against this vital species which is posing threat to the public health across the globe.

Uncovering Genetic Relationship of Mosquito-Microbe-Parasite Interactions and Malaria Transmission in Indian Malarial Vectors

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The evolution and adaptation to blood feeding behaviour is key to the success of the adult female mosquitoes to adapt, feed and support transmission of many pathogen. Thus, to track and crack the secretes of the mosquito success is prime important to identify novel molecular targets to design novel strategies for vector control. Indian populations, especially residing sub-urban and rural areas, are one of the major victims of these vectors borne disease, mainly malaria and dengue. Two mosquito species *Anopheles culicifacies* and *A. stephensi*, which have been identified as major malaria vectors in India, transmitting more than 65% rural and 17% urban malaria, respectively. At NIMR-ICMR we have initiated an integrated meta-transcriptomic research program focused to decode and identify key genetic factors, regulating complex biology of (i) host-seeking and blood feeding behavior; (ii) immunity and resistance to pathogen; and (iii) reproductive success in Indian malarial vectors. We utilize high throughput multi-tissue RNAseq based transcriptomic and metagenomic studies to catalogue; identify unique mosquito tissue specific transcripts and/or dominant endosymbiotic bacteria affecting distinct biology mosquito immunity, parasite development and its transmission in Insectary reared mosquitoes.

In our recent studies focussed on decoding blood feeding associated molecular complexity of neuro-olfactory regulation and salivary gland responses, we demonstrated that (a) adult female mosquitoes are evolved with unique ability of salivary gene expression switching to manage meal specific responses (<https://atlasofscience.org/how-adult-female-mosquito/>); (b) mosquitoes hijacked large scale genes from plants useful for their survival in diverse ecologies (<http://www.pr.com/press-release/669820>); (c) salivary glands harbor more diverse microbial community than gut in the mosquito *An. culicifacies*. An ongoing RNASeq analysis further demonstrate that a smart molecular relationship with individual tissues such as midgut, hemocyte, salivary glands, and a strategic genetic makeup changes favours *P. vivax* survival in the mosquito host. Importantly, our data unravel that a direct interaction of hemocyte with free circulating sporozoites significantly modulates the hemocyte specific global expression in a way to impart hyper immune response for rapid clearance of vast Sporozoite population. A transcriptional profiling data on salivary glands further suggested that for its survival, the invaded Sporozoite restricts metabolic responses, possibly to counterbalance the salivary defense, till it passes to the next host.

We believe future experimental studies clarifying tissue specific molecular and functional relationship against microbial pathogens such as *Plasmodium vivax*, virus may be valuable to identify novel molecular targets to design new molecular strategies for vector borne disease management.

Vectors monitoring & control using Bogorchid (Smart Machine) and application of cutting edge technologies to protect our communities from vector borne diseases & their epidemics

Hemachandra Bhovi

MGH Labs Pvt Ltd (IIT Madras Bio Incubation Company), A9, First Floor, IIT Madras Research Park, Kanagam Road, Taramani, Chennai -600113, Ph# +91 044 33870034 / 9886728680 www.bogorchid.com

MGH Labs Pvt Ltd is a "Startup" company incubated at IIT Madras Bio-Tech Incubation center. MGH Labs has innovated Bogorchid which is a smart machine that Attracts, Catches & Kills mosquitoes naturally using pheromones which are non-toxic. The machine identifies & attracts vectors in homes as well as in communities & eradicates them for good. The machine applies multi-domain skills & deep technologies to achieve the goal. Machine Learning & Artificial intelligence is used to adopt the machine for various real time dynamics of vectors & their changing behaviors. Data analytics is used to extract information from varied parameters like spot data from the machine, weather, historical data of the geography, vectors species & their historical behaviors in similar terrains etc to forecast any potential epidemics and take appropriate preventive measures in such a way that people are safeguarded from any vector borne epidemics. The product is tested and certified for various product qualities both in terms of quality & non-quality. Several thousands of individual customers have benefitted so far & continuing the same and few corporate customers are leveraging the same!

HIL's Forthcoming Vector Control Tools – Committed to Eliminate Malaria

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HIL (India) Limited (A Government of India Enterprise),
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Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Nets (LLINs) continue to be two highly effective vector control tools for reducing Malaria transmission in most affected regions – Africa and South East Asia. Amongst the available IRS chemicals, DDT continues to play a pivotal role in control and elimination of disease from many states in the country and worldwide. Moreover, LLINs alone are responsible for 69% of all the malaria cases averted by interventions in Africa since 2011. Selection of right vector control tool based on available entomological and epidemiological data is crucial for defining an effective vector control strategy for the region. The Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) in 2001 identified DDT as one of the 12 POPs and exempted it for production and public health use for indoor application for control of vector-borne diseases, mainly because of the absence of equally effective and efficient alternatives. India is one of the signatory to Stockholm Convention and the Country has expressed its commitment to reduce reliance on DDT as IRS chemical. HIL (India) Limited (Formerly known as Hindustan Insecticides Limited), a CPSU under Ministry of Chemicals and Fertilizers, Government of India, the sole manufacturer of DDT globally, has already taken certain initiatives for developing alternatives to DDT. In the process, HIL in association with Institute of Chemical Technology, Mumbai has developed isosteric form of DDT which is environmentally safe, user friendly with equivalent efficacy. The product is under registration phase in the country. Secondly, HIL is coming up with its own manufactured LLIN with the trade name of "HILNET" with improved technology. Both the products shall be made available at a reasonable cost to the National Vector Control Programme and to other Countries very soon. With renewed focus of Malaria Elimination by 2030 at Global (WHO's Global Technical Strategy for Malaria) and National level (Government of India's National Framework for Malaria Elimination), HIL (India) Limited, the only Public Sector Enterprise in Public Health segment, is committed to manufacture and supply quality products at reasonable cost towards achievement of set Malaria Elimination targets.

ICON® 10CS – Advanced micro-encapsulated insecticide for long lasting indoor residual spraying (IRS)

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Vector control through Indoor Residual Spraying (IRS) program is a highly effective strategy for reducing Malaria transmission and is a key preventative strategy highlighted in the Global Malaria Action Plan. In the 1980s, Syngenta had provided Icon®10WP for IRS which was very successfully adopted by malaria control programs across Asia Pacific, Africa and Latin America. Syngenta is committed to improving the technology available for malaria vector control. We have introduced the first capsule suspension (CS) formulation recommended by WHOPES (now PQ) for indoor residual spraying program (IRS). Icon®10CS is a micro-encapsulated formulation which improves upon the established Icon®10WP. Icon®10CS delivers long lasting residual performance and an enhanced safety profile to suit the requirements of modern IRS programs. Icon®10CS is equipped with high potent pyrethroid, Lambda-cyhalothrin and it's a broad spectrum insecticide at exceptionally low rate for control of mosquitoes and other pests of public health importance. In Icon®10CS, the lambda-cyhalothrin is encapsulated in polymer micro capsules which remain intact on the surface after spraying. The active ingredient remains protected from the environment within the microcapsules and this protection delivers a long lasting residual effect, particularly on challenging surfaces like cement and mud. Insects moving over the treated surface pick up capsules on their bodies. Once attached to the insect's cuticle, the active ingredient rapidly moves out of the capsule into the insect providing rapid knockdown and quick kill. Icon®10CS has been successfully evaluated by WHO Pest Evaluation Scheme (WHOPES) for IRS program for malaria prevention and control. Both studies done in Uganda and Malawi, wall bioassays indicated over 80% control Anopheles spp. up to 6 months on surfaces like mud and plaster. Field evaluation conducted by National Institute of Malaria Research in India also revealed that Icon®10CS formulation was relatively more effective than malathion and deltamethrin in WP formulation. Icon®10CS has an excellent toxicological and environmental safety profile which categorized under relatively low hazard as according to WHO guideline. When label recommendations are followed and appropriate personal protection equipment (PPE) are worn, there is negligible risk to applicator. Icon®10CS provide a broad spectrum, long lasting residual control with proven efficacy on various surfaces, cost effective (reduce frequency of spraying) and safe to use.

Insecticide Resistance in Indian *Aedes aegypti*

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In India, *Aedes aegypti* is considered the primary vector of several arboviral infections like dengue, chikungunya and Zika. In absence of any specific drug or vaccine against these infections, vector control remains only option to control these infections. Development of resistance against commonly used insecticides, particularly pyrethroids, may jeopardies vector control efforts against this vector. As such there was no report of resistance from India against commonly used insecticides till the year 2015. Recent monitoring of insecticide resistance in some urban set up of India revealed widespread resistance against DDT and synthetic pyrethroids. The development of resistance against these insecticides in urban set up was surprising as these insecticides are not being used in urban areas. Recently we conducted a study to monitor knockdown resistance (kdr) in some urban *Ae. aegypti* populations of India. DNA sequencing of domain II, III and IV of the voltage gated sodium channel—the target site of action for DDT and pyrethroids, revealed presence of a total of five kdr mutations, i.e., S989P and V1016G in domain II, T1520I, F1534C and F1534L in domain III. We reported presence of two novel mutations in *Ae. aegypti*, i.e., T1520I and F1534L. The role of novel mutation T1520I in insecticide resistance could not be established as because this is tightly linked to F1534C, while other novel mutation F1534L has shown significant protection against pyrethroids. The distribution of these kdr mutations was found to vary in different locations. Simple PCR-based methods were developed for all the five kdr mutations which was used for monitoring of kdr mutations in some urban areas which revealed a high frequency of F1534C mutation in Delhi, Kolkata and Bengaluru. Mutations S989P and V1016G which are linked and was found in Kolkata and Bengaluru but not in Delhi. The novel mutation F1534L was found in Bengaluru only. Still there is no any report from India about metabolic mediated insecticide resistance which is urgently required to be studied. Currently we are performing RNAseq based transcriptome analysis to understand mechanism which involves over-expression of insecticide metabolizing enzymes.

Insecticide Resistance And Its Management-Current And Future Perspectives.

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Vector control with the use of insecticides remains the most effective measure to prevent transmission of vector borne diseases (VBDs), particularly malaria. In India, currently, malaria vector control relies largely on wide scale use of insecticide-treated bed nets and indoor residual spraying (IRS). However, one of the major impediment for effective vector control is development of resistance in vectors to the insecticides, which is threatening to slow and even reverse the gains made in malaria control. Resistance development is a complex process and influenced by many factors. In areas, where insecticides are continuously used, the frequency of resistant insects in a vector population is likely to increase, reducing the efficacy of the treatment, sometimes to a level that would require replacement of the insecticide with another one. Therefore, detection and monitoring of insecticide resistance in the disease vectors at an early stage is crucial so that the resistant management strategies could be designed and introduced in appropriate time for rationalizing the field operations to control malaria and other VBDs. Besides, the level of resistance to the selective compounds, the type of resistance mechanism selected and consequently the spectrum and levels of resistance conferred to other compounds need to be considered to formulate an effective resistance management strategy.

Resistance management can be practiced through insecticide-based approaches in combination with other non-chemical/biological vector-control methods in the context of integrated vector management (IVM). Insecticides with novel mode of action and optimum biological properties will also have a role in resistance management. There are instances that integrated control programmes faced practical constraints when implemented in large scale as long-term control programmes. Therefore, operationally, resistance management is likely to be insecticide-based such as rotations, mosaics, mixtures or combinations and for successful implementation of these resistance-management strategies, it is vital to have information on the mode of action, target sites in insects, chemical properties and residual life of the insecticide products/formulations.

As suggested by the Insecticide Resistance Action Committee (IRAC), insecticides should be applied at the recommended dosages, only when and where needed, avoiding over-dosages, which are potentially harmful, as well as under-dosages, which may accelerate the development of resistance. Insecticides of the same chemical group, acting on the same target site, should be considered as a single product as far as resistance is concerned. As soon as resistance is detected in a target vector population, an unrelated insecticide should be selected and introduced, either alone or in combination.

Strategies On Insecticides Resistance Management

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Insecticide resistance is the term used to describe the situation in which the vectors are no longer killed by the standard dose of insecticide or manage to avoid coming into contact with the insecticide. Use of insecticides in both public health and agriculture can contribute to the evolution of resistance in malaria vectors. The four main Insecticide Resistance Management (IRM) strategies for malaria control are Rotations of insecticides, Combination of interventions, Mosaic spraying and Mixtures. IRM strategies can have different effects on resistant vector populations. Five pillars of the Global Plan for Insecticide Resistance Management in malaria vectors are (1) Plan and implement insecticide resistance management strategies in malaria-endemic countries (2) timely entomological and resistance monitoring and effective data management (3) Develop new, innovative vector control tools (4) Fill gaps in knowledge on mechanisms of insecticide resistance & (5) Ensure that enabling mechanisms are in place. Some of the IRM strategies are use of non-chemical control methods, limitation of pesticide use to areas with high levels of disease transmission, use of adulticides, which kill only adult females, rather than larvicides, which kill both sexes, resulting in approximately half the selection pressure for resistance, rotation among unrelated insecticides according to a pre-arranged plan based on knowledge of the likelihood of resistance developing to each compound, choice of a compound that has been found by experience to select for a narrow spectrum of resistance rather than a broad one; and use of mixtures or mosaic treatments with unrelated compounds, so that individuals resistant to only one of the components are killed by the other.

Public Education and Vector Control: Educate, Empathize, Engage

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Public Education and outreach are vital pieces to gaining public access and acceptance for vector control activities. Many initiatives die before they have an opportunity to start because communities are not properly informed. There are a variety of reasons why these initiatives achieve success or are destined to fail. As scientists, we can overlook the importance of engaging our stakeholders for improved success. This can include the general public, civic leaders and politicians. Sculpting public perception is an important piece to vector control success. Social media remains a staple for reaching a mass audience, but it can have negative consequences as well. It must be used with wisdom and restraint. Public education is a key facet to vector control success.

Vector Control Products And Technologies Of Drdo In Protecting Indian Armed Forces From Arthropod Vectors

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Indian Armed forces face a lot of threat due to many arthropod vectors and vector borne diseases in different terrains of our country while protecting it. Their protection from arthropod vectors is very important for all successful military operations. Entomologists of DRDO, Ministry of Defence, in collaboration with other scientists are working since 1972 and developed many products and technologies for protecting Indian Armed Forces for protecting them from many arthropod vectors and vector borne diseases. The products include a multi insect repellent Diethyl Phenyl Acetamide (DEPA), ready to use Slow Release Insecticidal Paint (SRIP) formulation, ROACHLINE and ROACHTOX against crawling insects like cockroaches, a lure and kill technology ATTRACTICIDE against dengue vectors etc. We have also developed other products like WOOLCARE for protecting woolen materials, RATOX to protect their stores, materials from damage due to rats and Nicotinilide (a potent Molluscicide against freshwater snails transmitting animal schistosomiasis) for protecting horses & mules which are used for transporting armed forces stores and materials to high altitude unmotorable areas. Many of these DRDO products and technologies do have spinoff benefit to civilians also. Research is continued in DRDO for developing newer products and technologies for protecting Indian Armed Forces from vector and vector borne diseases.

Key words: DRDO, Arthropod vectors, Entomologist, DEPA, WOOLCARE, SRIP, ATTRACTICIDE, Nicotinilide

Advancing Public Health and Well Being with Better Vector Management

Jitender Gawade and Ashish Dokras

Member Society for Vector Ecology (Indian Region)

Globally, vector-borne diseases account for 17% of all infectious diseases, causing more than 700,000 deaths every year. More than 3.9 billion people in over 128 countries are at risk of contracting dengue, with 96 million cases estimated per year. Malaria causes more than 400,000 deaths every year globally, most of them children under 5 years of age.

Vector-borne diseases (VBDs) adversely affect communities with poor living conditions and the financial burdens of such diseases worsen poverty. In recent years, VBDs are spreading rapidly irrespective of climatic conditions and geographies. Humankind has more diseases to deal with than before and with the evolving vector complex, the traditional solutions and approaches would not be sufficient to address the seriousness of these diseases.

Vector control has been an important and effective intervention for keeping vector borne diseases in check. It focuses on prevention and can control the spread of disease in a relatively short period by working at community level.

Modern approach to vector control would play a key role in combating many well-known as well as neglected vector borne diseases in this evolving situation. Residual spray is an important intervention for suppression of vector population. However, sprays with longer residual efficacy that will fit the public health budget of rural setup and enable protection from vector over entire duration of peak vector activity is the need of time. Space sprays that can break the vicious epidemic cycle of VBDs need to be more eco-friendly, less polluting and able to provide wider coverage than before. More innovations that would bring new mode of actions to address the challenge of resistance looming largely over prolonged used molecules.

VBDs are more concentrated in impoverished populations in the developing world where public health priorities are at low. Development of suitable framework for collaboration between government, public and private for early adoption of innovations, science and technologies would make vector control more effective and safe for the environment in which we all live, work and play.

Role and Support of Indian Pest Control Association (IPCA) in Vector Management

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As this is vector related conference, presentation highlights will be mainly on the important work we as Indian Pest Control Association (IPCA) are carrying out. IPCA is an apex association in India existing for more than 51 years having more than 350 active members and Allied members. IPCA being founder member of Federation of Asia Oceania Pest Managers Association (FAOPMA) recently joined as a member of Global Pest Management Council (GPMC). Awareness campaigns are the need of the hour and looking at the seriousness of deaths due to Dengue, Malaria that have taken place, INDIAN PEST CONTROL ASSOCIATION in joint venture with GPMC have organised PUBLIC AWARENESS – VECTOR MANAGEMENT, a CAMPAIGN to educate & involve COMMON MAN in the fight to manage the Vector borne diseases. IPCA also provides a completely free, informative presentation and actual field demonstration to all members of Schools, Colleges, public in Housing associations / societies etc. with easy and “Do It Yourself” approach. My Presentation also includes important role IPCA recently played in flood affected Kerala region. IPCA with support from Bayer also help with Awareness posters, Leaflets, Demonstrations, Interactions etc. We are emphasising on Do’s & Don’t’s, what a common man should do.

Sourcing and evaluation of the efficacy of mosquito pathogenic bacilli from Coastal environment in Goa, India

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Vector-borne diseases, account for about 17% of all infectious diseases. Malaria remains a human parasitic disease with highest burden and with risk of re-emergence in several areas worldwide. Additionally, since 2014, major outbreaks of dengue, malaria, chikungunya, yellow fever and lately Zika have surfaced and afflicted populations far and wide, claiming lives and overwhelming health systems in many countries. Control of vectors is imperative to achieve disease control. In our efforts to discover new vector control tools, 9 bacterial isolates belonging to *Bacillus* spp. were collected from 3 different zones i.e. tidal, intertidal and sea shore of Goa, India. These were further tested for mosquito larvicidal activity against *Anopheles stephensi* (vector of malaria), *Culex quinquefasciatus* (vector of filariasis) and *Aedes aegypti* (vector of dengue, chikungunya and zika). Colony, cell morphology and biochemical/physiological characteristics of the isolates were determined. Bioassay showed that 6 of the 9 isolates (B1C, C1A, C2A, C2C, C2D and C2E) were highly to mildly toxic (mortality ranging from 60-100%) against larvae of all 3 mosquito species and their LC₅₀ and LC₉₀ values following following treatment for 24 hours and 48 hours were worked out. Phylogenetic analysis showed that all 6 active isolates clustered around other known and some previously unreported strains of *Bacillus* spp. as mosquito-pathogenic. These findings suggest that coastal environment is conducive to the growth of bacilli that could be further developed and evaluated as vector control products for public health use.

Role Of Gene Duplication In Insecticide Resistance In *Anopheles stephensi*

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Insecticide resistance is a major threat for the success of vector control programme which currently rely heavily on insecticides that is used in insecticide impregnated materials and in indoor residual or aerial spraying applications. Understanding of mechanisms of insecticide resistance is essential for effective integrated vector management (IVM). One of the major mechanisms of such resistance is increased metabolic detoxification of insecticide which is achieved through transcriptional overexpression of genes in resistant insects. We proposed a hypothesis that gene duplication may be one important event leading to overproduction of metabolizing enzyme followed by fixation in response to insecticide selection pressure. To test the hypothesis, we attempted to identify such gene duplication events in laboratory selected deltamethrin- and DDT-resistant *Anopheles stephensi* (a major malaria vector in India) through whole genome sequencing (WGS) and establish that such gene duplications are actually responsible for insecticide resistance. WGS of deltamethrin and DDT resistant *An. stephensi* revealed at least two major duplication events in the genome that is associated with insecticide resistance. One such event shows duplication of 6.4 kb of genome encompassing two GST epsilon genes along with a pseudogene and another event showing duplication of 15.67 kb of genome encompassing two CYP450 and one carboxyl ester genes. Attempt were made to identify break-point and orientation of duplication through Sanger's sequencing which confirmed that duplicated genes in both duplication events are tandemly repeated. Among the duplicated genes, atleast two CYP450 genes and two GST epsilon genes have shown high copy number at transcriptional level in resistant mosquitoes as compared to susceptible confirming their role in insecticide resistance. Detailed results will be discussed.

Are Vector Habitats Source Of Gut Microbiota?

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²

Malaria affects approximately three billion people worldwide each year. *Anopheles stephensi* is an important urban malaria vector in India and the neighbouring countries of South Asia. In vector bioecology, breeding sites play a crucial role, where oviposition, larval and pupal development and emergence of adults take place. In their aquatic environment, mosquito larvae actively feed on microorganism through filtration process, thus acquiring microorganisms from the breeding habitat some of which have the ability to thrive in the host mid gut. Literature reveals that microbial communities harboured in the mosquito midgut perform vital functions which impact vectorial capacity of adult mosquitoes. Considering this, the midgut diversity of *Anopheles stephensi* was explored in this study. Microbes were isolated from the midgut of larvae, pupae and adult *Anopheles stephensi* using five different media. The diversity in microbiota in breeding habitats and midgut was studied using biochemical approach and 16S RNA gene sequence analysis followed by blast with their type species, manual curation, editing and alignment using BioEdit software and finally submitting to the GenBank. Around 133 sequences obtained in our study were compared with GenBank database using the BLAST algorithm. The diversity of the gut microbiome showed great variation between immature stages and adults and also different population, with laboratory population showing the least diversity. The bacterial isolates belonged to Actinobacteria, Bacteroidetes, Enterobacteriaceae, Firmicutes, Microbacteriaceae and Staphylococcaceae. The abundance of the isolates in each stage of the mosquito life cycle was calculated, with respect to different media. Our study provides information on the microbiome in the breeding site and that harboured in the vector gut and the similarity and differences between the two.

Identification Of Mosquito-Plasmodium Associations Using Proteogenomics

Dey G.¹, Mohanty A. K.², Sreelakshmi S. K.¹, Kumar M.³, Kumar A.², and Prasad T. S. K.⁴¹Institute of Bioinformatics, Bangalore, India²National Institute of Malaria Research, Field Station Goa, Panaji, India³Department of Immunology & Infectious Diseases Harvard School of Public Health, Boston, MA 02115 USA⁴Center for Systems Biology and Molecular Medicine, Yenepoya Research Center, Yenepoya (Deemed to be University), Mangalore, India

Vector-borne diseases are a global health hazard and account for 17% of all infectious diseases causing around 700,000 deaths worldwide (WHO, 2017). Most control measures undertaken to prevent vector-borne disease involve either by targeting the disease causing pathogen or the vector that transmits the pathogen. However, the emergence of drug-resistant pathogenic strains and insecticide resistant vectors threaten the present-day control efforts. Therefore, despite the considerable emphasis on the development and deployment of control methods, the vector-borne disease such as malaria remains a significant threat. In-depth understanding of the interactions between vector-parasite interactions, therefore, would provide vital information that can be utilized to reduce the disease burden. The availability and accurate annotation of the genome assembly is the first requirement towards understand of the underlying molecular processes inside an organism. However, newly sequenced and assembled genomes use a computational pipeline to annotate protein-coding potential of the genome. Such pipelines have their own limitations that lead to erroneous or missed out genome annotations that would in turn affect molecular studies on the concerned organisms. It is therefore, advantageous to have transcriptome and proteome data that can complement the genome sequencing projects.

Our study provides insights into the biological processes occurring in the essential organs of vector *An. stephensi* that are associated with the developmental and transmission potential of this vector. Besides refining the current *An. stephensi* genome assembly, our study also confirmed the presence of several essential genes having integral roles in vector-pathogen interactions but were missed out by conventional computational annotation approach.

Assessing the diversity, evenness and infectivity of mosquitoes in closely situated city zones in Goa, India

De Souza CR¹, Deepika Verma¹, Nikita Fernandes¹, Mohanty AK¹ and Kumar A¹

¹ICMR-National Institute of Malaria Research (ICMR), DHS Building, Campal, Panaji, Goa – 403001, India

Mosquitoes are the most significant insects of public health importance due to the number of vector borne diseases they can transmit. Apart from transmitting diseases, some species also show relevance in maintaining low levels of pathogens while vector species lay dormant. Also, certain species cause nuisance biting and disturbance to the human activities and thus lower quality of life. Ecological disturbances like changing landscape have proven to decrease diversity of mosquitoes, biasing relative abundances of species to those that can quickly adapt or have higher tolerance ranges. Studies conducted elsewhere strongly suggest that the dependence of, the prevalence and spread of vector borne diseases are based on factors like species richness and various other mosquito community features as well as human land use patterns. To address the composition of mosquito communities, mosquito surveillance was carried out from December 2015 to May 2016 in Panaji, Goa as part of NIH-UW sponsored International Center of Excellence for Malaria Research. Mosquitoes were captured by CDC-UV light traps from two zones, each containing three types of sites namely, developing site-DS, moderate to high human population density site-MHDS and low human population density site-LHDS. Altogether 15054 mosquitoes belonging to 22 species were captured in 1068 traps which were identified using standard taxonomic keys and the recorded data was analysed to address mosquito species diversity and evenness as well as fortnightly trapping densities of each species.

Results revealed that mosquito diversity of spatially proximal sites (DS and MHDS) in both zones shared similar diversity. LHDS however, showed higher diversity and even species richness. Special emphasis on the diversity analysis of Anopheline species revealed an observably low Shannon-Wiener's evenness in LHDS (located near fields and brackish waters) relative to other sites, due to greater abundance of *Anopheles subpictus*.

Fortnightly trapping densities reveals that there is a general peak in densities of all dominant *Anopheles* species in the second fortnight of February, highest being of *Anopheles subpictus*. Dominant *Culex* species showed varied peaks, *Culex quinquefasciatus* peaking in the first half of January and *Culex pseudovishnui* peaking in the first half of April. *Mansonia* species generally peak between February to March, *Mansonia uniformes* of which, also peaks in beginning of May. *Armigeres subalbatus* showed a relatively consistent density during the study period.

Curiously, 6 out of 9 *Anopheles* species assayed with nested PCR showed the presence of *Plasmodium falciparum*, *P. vivax* or both in the abdomen of 41 *Anopheles* mosquitoes. Most (29/41) infections were in *Anopheles subpictus*. This finding is of significance for xeno-monitoring of *Plasmodium* presence in human hosts using vectors and non-vector mosquito species as a proxy for assessing levels of ongoing transmission particularly during malaria elimination and post elimination sustenance phases.

Climatic and Ecological attributes of Kyasanur Forest Disease in India

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ICMR-National Institute of Malaria Research, Sector 8, Dwarka, Delhi-110077, India

Introduction: Kyasanur Forest disease (KFD) is caused by Kyasanur Forest disease virus (KFDV) and transmitted by the bites of *Haemaphysalis spinigera* ticks. The maximum number of cases has been reported from the state of Karnataka. In recent years, in addition to Karnataka, KFD outbreaks have also been reported from other states like Kerala (2015), Maharashtra (2016), Goa (2016) and Tamil Nadu (only monkey positive in 2013). Studies have been undertaken on aspects of epidemiology, outbreak investigation, emergence from new area, viral positivity of ticks and monkeys, etc. However, the role of climate and ecological changes as the main reasons of emergence of KFD in new areas remain unknown.

Objective: The present study was undertaken to find out the relationship between climatic and ecological parameters and transmission of KFD.

Methods & Materials: Study was undertaken in Shivamogga district of Karnataka. For analyzing the role of climate and ecology as the potential risk factors for KFD, climate i.e. temperature and rainfall was procured for study site from IMD, Pune and for whole country, was extracted from National Oceanic and Atmospheric Administration, (NOAA) and disease data from concerned district health authorities. Based on 32 occurrence points, the current climatic sensitivity and suitability of the KFD in India using Maximum Entropy (MaxEnt) modeling was determined. On the basis of location of current occurrence of cases and bio-climatic variables, (described by IPCC, fifth assessment), the potential areas for KFD have been predicted in India. Climatic variables contributions were assessed using Pearson correlation coefficient.

Results: Results of the analysis revealed that KFD cases and temperature have a significant relationship with 1-2 months lag period while with rainfall, there is 4-5 months' lag effect. Based on the occurrence of monthly cases, the suitable average temperature was found from 22-30°C; cumulative rainfall >1000 mm in non-transmission season, and <100mm rainfall at the threshold of transmission months (November/ December). Rainfall with temperature shows transmission suitability for 5-8 months in the study site. MaxEnt result shows that the probability of KFD presence is related with mean warmest quarter temperature ranges between 14°C-30°C. However, more than 30°C was not found suitable for KFD tick. The risk of KFD ticks presence increases considerably when the mean diurnal temperature ranges between 5.0-8.3°C, while more than 8.3°C mean diurnal

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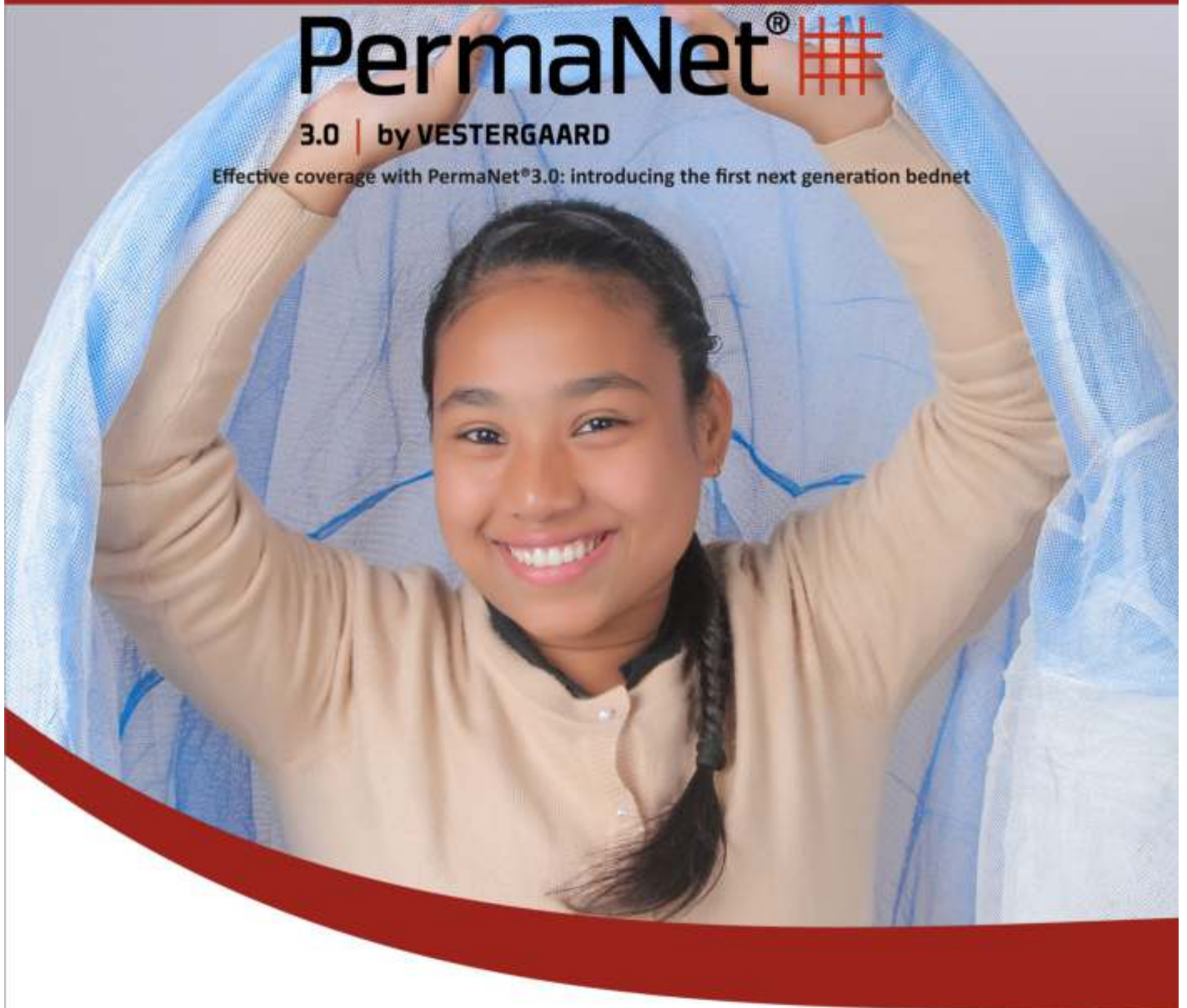
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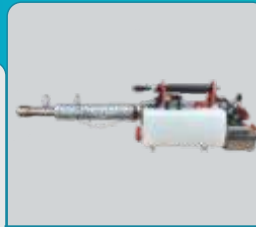
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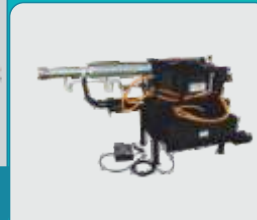
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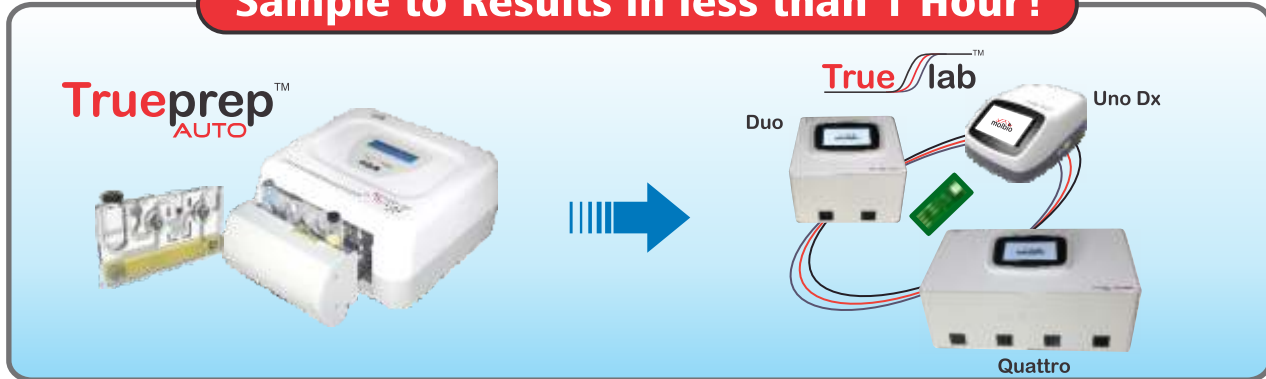
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