Tracking down microbes – *Laboratory experiences to point of care*

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The Chief Guest, Vidyajyothi Prof. Lalitha Mendis - Emeritus Professor of University of Colombo, the Guest of Honour Professor Carlo Fonseka –Emeritus Professor of Physiology, University of Kelaniya, Members of the Council of Sri Lanka College of Microbiologists, Distinguished Invitees, Colleagues, Friends, Ladies and Gentlemen. It is customary for the President to address on a topic on aspects of Microbiology highlighting the importance of pathogenic microorganisms in the human disease. Today in keeping with the theme **"Iatrogenic infections- portal of sepsis- a major challenge"** I **begin with** some facts through the lens of history and wish to talk on the topic "Tracking down microbes – Laboratory *experiences to point of care"* through some highlights from my own research carried out at different laboratories in Sri Lanka and in the Overseas.

Main points of my address include; land marks in tracking microbes through the lens of history and experiences on the pathogenesis & Immunity to viruses, confronting problems of Microbiology services, research on virus infections of public health importance to Sri Lanka and the messages from such studies with future directions.

Tracking microbes through the lens of history:

About 330 years ago in Holland an indistinct man named Anthony Leeuwenhoek (1632-1723)-an amateur lens grinder looked for the first time into the mysterious new world populated with a thousand different kinds of tiny beings, some ferocious and deadly, others friendly and useful, many of them are more important to mankind than any continent or islands. To Leeuwenhoek the world of those little animalcules represented only a curiosity of nature and their role in disease was not realized.

Lazzaro Spallanzani (1729-1799) born in Northern Italy proved that sub-visible animals arising spontaneously was wrong. He too said nothing about possible deadliness of microbes. After the his death, microbe hunting had come to standstill once more while other sciences were making great leaps ahead.

Ignaz Sernrnelweiss (1818-1865) was an assistant physician in the General Hospital, Vienna. There were two maternity wards and in ward 1 where babies were delivered by medical students, the death rate from 'child bed fever' was horrifying, some times up to 30%. In ward 2, where deliveries were conducted by midwives the rate was 3%. Child bed fever was a shocking condition in which young women often in perfect health developed high fever, sepsis, coma and death. Pregnant women begged and prayed not to be admitted to the notorious ward 1. At this time nothing was known of the real cause of infectious disease. Germs were unheard of.

Sernrnelweiss carefully notice that women admitted just after their baby had been born rarely suffered from the deadly disease. He also deeply affected by his friend and colleague a Professor of Forensic Medicine who died after cutting his finger during a post-mortem examination and noticed the changes found in his body were identical to women died of child bed fever. Based on these observation Sernmelweiss decided that medical students must be carrying some poison from the corpuses in the autopsy room to the labour ward. So he insisted that anyone delivering a baby must first wash his or her hands in chlorinated water. Within a year the mortality rate had dropped to 1%.

Semmelweiss' chief and others resented this conclusion and suggestion that they might have been responsible for women's deaths. Like other doctors of the time, they were proud of the hospital odour they carried on their hands. Little attention was paid to his idea on hand hygiene. He was forced out of his job. His work was forgotten and maternal death rate rose to recorded heights again. 14 years after the death of Semmelweiss, Louis-Pasteur announced his discovery of the microbe responsible for childbed fever: Streptococcus bacterium.

Louis Pasteur (1822-1895) originally trained as a chemist, flashed up great wave of excitement about microbes. He showed the world how important microbes were to it, and in doing this he made enemies and worshipers. He showed that microbes are the real murderers of the human race. He invented Paste —— on, sterilization, attenuation of microorganisms to immunize. His most hard and dangerous laboratory & animal experiments with the unseen microbe of rabies were a great success.

As a Microbiologist, I spent most of my time working on viruses. What is a virus? Viruses are obligatory intra-cellular parasites of man and animals. They are electron microscopic infectious agents which carry their genetic message in nucleic acids coated by a protein capsid. For the terms of easy understanding, the Nobel Laureate Peter Medawar stated that "the virus is a bad news wrapped in protein".

The land marks in virus research include the discovery of 1st virus - a plant virus TMV by Ivanosfky in 1892, First animal virus FMD by Loeffler & Frosch in 1898 and the first human virus – the yellow fever virus which killed millions over several centuries by Reed et al in 1901. Eradication of small pox, the terrible human plague in the 20th century is the first planned extinction of a human virus. We are going to witness the planned extinction of a second virus – Poliovirus, one which has caused untold number of deaths and deformities through the ages.

Laboratory diagnosis of viruses

Laboratory diagnosis of virus infections were evolved in three stages; 1. Inoculation into embryonated eggs or animals, 2. Evolution of cell culture facilities in 1950s and 3. Provision of diagnostic virology services in recent decades. Important clinical decisions made based on prompt and accurate viral diagnosis, i.e. neonatal herpes and maternal genital herpes, genital herpes in non pregnant women. Availability of paired sera for serodiagnosis, antiviral drugs for treatment of HSV encephalitis, many cell lines, immunological tests for Ag detection and electron microscopy stimulated more request for virus diagnosis. Identification of viruses at present is a complex area, approximately based on 4 categories, namely Structural properties, Biological properties, Antigenic or immunological properties and Molecular properties.

Animal experiments in virology

Experiments using animals have played a crucial role in the development of modem medical treatments against infections. Similar work happens this day & continues to be necessary to minimize existing illnesses and respond to emerging diseases. I quote the following message extracted from the Prestigious Journal – Science which explains the importance of basics in Virology which I have told you up to now.

"Old guard urges virologists to go back to basics"

"The message to the younger generation, with its sleek polymerase chain reaction (PCR) robots, DNA sequencers and high -speed computers: Without bricks-and-mortar virology, it will be much harder to understand and fight the next dangerous virus that comes along."

Science, 6 **July 2001 (293: 24-5)**

Research at the University of Peradeniya: ***Studies on Viral gastroenteritis***

It was well known that the majority cases of infantile gastroenteritis leading to infant morbidities and mortalities were due to rotaviruses. These viruses have been associated with diarrhoeas in several species of young animals including cattle but there was nothing known about existence of buffalo rotaviruses at that time. I was able to detect and isolate buffalo rotaviruses in cell culture in Sri Lanka (1, 2, 3). That finding motivated me to continue studies on pathogenic microbes in particular the **viruses** - the obligatory intra-cellular parasites of man and animals.

The message I can give from that experience is if anyone try to establish cell culture work for virus isolation and serial passages he requires a lot of patience and hard work to be successful in virus research in this country.

Research at the University of Cambridge: ***Herpes* virus *pathogenesis***

Animal models have been of great importance to herpes virus pathogenesis by providing systems to investigate basic virological and immunological aspects of acute & latent infection also to evaluate chemo-therapeutic & vaccination regimens.

The need for an amenable animal model to study EBV stems from the role of this virus in Infectious Mononucleosis (IM) including fatal IM of X-linked Lympho-Proliferative Disease and its association with 3 human cancers – Burkitt's Lymphoma, Nasopharyngeal carcinoma, Hodgkin's disease, lymphomas in post-transplant and AIDS patients.

I studied biological properties of a murid gamma-herpes virus (MHV68) in laboratory mice to *see* whether it can mimic the human infection by EBV. I summarize four of such experiments.

Experiment -1: Virus replication & sites

Inoculation of Balb/C mice by the intranasal route resulted in a productive infection in the lung, with virus present in alveolar epithelium and mononuclear cells. The virus titre (pfu) increases between days 1 and 3 reaching peak levels from 3-7 days post infection with the elimination of infectious virus by days 10-15, indicating active virus replication within the lung (4). As with other gamma-herpes viruses, lymphocytes are the major site of virus persistence. The lymphocyte harbouring virus during latency was identified as an Ig positive B lymphocyte (5, 6).

Experiment -2: Role of T cells & disease

Little is known about the immunological responses to primary gamma herpes virus infection. The role of T cells in a primary infection in mice was investigated using monoclonal antibodies to deplete lymphocyte subsets *in vivo*. Results clearly demonstrated CD8 (but not CD4) cell depletion led to an uncontrolled virus replication in the lung and eventual death (7).

Experiment -3: Association with LPD & lymphomas

It was observed that 9% of chronically infected mice developed LPD similar to that seen in patients infected with EBV and 50% were displaying high-grade lymphomas. LPD was greatly increased with cy closporine A treatment. Based on these studies, many virological and pathological features of this virus i.e., splenomegaly, B cell latency, and association with LPD suggest that this novel murine model provide a system to study antiviral strategies for gammer herpesviruses (8, 9).

Experiment - 4: Efficacy of antiviral compounds

ACV is widely used in clinics as an anti-herpetic agent. Action of ACV on this novel murine gamma-herpesvirus was studied in vitro by a plaque reduction assay in BHK cells showed the virus is sensitive with a 50% effective dose (ED_{50}) of 0.2mg/ml tissue culture medium indicating that the virus shares similarities with thymidine kinase (TK) activity (10). Therefore, animal model permits testing of novel antiviral compounds that target TK (11).

The messages that I can give from these animal experiments are; 1. Animal research has been essential in the past and it will be vital in the future too, 2. Some questions can be answered only by animal research, 3. Studies on the pathogenesis and immunity of viruses or the antimicrobial efficacy of new drugs cannot be studied in humans, 4. The use of animal models to study human infectious diseases is one of the major trends of current research in medical microbiology.

Research at the Faculty of Medicine, University of Kelaniya

(A). Confronting problems of Microbiological services:

I picked three interesting problems which I investigated for the period of 2001-2003.

Study -1: *Bio-burden of doctors' hands & stethoscopes:*

The first study was focused to understand the bio-burden of hands of doctors and other healthcare workers and medical devices i.e. stethoscopes. Special Care Baby Unit (SCBU), Labour room, surgical wards and Theatre and Intensive Care Unit (ICU) were selected for the study. 30 hand imprints of 13 Doctors and other 17 HCW and 85 swabs taken from 6 stethoscopes and 31 common medical equipment were cultured for bacteriology.

Growths of *S. aureus* were isolated from hand imprints of both doctors and HCW (i.e. nurses). Some of the *Staphylococcus aureus* strains isolated were found to be MRSA.
Microorganisms isolated from hand imprints include; *S. aureus* (43%), *Klebsiella*, *E coli* and other coliforms (27%), and Candida (3%).

Therefore, a total of 97%(29/30) hand imprints were contaminated. Complete elimination of resident organisms was observed in the control group when proper hand washing was introduced. Among all investigated equipments 73% (51/85) were contaminated. Type of organisms isolated and their frequencies were similar to that of hand imprints. Complete elimination of resident organisms was observed in the control group when proper hand washing was introduced (12).

Study -2: *Outbreak or pseudo-outbreak of TB?*

An unusually increased numbers of AFB- stain positive broncho-alveolar lavage samples received from NCTH was observed during microbiological investigations. Was it an outbreak? Was it a sterility problem leading to a pseudo-outbreak? I wanted to investigate this problem. Endoscopes represent the medical devices most commonly linked to nosocomial outbreaks of infections

AFB staining & TB culture of 107 sputum and 60 bronchial wash samples received from patients suspected of tuberculosis & saline wash - specimens of the fiber-optic bronchoscope of the NCTH from January 2001 to October 2002 were studied. All specimens were stained by Ziehl-Neelsen stain and out of that AFB stain positive samples were cultured for AFB. Results revealed a pseudo outbreak of acid fast bacilli amongst patients with suspected of tuberculosis by a contaminated fiber-optic bronchoscope. Therefore,

the value of positive AFB stain in bronchial wash samples for predicting pulmonary tuberculosis is questionable. Failure to eradicate contamination of bronchoscope can leads to transmission of Non-tuberculous mycobacteria or Mycobacterim tuberculosis (13).

Study -3: Postpartum sepsis in modern times: Is it same as in the case ofpre- microbiologyera?
Blood culture specimens from two patients with severe post partum sepsis which had challenged empirical antibiotic therapy were my concern for this study. The etiology and the nature of antibiotic sensitivity were the first information to assist the situation.

Patient No. 1:

A primigravida developed septic shock following an elective caesarian section for twins from the first day. The patient had received intensive care and empirical therapy with cefuroxime, metronidazole and gentamicin which was continued despite the negative initial blood culture results from a different laboratory. As the patient was not responding until the 7th day, additional blood culture was collected and sent to my laboratory. Although, we were informed that the patient had died on the 7th day itself, blood cultures were processed to trace the killer organism.

Findings of patient No. 1 showed that 2nd subculture exhibited a heavy growth of Gram negative diplo-cocco-bacilli on the 9th day. The organism was identified as a strain of multidrug resistant but netilmicin sensitive *Acenetobacter lwoffi*.

Patient No. 2:

A primigravida at 37th week developed postpartum haemorrahage following normal vaginal delivery. Total abdominal hysterectomy was performed. This patient too showed evidence of sepsis from day 2 and received intensive care and empirical therapy with metronidazole and ciprofloxacin.

Findings of patient No. 2 showed that the blood culture sent to my laboratory on 3rd day of surgery yielded a growth of Gram negative diplo-cocco-bacilli which was identified as another strain of multi-drug resistant but imipenem sensitive *Acenetobacter lwoffi*. Patient did not respond to the empirical antibiotic therapy but responded to imipenem with an uneventful recovery. These case studies are clear examples of highly virulent multi-drug resistant strains of *Acenetobacter lwoffii* that *had* emerged in a Sri Lankan Hospital (14). Therefore, collection and transport of specimens in time, 2. Simultaneous testing by another laboratory, 3. Prompt microbiological reports consists of sensitivity patterns to a wide range of antibiotics are critically important for the fight against these deadly pathogens.

The Message from hospital microbiology urge us to 'Stick to Basic rules' including Proper hand hygiene, Sterilization and disinfection of medical devices, Prompt specimen collection, transport and laboratory diagnosis, Strict antibiotic policy and Proper waste disposal.

(B). Understanding Viral infections of the Sri Lankan community:
Rabies – as a major zoonotic problem, sero-prevalence of Herpes Simplex virus1 & 2 (HSV1 & 2) in the community, Hantavirus disease with occupational risk and the Need for Point of care diagnosis of dengue are four research studies carried out by me.

Rabies and Public health implications in Sri Lanka

A fatal acute viral disease of the CNS in all warm blooded animals and humans.
Human Rabies is *"The most severe of all communicable diseases". In* Sri Lanka, majority of animal rabies cases (92%) are recorded in dogs, also few cases of confirmed wild animal cases. Based on studies in Sri Lanka, approximately 100,000 persons annually undergo post exposure treatment for rabies. This is an enormous amount of psychological stress and loss of millions of man hours work. At least 50-100 deaths are recorded from the disease in the country each year.

A study on the appropriateness of current rabies post-exposure therapy in a Government hospital of Sri Lanka was carried out on 500 dog bite victims reported for RPEP at the North Colombo Teaching Hospital. This study reveals that there were 413 normal & 87 abnormal animals involved in the exposure. 418/500 (84%) eases were exposed to dog bites. 3831500 (77%) animals were observable. 50% dog bites were due to provocation.
Further, 82 dogs were vaccinated, 148 dogs were unvaccinated and 188 were of unknown vaccination status (15).

The Message on the use of RPEP is often inappropriate. In addition, antirabies antibody level in a vaccinated animal help in the risk assessment for RPEP and in-house tests are needed for monitoring antibody responses of canines following anti-rabies vaccination.

Herpes simplex virus 1 & 2 in the Sri Lankan community
70% HSV infections are clinically unrecognized worldwide. HSV-2 usually causes genital herpes. It is also a common cause of genital ulceration, a marker of sexual behavior. The virus facilitates HIV transmission / acquisition. There are serious consequences due to the infection in pregnancy and in neonatal herpes. However, there were no information on the sero-prevalence of HSV-1 and HSV-2 amongst Sri Lankans. This study revealed the overall seroprevalence (%) by target group amongst Sri Lankans as shown in the Table 1.

Table1: Sero prevalence of HSV-1 and HSV-2 amongst Sri Lankans

Target group	HSV-1	HSV-2
Children	50%	5%
Antenatal women.	76%	8%
Blood donors	79%	11%
Adult in patients	77%	21%
STD attendees	78%	39%

Acquisition of HSV 1 & HSV-2 among Sri Lankans was found to be 34% and 9% day 1 to 1 year of age children were positive for antibodies to HSV-1 and HSV-2 respectively indicating maternal transfer of antibodies at this age. Seroprevalence to HSV-1 increases with age but for HSV-2 declines to 4% by the age of 10-12 years. Acquisition of HSV-2 occurs from teenage to adulthood (16).

The results also revealed that the overall HSV-2 sero-prevalence is lowest in children (5%) and highest in STD clinic attendees (39%) as expected. However, non-high risk adult males (hospital in-patients) over

40 years of age have also reached the sero-prevalence rate of male STD attendees of 20-29 years age. In addition, the highest HSV-2 sero-prevalence amongst male blood donors was 20% observed at the age of 40 years or more and antenatal women of Sri Lanka had the lowest HSV-2 sero-prevalence (8.6%) among non-high risk adults (17).

The messages comes from this study include that ; HSV2 seropositivity is a marker of sexual behavior amongst Sri Lankans as indicated by high percentage among STD clinic attendees and the blood from donors of over 40 years has higher chance of transmitting other STDs such as HIV. Therefore, Screening of donors' blood for HSV-2 IgG may be a useful approach for preventing HIV transmission.

Role of Hantaviruses in patients with leptospirosis-like illness:

Leptospira *(Leptospira interrogans)* & Hantaviruses *are* worldwide zoonoses with similar epidemiology & disease forms. A prospective study carried out among patients hospitalized with leptospirosis-like illness in Sri Lanka in 1997.

Findings of this study indicate that there is mixed / co-infection of Hantavirus and leptospira (22.6%), Some patients were found infected with either virus (6.5%) or leptospira (29%) only and at least three different Hantavirus serotypes circulating in Sri Lanka i.e., Hantaan-like strain, Puumala-like strain and Puumala strain (18).

The Message comes from this study is that the leptospirosis-like illness due to hantaviruses is an example of viral zoonoses in Sri Lanka in which true public health implications to the Sri Lankan community have not been recognized to date.

DENGUE - Meeting the need for dengue diagnosis

Dengue is the most significant mosquito borne viral disease affecting nations from Asia to the Americas including Sri Lanka. Speedy diagnosis save dengue victims due to the fact that there is no dengue vaccine is available and there is no specific treatment for dengue. Appropriate clinical case management can save many lives. Therefore, early and speedy diagnosis is critical for prompt management of the patient.

Challenges in dengue diagnosis include; 1. Differentiation of primary and secondary dengue infection is particularly important in situations such as outbreaks where the allocation of resources needs to be directed to those at greatest risk, 2. One area that has been challenging in the diagnosis of dengue is remote areas or clinics of general practitioners where the amenities of collecting samples and transporting to an appropriate testing centre are lackiig. Therefore, the application of Point of care (POC) dengue diagnosis is studied to meet these unmet demands by a multi centre study carried out in 331 dengue suspected patients of the Gampaha district of Sri Lanka. In this study, a novel rapid assay was evaluated for the point of care (POC) diagnosis of dengue fever using a finger prick drop of blood as the analysate.

Findings of Point of Care testing of Dengue patients in Sri Lanka showed that the use of whole blood dengue lateral flow device is a valuable field based assay to support the clinical evaluation of patients and it has a high clinical utility as a field device which has the ability to rapidly and accurately detect and differentiate dengue infections.

The final Message from these studies highlight the importance of constant surveillance on infections, development of infra-structure and capacity building in laboratories for research, and that the joint work of both Medical and Veterinary communities in the control of zoonoses and tracking down emerging virus infections are the ways forward to 'Tracking down Microbes at present times in Sri Lanka

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