BIO NEWS

Quarterly e-newsletter of the Institute of Biology, Sri Lanka

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EDITORIAL COMMITTEE

COVER STORY

Our cover page depicts a photograph of plum Judy (*Abisara echerius*), the only species of Family Riodinidae in Sri Lanka. It inhabits forests and well wooded areas, common around *Ardisia elliptica* stands in wetlands- known as one of its larval food plants.

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IOBSL News and Events

Feature Articles

Updates on the Latest Research

Corner for Young Biologists

Nature Captures

Inter University Biology Quiz Competition

The Inter-University Biology Quiz Competition was organized by the IOB for the 5th consecutive year for undergraduates of State universities in Sri Lanka. The competition comprised of two stages and the entire competition was conducted virtually. The stage I of the competition was held on 18th June 2022 with the participation of 100 undergraduate students representing 13 State universities. Based on their performance, 15 students were selected for the stage II of the quiz competition which was held on 8th September 2022. The Centre for Education Technology and Media (CETMe) of the Open University of Sri Lanka assisted with conducting the online examination through the Learn OUSL.

The first place was secured by Mr. I.S.S.C. Uyangoda of University of Sri Jayewardenepura, second place by Ms. M. Pirunthini from University of Jaffna and third place by Ms. P. V. S. Iresha from University of Colombo. Medals and certificates were awarded to the winners of the competition. The overall coordination was performed by Dr. Devanmini Halwatura and Dr. Uthpala Jayawardena with the assistance from Dr. Sameera Ariyawansa, and Prof. Suneetha Gunawickrama as members of the organizing committee.

1st Place
Mr. I.S.S.C. Uyangoda

3rd place
Ms. P. V. S. Iresha

Young Scientist Award of the Institute of Biology Sri Lanka

The Young Scientist Award of the Institute of Biology Sri Lanka was introduced in 2022 to recognize the accomplishments and contributions of young biologists who excel in research, leadership and innovations in the field of Biology in Sri Lanka. Prof. Nayana Gunathilake, from the Faculty of Medical Sciences of the University of Kelaniya was recognized as the awardee of 2022 for his outstanding contribution to the field of Medical Entomology and Parasitology. Prof. Nissanka De Silva and Dr. Hiruni Harischandra coordinated the event.

Institute of Biology e-Photography Competition

The e-Photography competition was conducted on the theme "Nature's battle: the struggle to thrive in a polluted world". A photograph submitted by Mr. W.M. Yohan Fernando was selected as the most popular photograph. Mr. Ahamed Jazif was announced as the best biology photographer of the year. Consolation certificates were awarded to Ms. P.W. Deepa Wasanthi, Mr. Wasiri Gajaman, Mr. Tilan Weerasinghe and Mr. W.M. Yohan Fernando. All submissions were judged by an eminent panel of judges. Dr. Devanmini Halwatura, Dr. Uthpala Jayawardana, Prof. Suneetha Gunawickrama and Dr. Vindhya Kulasena coordinated the event.

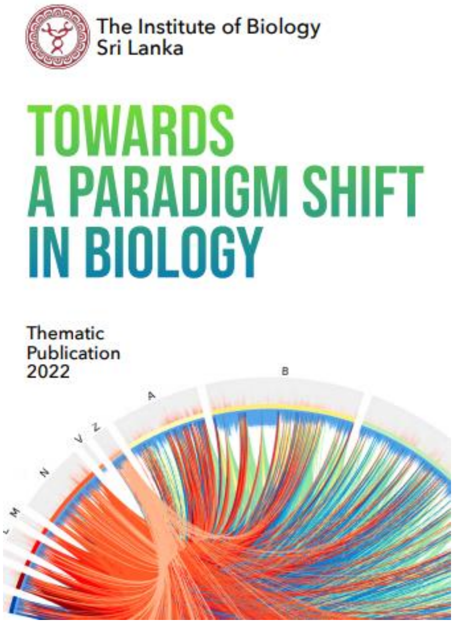
Best Biology photograph
Mr. Ahamed Jazif

Most popular photograph
Mr. W. M. Yohan Fernando

Certificates were handed over by Emeritus Professor M. J. S. Wijeyaratne.

Institute of Biology Thematic Publication

The thematic publication for the year was titled "Towards a Paradigm Shift in Biology". It consisted of five chapters on different aspects related to the theme and written by experts in the field. The information provided in this book would be valuable to academics, researchers, teachers, and students in the field of biology and allied disciplines. Dr. Gayani Galhena and Dr. Rinukshi Wimalasekera served as the technical editors of the book.
http://www.iobsl.org/publications/books/towards-a-paradigm-shift-in-biology-thematic-publication-2022



The Institute of Biology
Sri Lanka

TOWARDS
A PARADIGM SHIFT
IN BIOLOGY

Thematic
Publication
2022

Annual Sessions 2022 (30th September 2022)

Inauguration Ceremony

The Inauguration ceremony of the 42nd Annual Sessions of the Institute of Biology was held on 30th September 2022, at the Marino Beach Hotel, Colombo 03 with 120 participants. The Keynote speaker of the event was Mr. Rohan Pethiyagoda, the first Sri Lankan scientist to receive the prestigious Linnean Medal by the Linnean Society of London. Mr. Rohan Pethiyagoda was also felicitated at the same event, in recognition of his outstanding contribution towards the conservation of biodiversity, discovery of almost hundred new vertebrates from Sri Lanka and taxonomical work. Dr Gayani Galhena (President IOBSL 2021/2022) delivered the presidential address on "Human Genetic Research in Sri Lanka – Biologists' Standpoint". Winners of the IOB Quiz, Nature photography competition, Young scientist award and the Biology Olympiad were also presented with awards at the ceremony.

Technical Sessions

Altogether 50 abstracts were received this year and following the peer-review, 42 abstracts were presented at the technical sessions in four parallel sessions on 30th September 2022. The theme was "Towards a Paradigm Shift in Biology" The track chairs were Dr. I. A. J. K. Dissanayake, Dr. K. W. Samarakoon, Dr. T. Mathiventhan, Dr. Vindhya Kulasena. Further, B. S. Panchasika, Sudesh Ruvinda, R. D. S. M. Gamlath and M. A. Sandaru were awarded as the best presenters of the parallel technical sessions.

Transgenic Plants: Applications and Benefits

Prof. S. R. Weerakoon (Ph.D, Australia, F I Biol)
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Introduction

In transgenic plants DNA is modified using genetic engineering techniques to introduce a new trait to the plant, which is not present naturally in the species. Therefore, transgenic plants include a gene or genes that have been artificially inserted.

The inserted gene sequence is identified as the transgene and it can come from a distantly related plant species or from an unrelated species. The purpose of inserting a combination of genes into a plant is to make it as useful and productive as possible. This includes properties such as improved shelf life, higher yield, improved quality, pest and herbicide resistance, tolerance to heat, cold and drought as well as resistance to various biotic and abiotic stresses. Transgenic plants can also be created to express foreign proteins which are valuable in diverse sectors. This includes industrial, medicinal, and pharmaceutical applications etc.

The first transgenic plants were reported in 1983. Thereafter, many recombinant proteins have been expressed in several important agronomic species of plants such as tobacco, corn, tomato, potato, banana, alfalfa and canola.

Developing transgenic crops

Transgenic plants are generated in vitro by altering the genetic make-up of a plant's genome. Genetically engineered plants are generated by the "Gene Gun" method or by Agrobacterium tumefaciens mediated transformation method.

The "Gene Gun" method, also known as the "Micro-Projectile Bombardment" or "Biolistic" method is most used in monocotyledonous plants like corn and rice. This method has been applied successfully to many crops such as wheat and maize.

"Agrobacterium" method involves the use of a specific soil-dwelling bacteria, known as *Agrobacterium tumefaciens*. It can infect plant cells with a piece of its DNA, which is integrated into a plant chromosome, through a tumor inducing plasmid (Ti plasmid). The Ti plasmid can control the plant's cellular machinery and use it to make many copies of its own bacterial DNA (Figure 1). This method is especially applied to dicotyledonous plants like potatoes, tomatoes, and tobacco.

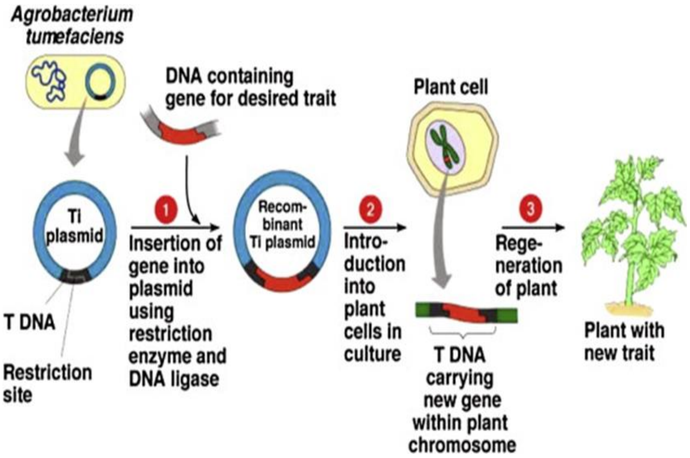

Figure 1. Agrobacterium mediated transformation (Source: Sabu, Khanam, & Subitsha, 2020)

Transgenic plants have genes deriving from other species inserted into them. The inserted genes can come from species within the same plant kingdom (i.e. plant to plant) or between kingdoms (e.g.: bacteria to plant). Transgenic plants are used to express proteins, like the CRY toxins from *Bacillus thuringiensis*, herbicide resistant genes and antigens for vaccinations.

Applications and benefits of transgenic plants

Genetic Modification is a technology which involves inserting DNA into the genome of an organism. To produce a Genetically Modified (GM) plant, new DNA is transferred into plant cells. The cells are then grown in a tissue culture medium to develop into plants. The seeds produced by these plants will inherit the new DNA. This technology has been used to produce a variety of crop plants to date. With the expanding global population, genetically engineered foods offer significant benefits.

Some benefits of transgenic plants are as follows;

Improved yield
Herbicide resistance
Insect resistance
Pest resistance
Virus resistance
Nutritional benefits

Tolerance to biotic stresses (insects, pests, virus, weeds etc.)
Tolerance to abiotic stresses (drought, salinity, flood etc.)
Use of marginalized Land
Reduced environmental impact
Pharmaceuticals and Vaccines

Improved yield:

Genetically Modified crops, including transgenic plants currently harvested at the commercial level have not been modified to directly increase agricultural yield, such as increasing the number of grains, pods, fruits or size of the plant. The GM crops that have been commercialized since 1996 were designed for two main traits: resistance to insects (or diseases) and/or tolerance to herbicides.

The first review on GM crops was published in 2014. It revealed that GM crops have allowed an average increase in agricultural yield by 22 percent and increased farmers' profits by 68 percent, with profit margins even larger in developing countries. Another review published in 2017 revealed that between 1996 and 2015, GM crops increased global production by 357.7 million tons of corn, 180.3 million tons of soybean, 25.2 million tons of cotton fiber, 10.6 million tons of canola and about a ton of sugar beet.

Herbicide resistant plants:

Glyphosate, Sulphonylurea and Atrazine resistant transgenic tomato, potato, tobacco, cotton have been developed and commercialized.

Insect resistant plants:

The bacterium *Bacillus thuringiensis* is pathogenic for a number of insect pests. Its lethal effect is mediated by a protein toxin it produces. Through recombinant DNA methods, the toxin gene can be introduced directly into the genome of the plant, where it is expressed and provides protection against insect pests of the plant.

Virus resistant plants:

Tobacco Mosaic Virus (TMV) resistant transgenic tobacco and tomato plants, Potato virus resistant potato plants , RSV resistant rice, YMV resistant black gram and YMV resistant green gram have been developed and commercialized.

Pest resistant plants:

If transgenic plants are developing a resistance to specific pests, it is beneficial to farmers. Papaya-ring-spot virus resistant papaya has been commercialized and grown in Hawaii since 1996.

There may also be a benefit to the environment if the use of pesticides is reduced. Transgenic crops, containing insect resistance genes from Bacillus thuringiensis, have made it possible to reduce significantly the amount of insecticide applied on cotton in the USA.

Nutritional benefits:

Traditional breeding methods were unsuccessful in creating a rice crop containing a high concentration of vitamin A . Researchers introduced three genes into rice: two from daffodils and one from a microorganism. The transgenic rice exhibits an increased production of beta-carotene as a precursor to vitamin A and the seed is yellow in color. Yellow or Golden, rice is a useful tool to treat the problem of vitamin A deficiency in young children living in developing tropical countries.

Use of marginalized land:

A large area of landmass across the globe has been marginalized due to excessive salinity and alkalinity. A salt tolerance gene from a mangrove species (*Avicennia marina*) has been identified, cloned and transferred to certain other plants. The transgenic plants were found to be tolerant to higher concentrations of salt. The gut D gene from Escherichia coli has also been used to generate salt tolerant transgenic maize plants. Transgenic tomatoes that grew well in saline soils have also been developed.

Reduced environmental impact:

Soils subjected to extensive tillage (plowing) for controlling weeds and preparing seed beds are prone to erosion, and loss of water content. Low tillage systems have been used for many years in traditional communities. There is a need to develop crops that thrive under such conditions, including the introduction of resistance to root diseases currently controlled by tillage and to herbicides which can be used as a substitute for tillage.

Pharmaceuticals and vaccines:

The proteins produced in transgenic plants for therapeutic use are of three types; antibodies, proteins and vaccines. Antibodies directed against dental caries, rheumatoid arthritis, cholera, E. coli diarrhea, malaria, certain cancers, HIV, rhinovirus, influenza, hepatitis B virus and herpes simplex virus are known to be produced in transgenic plants.

Vaccines against infectious diseases of the gastrointestinal tract have been produced in plants like potato and bananas.

Other benefits of transgenic plants:

An important advantage of GM food is the enhanced ability to withstand long-distance transportation. The GM crops are picked when still green can ripen during transportation, yielding a longer shelf life. Even with prolonged shipping and storing periods, the product reaches its destination without spoiling. In addition, this leads to several other benefits such as increased yields and decreased costs.

Although several applications and benefits are linked with transgenic plants, there are some potential issues of concern for plant protein production in these plants. These include,

- Allergic reactions to plant protein glycans and other plant antigens.
- Plant and product contamination by mycotoxins, pesticides, herbicides and endogenous metabolites.
- Regulatory uncertainty, particularly for proteins requiring approval for human drug use.

The declared advantages and disadvantages of transgenic crops need to be combined with each other to provide a crop which is environmentally sound and non-hazardous. Producers of transgenic crops and the agencies that study their effects are aware of this point. However, to date, there has been little evidence to support either case. More research is required in this field to determine the true safety of these plants and to decide whether they are safe for both the environment and for those who consume these products over the years.

Future of Transgenic Crops

The commercial success of transgenic crops for the past two decades has demonstrated that significant benefits are going to ensue from the use of transgenic crops for commercial cultivation.

Significant benefits will include; improved and more efficient weed control, decreased losses due to insect pests and viruses and decreased need of insecticide, decrease in post-harvest losses due to better shelf life and marketing flexibility due to resistance against storage pests, increase in nutritional quality, more effective production of hybrid seed. These benefits will not only help in a sustainable food security system, but also a safer environment, due to reduced use of insecticides and pesticides.

In future, transgenic crops will be used for improved agronomic traits as well as for traits involving food processing, pharmaceuticals (including edible vaccines) and specialty chemicals. The public and farmers will have to respond to this changing scenario.

Conclusion

By the year 2050, world population will possibly reach nine billion. Food production will need to increase at the same rate or more to satisfy the needs of this large number of people. Therefore, there is a need to use genetic techniques to improve crops over the coming decades. Using transgenics, one can produce plants with desired traits and even increased yields. The transgenics would allow for more crops that last longer and withstand pests and diseases. Transgenic plant production will allow us to feed the growing population and to produce more desirable products. The future of GM crops remains a vital debate, as its applications have several advantages and disadvantages as well as a huge impact overall on humans.

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Arbovirus Transmission – Urbanization and Globalization

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Arboviruses

Arthropod-borne viruses or arboviruses are the causative agents of infectious diseases transmitted to people through the bite of infected hematophagous arthropods, predominately mosquitoes and ticks. Most common arboviruses that affect humans can be categorized in the families Flaviviridae (West Nile virus (WNV), Zika, Yellow Fever Virus (YFV), Dengue Virus (DENV)), Togaviridae (Chikungunya Virus (CHIKV)) and Bunyaviridae (Rrift valley fever). The arboviruses replicate inside the arthropod vector and transmit through saliva. Transmission between the vectors can happen in two ways, namely vertical and horizontal transmission. During vertical transmission the virus moves from infected female vectors to their offspring via eggs and in horizontal transmission male vectors can become infected via female vectors during copulation and most importantly vertebrate hosts are infected via saliva during the blood feeding. Dengue, chikungunya, zika and yellow fever are some commonly reported arboviral diseases currently in the world.

Historically, arboviral diseases were not given much consideration and regarded only to be minor contributors to global health and infrastructure. Many arboviruses have been circulating unnoticed in Africa before they emerged and spread internationally. In their natural cycle, arboviruses circulate between mosquito vectors and their vertebrate hosts, and in the event of a spill over to humans, they cause severe diseases and epidemics. Epidemics are associated with the spread of arboviral disease to new areas and thus, the native vertebrate and arthropod hosts become susceptible to the virus. During the last few decades, exponential growth of these arbovirus infections in human populations has alarmed the health authorities. For instance, Dengue virus has witnessed an unprecedented growth enabling it to become the most common vector-borne viral infection in the world. Apart from DENV, Zika, CHIKV, YFV and WNV have also resurged posing threats to global health.

History of arbovirus spread and epidemics

Since historic times human populations have played an important role in spreading arboviral diseases. Commercial globalization, human population movements, passive transport of vectors through human populations and environmental changes due to unplanned and rapid urbanization are the main factors favoring the global spread of arboviral diseases.
The increase in global travel has been vastly associated with arbovirus epidemics. The magnitude of international travel has increased during the past decades with the number of travelers in the world arising from 450 million in 1990 to 1.4 billion in 2018. An infected individual may travel to a new geographic location and could introduce an arboviral disease to them. Air travel may contribute to the increase of epidemics due to new virus serotypes/pathogen strains been introduced to new geographic region.

Commercial globalization, urbanization and changing lifestyles also play an important role in the resurgence of arboviral diseases. The global automobile trade provides oviposition sites and larval habitats for the mosquito vectors. This has become a main mechanism of the geographic spread of arthropod vectors, particularly mosquitoes. Also, the increased use of nonbiodegradable materials such as plastic containers, have provided ideal larval habitats as they are discarded into the environment.

Unplanned urbanization particularly in the tropics have provided ideal mosquito breeding habitats thus, increasing the mosquito population densities and increased arboviral epidemics.

Dengue, YFV and CHIKV are some major viruses that evolved as forest dwelling viruses and have spread in the world widely due to urbanization and globalization.

Dengue virus first occurred as an epizootic virus involving forest dwelling primates (E.g. *Macaca sp*. and *Presbytis sp*.) and canopy dwelling mosquitoes (*Aedes furcifer*, *Ae. taylori* and *Ae. luteocephalus*). It has been suggested that, at some point in history, DENV moved out of the forest as a result of the development of human dwellings, migration of people and commerce. The virus was first introduced to the new world via trans-Atlantic trade and expanded its geographic distribution during World War II to the tropical regions of the world. Also, the introduction of highly anthropophilic African Aedes aegypti mosquitoes to the Asian region via slave trade facilitated the rapid spread of the DENV in the region. Extensive urbanization, increased commerce and travel led to an explosion of urban dengue fever after World War II. Recent research carried out in Sri Lanka have revealed passive migration of Ae. aegypti mosquitoes inside the country as well as into the country identifying possible DENV spreading routes (Fernando et al., 2020a, Fernando et al., 2020b)

Yellow fever virus is another virus that has spread due to urbanization and globalization. It is a highly virulent virus with the case fatality rate occurring between 20-50%. However, as an effective YFV vaccine is available, this disease is only currently infrequently reported in Latin America and some parts of the Africa due to incomplete coverage of vaccination in these areas.

Yellow fever virus also originated in Africa. The transmission cycle consisted of mosquito vectors and non-human primates. During the days of the slave trade, YFV developed enzootic transmission cycles and during the early 17th to 20th century YFV caused periodic epidemics in the port cities in temperate regions of the world. Its spread to Asian parts of the world has never been reported.

Chikungunya virus (Togaviridae: Alphavirus) originated in Sub-Saharan Africa and was maintained in an enzootic transmission cycle between non-human primates and arboreal Aedes mosquitoes and spread to the port cities during 18th and 19th centuries via trade. Unlike YFV, CHIKV has spread to Asia from Eastern Africa and have established permanent urban transmission. It is believed that the emergence of CHIKV occurred mainly due to infected travelers.

Similarly, WNV and Japanese encephalitis virus (JEV) and most recently Zika virus have also been introduced unintentionally to new areas via commerce.

Prevention and control

Prevention and control of arboviral diseases have become increasingly difficult due to the rapid and unpredictable spread. Mitigating arbovirus urbanization involves, intervention and prevention of enzootic spill over and prevention, reduction, or elimination of urban transmission. Intervention and prevention of enzootic spill over is highly challenging due to the widespread of sylvatic possibilities, deforestation and increased human settlements in forest areas. Thus, more emphasis is being given in preventing or reducing urban transmission.

Control programs must incorporate new tools for surveillance as well as tracking of imported cases is of utmost necessity. The most effective control programs should include an integrated approach which will combine enhanced mosquito control through surveillance and improved clinical management with new antiviral and therapeutic drugs.

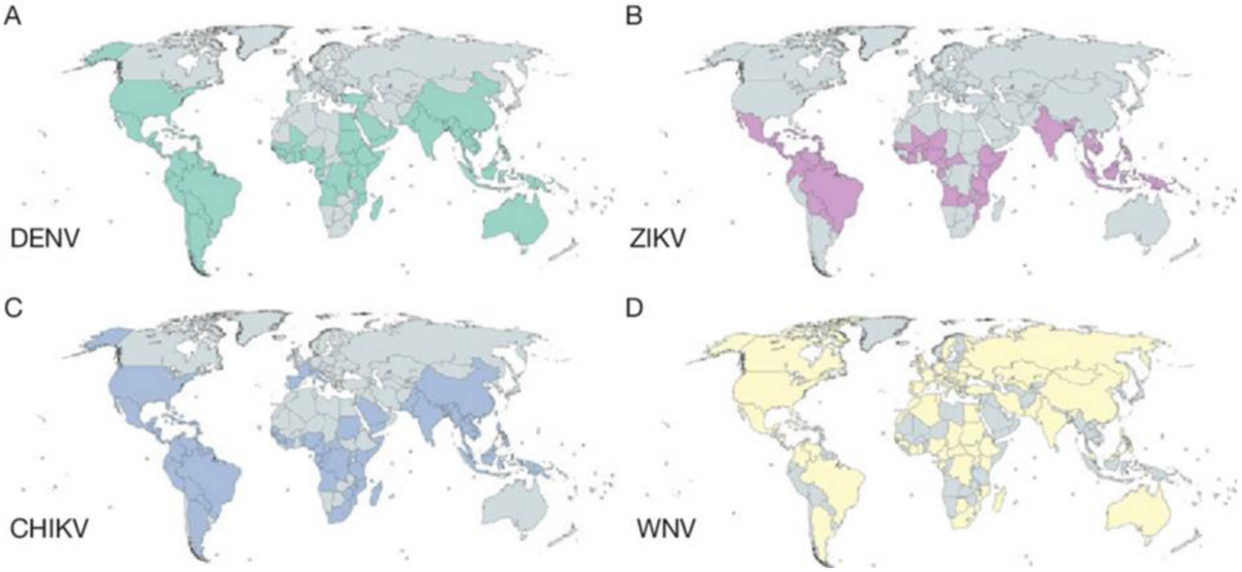

Figure 1. Global distribution of arboviruses. The map depicts countries or territories with previous or current transmission of dengue virus (a), Zika virus (b), chikungunya virus (c) and West Nile virus (d) (Source: Wu et al., 2019).

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Assessment of SARS-CoV-2 specific antibody levels induced by natural infection or vaccination using an in-house serological tool

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the corona virus causing the COVID-19 pandemic was first reported in Wuhan City, the capital of Hubei province, China in December 2019 and spread to many other countries within a few months. Cases of SARS-CoV-2 has been reported from 230 countries and territories around the world with more than 615,000,000 confirmed cases and more than 6,500,000 deaths reported by September 2022. Sri Lanka reported more than 670,000 confirmed cases and more than 16,000 deaths. People with SARS-CoV-2 generally develop signs and symptoms in an average of 5-6 days after contracting the infection with a range of 1-14 days.

SARS-CoV-2 infection demonstrates a wide range of disease manifestations, from asymptomatic to mild, severe-complicated and fatal. There is a high prevalence of asymptomatic or mild infections, however they are undetectable with the currently available diagnostic tests. Due to having a high prevalence of asymptomatic or mild infections, the true extent of virus spread within the population is underestimated, making containment of the infection and the decision making for the fight against SARS-CoV-2 difficult. Therefore, rapid detection of the infection and the seroconversion levels is important for decision making for control and containment of COVID-19.

There are few studies conducted so far on evaluating serum antibody levels in COVID-19 vaccinated individuals in Sri Lanka. They evaluated serum antibodies to receptor binding domain (RBD) protein of SARS-CoV-2 in Covishield (ASD1222) and Sinopharm (BBIBP-CorV) vaccinated individuals using commercially available antibody detection kits. According to the findings of this study, Covishield vaccine induced high levels of antibodies in naive individuals which was greater than the immune responses induced by natural infection. Comparatively, Sinopharm vaccine induced a similar level of antibodies as observed following a natural infection.

Commercially available diagnostic kits are expensive, have a high demand and the sensitivity of some test kits is limited. The development of an in-house assay which is cost-effective and having higher sensitivity would thus be important. Since the rapid development of more effective vaccines and treatments are expected, it is very important to assess the prevalence of the disease by estimating the proportion of individuals who were previously infected, recovered or who are having vaccine induced immunity. Antibody assays can be used to estimate the true extent of seroconversion levels that represent true transmission of the disease and are important to understand the infection risk and fatality rates. This will be immensely helpful for guiding public health policies as well as disease control and surveillance activities.

Performance of serological assays depends on several factors such as assay design, antigenicity of viral epitopes/heterogeneity of antigens used, secondary antibody isotypes used etc. Choosing the best antigen or antibody needs to be done carefully. Coronaviruses are enveloped viruses with single-stranded RNA and four main structural proteins [i.e., envelope (E), membrane (M), spike (S), and nucleocapsid (NC) proteins].

The most commonly used antigens for immunoassays are S1 with receptor binding domain (RBD) and the NC protein. The antigens can be used alone or in combinations. Most of the serological assays reported so far used complete S protein or S1/S2 subunits or the RBD with high sensitivity and specificity. Serological tests allow for detection of antibodies from one to several weeks/months after infection or vaccination. However, use of this antigenic protein alone may underestimate the true antibody response. S protein usually mediates binding and entry of virus into cells and being a target for neutralizing antibodies. Therefore, compared to NC protein, S protein is more prone to acquire mutations due to continuous selective pressure and might affect the accuracy of the assays. NC protein also shows high accuracy comparable to S protein in immunoassays. Also, NC protein is easy to produce in prokaryotic expression systems due to its relatively small size and lack of glycosylation sites.

IgA and IgM are the earliest developed antibodies following a natural infection while IgG forms later. However, IgG denotes a higher specificity and guarantees a longer term protection than IgM. Due to significant variability of antibody expression patterns in an infection, use of both IgM and IgG simultaneously is recommended for serological assays. Total antibodies considering IgM and IgG together show high level of accuracy after the second week of infection, peaking at 2-3 weeks. In our recent study conducted at the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), we evaluated an in-house method for detection of anti-SARS-CoV-2 antibodies which can better estimate seroconversion levels.

We assessed anti-SARS-CoV-2 antibodies in individuals infected with SARS-CoV-2 using IgM and IgG enzyme-linked immunosorbent assays (ELISAs) developed in-house, which was validated against a commercially available ELISA kit (EuroImmune). Total IgG and IgM levels in sera (n=50) of laboratory confirmed SARS-CoV-2 patients from North Colombo Teaching Hospital were evaluated and compared with sera (n=50) collected from healthy individuals (pre-pandemic) as controls. Seropositivity of confirmed cases with either IgM or IgG was 94.0% (n=47/50) with the ELISA developed in-house. In contrast, the seropositivity of only 48.0% (n=24/50) was demonstrated with commercial ELISA kits for detection of IgG or IgM while 28.0% (n=14/50) demonstrated equivocal results.

Further we evaluated the effect of sampling time on diagnostic sensitivity. Accordingly, number of days from onset of symptoms in each patient were recorded. Correlations were determined by linear regression analysis. The sensitivity of IgM and IgG ELISAs ranged between 33.3-50.0% and 33.3-66.7% respectively during first week of post symptoms-onset and was increased to 81.3-87.5% (for IgM) and 68.8%-90.6% (for IgG) by the second week.

IgG ELISAs showed an approximately same sensitivity range (66.7%-83.3%) by third week while IgM ELISAs showed 100.0% sensitivity. Both ELISAs showed 100.0% sensitivity after three weeks from the onset of symptoms. Significant correlations ($p \leq 0.001$) was observed between Ab levels and sampling time further confirming these results. This shows that the detection level of serological assays is dependent on the sampling time from the onset of symptoms.

The developed assays may be used to estimate the true extent of seroconversion levels that represent true transmission of the disease which will be helpful for disease control and surveillance activities.

However, as recommended by U.S. Food and Drug Administration (FDA), more research is needed to carefully evaluate the positivity or negativity of an antibody test by analyzing different groups of people including people with a prior SARS-CoV-2 infection, people who received COVID-19 vaccinations and people who are not vaccinated or partially vaccinated. Further studies are ongoing for assessing serum antibody levels in vaccinated individuals using the developed ELISA.

Our research team:
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Could your pet be a threat?

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Zoonoses have been documented for centuries, yet still wreak global havoc as experienced by the recent COVID-19 pandemic. Changes in ecosystems due to climate change and urbanization, and social changes of human populations provoke zoonoses. Furthermore, genetic variations in the genomes of pathogens allow them to cross the animal-human barrier, adapt, survive, and evolve within humans, making zoonoses a rising threat to human and animal health. Zoonosis can be caused by viruses, bacteria, fungi, parasites and prions transmitted via air, consumption of contaminated meat and produce, close contact with infected animals and surfaces, and by arthropod vectors.

Many forms of zoonotic filariasis are spread by vectors worldwide. Zoonotic lymphatic filariasis, zoonotic onchocerciasis, dirofilariasis and Bung-eye disease are some such diseases, of which lymphatic filariasis and dirofilariasis, though not yet confirmed, are a rising concern in Sri Lanka. Lymphatic filariasis (LF), more commonly known as elephantiasis, has threatened 863 million people worldwide. The most notable symptom of LF is the disfigurement caused by thickening skin and swelling in calves and extremities, which gives its name elephantiasis. This, however, can be seen only in severe cases of LF. Other affected individuals may have episodes of acute inflammation of lymphatic vessels (lymphangitis) along with high temperatures, shaking chills, body aches, and swollen lymph nodes (Figure 1). Excessive amounts of fluid may accumulate (edema) in the affected areas (i.e., arms and/or legs) causing temporary swelling. Yet some others are asymptomatic.

Figure 1: Filariasis infected human with lymphedema in (A) legs and (B) hands

Lymphatic filariasis is caused by parasitic nematodes of Bancroftian and Brugian origins, namely *Wuchereria bancrofti*, *Brugia malayi*, *Brugia pahangi* and *Brugia timori*. These parasites are transmitted via infected mosquitoes. When an infected mosquito bites, the parasites fall on to the skin and enter the host via the puncture left by the mosquito. These L3 larvae then migrate to the lymphatic system where they mature into adults and can survive for about seven years.

These adults mate and produce millions of microfilariae during their lifespan. The microfilariae migrate to the bloodstream at night and enter mosquitoes during blood meals, where they molt into L1, L2 and finally the infective L3 larval stage and the cycle continues.

Treatment for LF entails chemotherapy with Diethylcarbamazine citrate (DEC), Albendazole and Ivermectin (IVM). There are no drugs effective against the adult stages of these parasites and therefore, strategies such as bandaging to reduce the edema is also an integrated part of disease management to reduce discomfort caused by this disease. Hindering transmission by reducing the vector population by destroying breeding grounds such as clearing aquatic vegetation which are required for *Mansonia* spp. mosquitoes to breed and Mass Drug Administration (MDA) to populations at risk is how this disease is controlled at the population level.

Lymphatic filariasis has been endemic for many years in eight districts in Sri Lanka, starting from Northwestern, Western and Southern provinces, bordering to the Western coast. This area is well known as the "filariasis belt". Of the two main types of Lymphatic filariasis, Brugian filariasis (BF) was eliminated from Sri Lanka in 1969 after many rounds of MDA and successful vector control measures. However, sporadic cases of BF have been reported over the past few years (Figure 2).

Several studies have reported a surge in filariasis among canine and feline populations in Sri Lanka over the past one and a half decades. This raises concern as to whether the increased microfilaraemic rates in humans in the recent past is reflective of the possibility of a parasite with a zoonotic origin. Other findings such as sub-periodicity of a human Brugian infection, unresponsiveness of Brugian infections to the Brugia Rapid Test (BRT) also suggest the same. Since there is a large population of domestic and stray cats and dogs in Sri Lanka, it is imperative to identify whether they can serve as reservoirs for the re-emergent Brugian parasite to prevent the resurgence of BF in Sri Lanka and this is what fueled the current study.

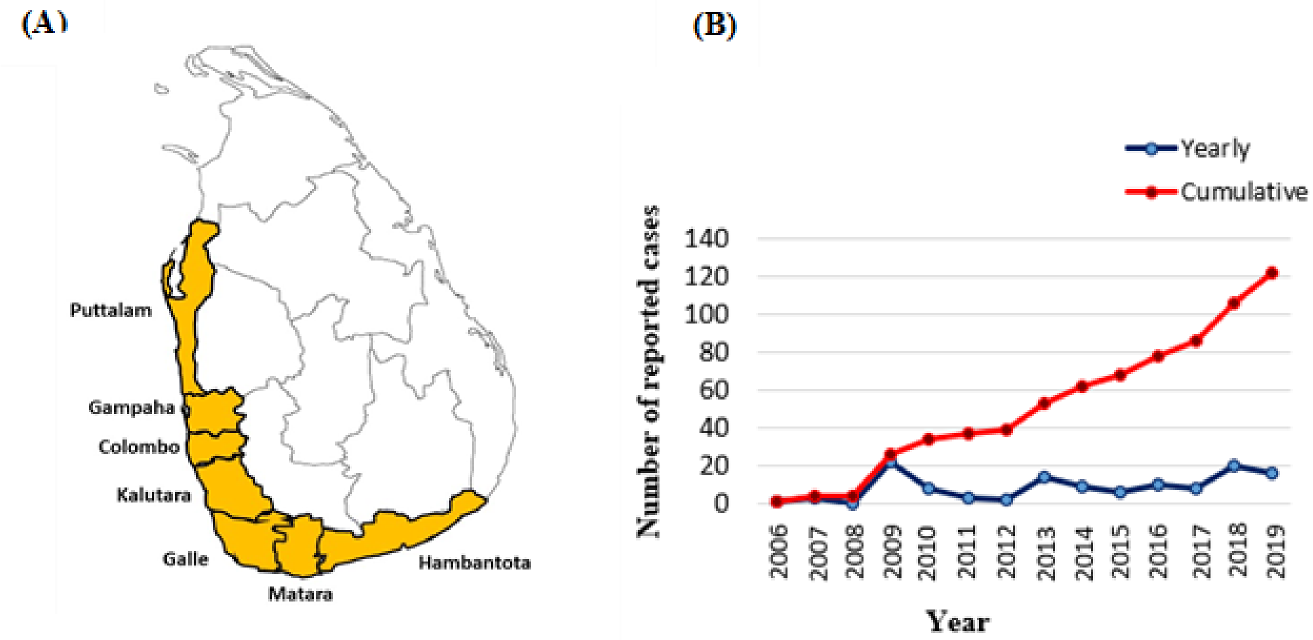

Figure 2: Brugian filariasis in Sri Lanka. (A) The Filariasis belt where filariasis is endemic in Sri Lanka. (B) Increasing incidence of Brugian filariasis in Sri Lanka.

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The gold standard for detection of filariasis is observing Giemsa-stained Thick Blood Smears (TBS) prepared from blood collected between 10 p.m.-2 a.m. for the presence of parasites since microfilaria enter the blood stream only at night. This method of detection is inconvenient due to the night blood collection and error prone as it does not detect early-stage infections due to the low titers of the parasites. Moreover, these infections can be detected only once the infections have established and the adults start producing microfilaria. This is unfortunate because there are no adulticides and the adults can produce millions of microfilariae during their life span of around seven years. Not only do these individuals have to live with the burden of the disease until the parasites outlive their life span, but they also serve as a source of microfilaria for further dissemination of the disease. Therefore, it is imperative to develop better diagnostics to detect infections early on and is one of the main focuses of our laboratory. Moreover, our research suggests that the actual prevalence of this disease within the country might be higher than that currently being reported and a proper detection method is crucial for assessing the actual height of this threat. Analysis of Brugian filarial parasites in animals, humans and mosquitoes around human BF cases in selected areas within the filariasis belt revealed that the parasite within all three are the same. This is alarming as it is suggestive of an active zoonotic transmission cycle between animals and humans via mosquito vectors, especially given the number of domestic and stray cats and dogs in Sri Lanka. The impact of zoonotic diseases is greater than we think due to the very fact that we cannot estimate the impact of it without knowing how much it has spread among the reservoir hosts. This adds a layer of complexity to the control of this disease because it is evident that not only humans but also animals should be surveyed to get an accurate understanding of parasite prevalence. Although filariasis is currently endemic only within the filariasis belt, there is a threat of transmission of these infections to other filariasis-free districts in Sri Lanka, if the canine and feline populations serve as reservoirs due to the increased mobility range of animals than arthropod vectors. This requires immediate attention and the implementation of instantaneous control measures.

Zoonosis is provoked by imbalances in the human, animal and environmental triad. It is a rising threat and, in some cases, we cannot predict the damage as was evident during the COVID-19 pandemic we just faced. Therefore, it is important to be mindful of the risks inherent in this tightly woven web.

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Animal Microbiomes: The Potential Role in Animal Ecology and Conservation

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Microorganisms of animals play a critical role in the ecological success and health of their hosts. For a long time, scientists considered microorganisms as pathogens, causing infectious diseases to the host. However, with the advances of new technologies, there is a radical shift in the understanding of the relationship between animals and their microorganisms. In fact, scientists have found that they are useful for informing and supporting conservation practices.

The heady mix of bacteria along with, archaea, fungi, and viruses – together dubbed the microbiome- are not mere hitchhikers. They contribute greatly to the adaptability and fitness of animal hosts by regulating processes related to digestion, reproduction, immunity, behavior, and even development. Since they are so critical to animal existence, many scientists consider animals to be symbiotic organisms, made up of the host, the microbiome, and the environment. This holy trinity is called the "holobiont".

Cumulative impacts of local stressors and rapidly changing climate affect this intricate balance- known as dysbiosis- diminishing their benefits and leading to infection and disease.

Ecological Impacts on Microbiome

Compositions of the microbiome have reached such a frenzied pace with global environmental change altering microbial diversity. Animals are often forced to adapt whenever there is a change in the environment, both through natural changes and anthropogenic threats, particularly endemic animals. As such, studying these microbial communities may provide useful insights into how dietary, behavioral, and/or environmental changes and disruptions, such as habitat loss and climate change, impact animal health.

Habitat Loss

Habitat loss is one of the leading ecological problems worldwide. The remaining habitat patches- and the population of species that still live in them- become fragmented. Research has confirmed that habitat destruction deeply influences the survival of the animal. It is not surprising that these effects are reflected by the animal microbiome. Often the animals in degraded habitats risk 'double jeopardy' from both external factors (reduced resource availability) and internal factors (diminished microbiomes).

Destruction of habitats and intensive livestock farming in the cleared lands have also brought wildlife into closer contact with humans than would naturally happen, such as in wildlife trade. The traded animals are held in cramped, stressful, unsanitary conditions with different species caged side by side. This increases the chances that viruses can jump from one species to another and ultimately from animals to humans, providing an opportunity for the emergence and spread of new viral variants from animals to humans, as recently observed with the COVID-19 pandemic.

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Figure 1: Coral bleaching due to heat stress

[https://fsmedia.imgix.net/01/19/42/2c/1b8b/49ba/bf0b/1ba437e7dbbb/a-bleached-coral-protects-itself-from-the-sun-with-a-glowing-blue pigment.jpeg?rect=0%2C227%2C2880%2C1440&dpr=2&auto=format%2Ccompress&w=650]

Climate Change

Climate change is happening and, despite growing global concern, is predicted to progress. Animal microbiomes not only shift in response to diet and health but also responds to other environmental variables, like temperature.

The best evidence comes from observed impacts of thermal stress on corals. The symbiotic relationship between coral and photosynthetic algae breaks down at high seawater temperatures - a process known as bleaching- this impairs the defense mechanisms of the host and increases the risk of pathogen attack. Australia's Great Barrier Reef, which is the largest coral reef system in the world appears to be suffering its fourth mass bleaching in the last seven years. If this continues over the next 10 years or so there will not be much of a Great Barrier Reef left.

Inbreeding

Breeding between closely related individuals can happen in small and isolated populations. In birds and animals, inbreeding has been linked to problems in reproduction, birth weight, survival and resistance to disease, predation, and environmental stress. This means the more closely related the breeding pair is, harmful genes are often expressed in their offspring. The microbiome will likely be affected directly or because of the compromised immune system resulting from the unpleasant effects of gene mutations.

Conservation and the Microbiome

Animals in captivity may be facing more than what they bargained for Although zoos and other conservation habitats provide a haven for injured animals, and critically endangered species, the artificial settings in captivity can still cause behavioral or physiological changes in animals.

Animals in the wild encounter a wider array of microbes than those in captivity, who live in small sterile enclosures, are given antibiotic treatment, and eat simple or altered diets potentiating microbiome change. Fecal samples from dugongs, red pandas, Tasmanian devils, and Andean bears show that wild critters harbor a higher microbial diversity than those in captivity. These changes might be linked with gastrointestinal disorders, immune deficiencies, and even reproductive success.

The critically endangered black rhinoceros are facing a deadly disease known as iron overload disorder that is affecting those in captivity. An overload of iron in vital organs increases their risk of diseases.

Black rhinoceros are considered specialized browsers in the wild, which makes it difficult to feed them in a managed conservation program. Dysbiosis of the microbiome thereby predisposes them to an impaired immune system.

Also, many of the microbiome changes show up in carnivorous animals in captivity. Interestingly, gut microbiomes of herbivorous animals such as koalas, giraffes, and cattle can be relatively stable and remain similar between captive and wild individuals. Although the richness of the gut microbiota is higher in herbivores than in carnivores, herbivores have a rather consistent diet throughout their life unlike carnivores. This further illustrates that the diet is one of the main reasons, if not the driver, in the changes to the microbiome.

The parallel changes in microbiomes seen in these threatened or captive animals are convincing patterns of dysbiotic disruptions because of captive conditions. Animals often struggle to thrive in their natural habitat after being released from captive breeding programs. It is very clear that microbiomes are susceptible to adverse change that can occur as the result of diet, disease, stress, and other factors.

As such, healthy microbiomes can potentially be engineered to improve host health by manipulating the composition of the microbial community such as through the use of prebiotics and probiotics.

Figure 2: Frog infected with *Batrachochytrium dendrobatidis* (Bd) fungus.

[https://allthatsinteresting.com/wordpress/wp-content/uploads/2019/03/frog-fungus.jpg]

Probiotics are usually appreciated for supporting intestinal health, but these supplements also enhance the microbiomes' ability to overcome disease and dysbiosis.

In amphibians, a healthy skin microbiome is warding off *Batrachochytrium dendrobatidis* (Bd), a fungus that callously kills amphibians by inhibiting their ability to breathe through their skin. Researchers are exploring how to use these probiotics in wild amphibians to slow down the spread of the fungus.

In support of this finding, another study found that corals treated with a probiotic concoction could mitigate coral bleaching, increasing their resistance to worsening marine heat waves. Another preliminary microbiome study on critically endangered hawksbill sea turtle eggs identified specific microbes with anti-pathogenic activity that could prove to be useful probiotics for hawksbill conservation.

Current Challenges, Perspectives, and Future Implications

Part of the challenge of studying animal microbiomes is generating data in the first place. Collecting samples from animals are quite difficult, even more so in remote tropical regions or in the deep oceans. Until recently, the diagnostic tests looked for a single microorganism, or at most a limited panel of microorganisms such as sequencing the 16s ribosomal gene for bacteria and archaea. Now, scientists use shotgun metagenomics to identify and characterize all microbes in their collected samples. This has paved the way for a new area of research in conservation biology focusing on the importance of host-microbiome associations for endangered species and the importance of conservation efforts.

However, so far, most research on animal microbiomes has been conducted on species that have been captive for decades or longer and are therefore, are organisms that are subjected to the selection processes that accompany both captivity and domestication.

The microbiomes of such organisms represent a small, and likely biased, sample of the diverse microbiomes yet to be discovered.

Since acknowledging the tremendous impact of the microbiome on animal health and fitness, scientists recommend conservation practitioners to incorporate microbiome monitoring as part of their species recovery programs and post-release monitoring to assess the impacts of translocation to the wild on animal microbiomes and understand its implication in translocation success.

Alternatively, scientists could design microbial health indexes – an early detection system with cheat codes that precede infection or disease. For example, the coral microbiome may show significant changes before visual signs of bleaching, which can be used to identify dysbiosis.

Microbial species or functions that could be used as biomarkers of health and disease can also be identified. However, this knowledge should not be extrapolated to all other species when we lack fundamental information regarding how microbiomes in even closely related species may respond differently under similar conditions.

Microbiomes of healthy hosts could be observed when exposed to stressors, with and without well-controlled manipulation of specific microbes. Such approaches will improve the understanding of the dynamics of the animal microbiome relationship and the best way to improve microbial therapy.

The impact of the microbiome is suggested by the way it has been described: “the second human genome”. Microbes can easily exchange genetic material and are subjected to changes that are likely driven by environmental factors. Therefore, the functional capacities of microbiomes and their host may not be highly constrained by the small number of evolutionary processes that affect how new traits appear in monogenomic eukaryotic organisms.

Along these lines, it is important to continue to be aware of how microbiome changes arise in response to the environment. Along with understanding the complexity of host-associated microbes and the many, yet undiscovered positive health aspects of these symbionts, it is critical to embrace a paradigm shift for how we approach protection of threatened wildlife along with their microbiomes. The animal microbiome is truly becoming the totem of the topic of conservation.

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