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Do we know enough of our own backyard ? Influenza surveillances in Sri Lanka over the last decade

The President and the Council of Sri Lanka College of Microbiologist, ladies and gentlemen, my dear colleagues. It is a great honor to have been invited to deliver this year's Sri Wickramasinghe oration.

The Late Dr. Rakkitha Sirimal Bandara Wickremesinghe, or Dr. Siri Wickremesinghe as we called him, was born on 28th November 1937 to Dr. Artie and Helen Wickremesinghe. He obtained his education at prestigious Royal College Colombo, and graduated MBBS in 1963 from the Faculty of Medicine, Colombo.

He started his medical career in the Dermatology Unit, General Hospital Kandy. He later joined the Medical Research Institute (MRI) and began his career in the Department of Bacteriology. He travelled to the University of Manchester to obtain his Diploma and Master of Science in Microbiology. Subsequently, he obtained MD in Microbiology from the Postgraduate Institute of Medicine, University of Colombo and was Board Certified in Microbiology in 1983. He continued to work at the MRI as the Consultant Microbiologist until he left to Australia with his family. Having worked in the Fairfield Hospital in Melbourne, he returned to the MRI and continued his service as the Consultant Microbiologist in charge of the bacteriology division until his retirement. He held the Director post in the MRI from 1996 to 1998.

He was a past President of the Sri Lanka College of Microbiologists and provided yeoman service as Secretary of the Board of Study in Microbiology at the Postgraduate Institute of Medicine. He was an exemplary microbiologist and a versatile teacher. I was one of the later generation microbiologists who immensely benefited from his oceanic knowledge in the field of bacteriology. Yet, he was a humble personality and I have travelled in a number of occasions in his ash "doctor" sunny from Ragama to MRI, when I was reading for the diploma in microbiology.

After retirement he worked as the Resident Pathologist and Laboratory Manager at Durdans Hospital, Colombo. At the age of sixty-six, on 08th April 2003, our beloved Dr. Siri Wickremesinghe departed from this world, leaving behind a legacy that to date stands unchallenged and unparalleled in the field of microbiology in Sri Lanka.

Background

Imbalance of the host pathogen homeostasis has led to various novel infectious diseases among humans. Infectious pathogens are arguably among the strongest selective forces that act on human populations. Most of these infections have been transmitted from wild or domesticated animals and co-evolved with human ancestors. Unarguably, hunter gatherers may have had no major infectious threats. However, cultural, agricultural and industrial revaluations narrowed the animal-human interface, which not only permitted spillover of pathogens, but also facilitated sustained transmission of infectious agents in communities leading to epidemics. Mass migrations exposed geographically confined novel pathogens.

Documented historical sources, "influenza like" outbreaks have appeared at intervals since ancient times. Hippocrates described a disease, which resembled influenza as early as 412 BC. A number of outbreaks

suggestive of influenza have also been documented in medieval Europe.

Influenza infections are reported globally with an annual attack rate estimated at 5% – 10% and 20% – 30% in adults and children respectively, leading to about 3 – 5 million cases of severe illness and about 250,000 to 500,000 deaths annually.

Introduction

Influenza viruses are enveloped, negative stranded segmented RNA viruses belonging to the Family *Orthomyxoviridae* and Genus Orthomyxovirus that in turn consists of five genera: ***Influenza virus*** A-C, ***Isavirus and*** *Thogotovirus*. Recent studies have identified a novel genus of influenza virus in cattle and dogs and have tentatively been named *Influenza virus D*. Of these, influenza virus types A and B cause regular epidemics, and type A occasionally causes pandemics. Influenza type C infects humans, but causes little or no disease. Influenza types A and B are grouped based on antigenic differences of their nucleocapsid and matrix proteins. Influenza A viruses are further subtyped based on the antigenic differences between two surface glycoproteins, the haemagglutinin (HA) and neuraminidase (NA). Eighteen (18) HA subtypes and 11 NA subtypes have been identified, and they are designated H1-H18 and N1-N11 respectively. Wild aquatic birds are considered to be the natural reservoir of influenza A viruses of subtypes H1-H16 and N1-N10. Recently, novel influenza A H17N10 and H18N11 viruses have been detected in bats. Nevertheless, limited number of influenza subtypes, 3 HA subtypes (H1-H3) and 2 NA subtypes (N1, N2) are so far known to have established themselves in the human population, although a number of other subtypes (e.g. H5-H7, H9, H10) have caused occasional zoonotic infections.

There are no known animal reservoirs of influenza B and influenza C viruses and these infections are mainly confined to humans, although infrequently isolated in non-human species. Only single subtypes of HA and NA are recognized in influenza B and C viruses. Influenza B has two designated virus lineages; Victoria and Yamagata that differ in their serological cross-protection.

The hallmark of influenza virus is its ability to evolve continuously through the mechanisms of antigenic drift and shift. Antigenic drift is a result of slow antigenic changes in the virus HA and NA genes through the accumulation of mutations in the error prone viral genome due to lack of "proof-reading" mechanism of the viral polymerase, under influence of positive selection pressure of the host immune system, which selects mutants that escape preexisting neutralizing antibodies. The emergence of antigenically drifted viral strains forces to change the constituents of influenza virus vaccine strains annually.

Influenza A virus occasionally undergoes swift changes of the antigenic properties of the HA and NA. These unpredictable events lead to dramatic antigenic changes in the major immunogenic surface proteins of the influenza A virus through genetic reassortment between a human and non-human influenza virus and is known as antigenic shift. If the reassortant virus has gained efficiency and sustains human-to-human transmission, it could lead to unprecedented spread in the immunologically naive human population leading to a pandemic. Four such human pandemics have occurred in the past 100 years: in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 2009 (H1NI).

The severity of annual seasonal epidemics is determined by serotype of the virus and immune status in the general population. The peak activities of influenza vary depending on the prevailing climate in a given region. For example, in Sri Lanka, influenza virus activity peaks correspond to a peak in rainfall. In general influenza viruses in tropical and subtropical regions could be detected at low levels outside the peak periods of viral activity, indicating possible circulation throughout the year with peaks during rainy seasons. In contrast, in temperate climates influenza shows a seasonal pattern with high incidence in winter months. Although the seasonality of influenza differs in the tropical and temperate regions, the overall disease burden and mortality remains comparable.

Influenza is an acute respiratory disease with incubation period ranging from 1-4 days that spreads by large droplet, small-particle aerosols or through contact with fomites. Peak virus shedding occurs from 1 day before onset of symptoms to 2-3 days after disease onset. Children younger than 2 years and the elderly population >65 years of age, and those with co-morbidities (respiratory or cardiac disease, diabetes and renal failure) have highest hospitalization rates. Acute influenza symptoms start abruptly with fever, headache, muscle ache, malaise and fatigue symptoms, which are caused by a number of inflammatory cytokines released during the early stages of the illness. These are followed by respiratory symptoms such as cough, sore throat and coryza. The clinical spectrum of infection could range from asymptomatic infection to primary viral pneumonia. In general, acute illness lasts for a week or so, although malaise and dry cough may continue for 2-3 weeks or much longer. Known pre-existing medical conditions (respiratory or cardiac disease, diabetes and renal failure), pregnancy and smoking could worsen the clinical outcome. Secondary bacterial pneumonia and exacerbation of underlying chronic health conditions are known complications of influenza. Myositis, myocarditis, toxic-shock syndrome and Reye's syndrome in children have also been infrequently reported.

Antigenic properties of influenza HA are the key determinant of virus tropism and host range as well as pathogenesis of influenza virus. The HA molecules of influenza viruses that have been isolated in avian species

and horses showed preference for binding to sialic acids with α2, 3 configurations. In contrast, HAs of influenza viruses from humans and other mammalian species show enhanced binding to α2, 6-linked sialic acids configuration. Additional human studies have shown that α2, 6 receptors are predominant on respiratory epithelial cells in the nasal mucosa, paranasal sinuses, pharynx, trachea and bronchi and α2, 3 receptors are abundantly found on non-ciliated cuboidal bronchial cells at the junction between the respiratory bronchial and alveolus and on type II cells in the alveolar walls. For influenza virus to be efficiently transmitted among humans, they need to infect the upper human airways, which predominantly contain a2, 6 receptors. This explains, that avian viruses that bind to α2, 3 receptors unlikely to replicate in the upper human airways and fail to be transmitted efficiently between humans. Thus if a highly pathogenic avian influenza (HPAI) H5N1 viruses were to gain access to the human respiratory tract, it could potentially infect and replicate in the lower respiratory tract possibly leading to severe life threatening pneumonia, but shows limited human-to-human transmission.

In contrast, pig trachea contains dual receptors (α2, 3 and α2, 6) and has been recognized as a host where co-infection of avian and human influenza viruses may occur. Thus facilitates genetic reassortment and subsequent adaptation of novel influenza virus that leading to pandemics.

Despite the common belief that influenza viruses exhibit tight species barrier, zoonotic infection of HPAI have been reported; H5N1 in Asia, H7N7 in the Netherlands and H7N9 in China. A number of low pathogenic avian influenza viruses such as H9N2, H6N1, H10N8 and H5N2 have also been sporadically isolated from humans in Asia. Adaptation of HPAI H5N1, emergence of H1N lpdm09 and a novel swine-origin human A H3N2 variant viruses [A(H3N2)v] in the USA have raised pandemic concern.

A number of different antivirals have been used against human influenza infections. The adamantanes (amantadine and rimantadine) act by blocking influenza A virus uncoating. However, many contemporary influenza virus strains are resistant to these group antivirals. Neuraminidase inhibitors (oseltamivir and zanamivir) block the activity of the virus neuraminidase in releasing the virus after replication in infected cells and provide clinical benefit when used within the first 48 hours after onset of disease. Current seasonal and zoonotic influenza viruses are generally sensitive to neuraminidase inhibitors. Occasional resistance to oseltamivir has been reported in individual patients but such resistance is not currently widespread. Early treatment with antivirals could reduce the duration of illness, antibiotic usage and lower the risk of complications.

Vaccine remains the cornerstone for prevention of seasonal influenza. Such vaccines contain two influenza A subtypes H1N1 and H3N2 and one influenza B virus lineage, viz the one assessed to be the lineage that is likely to become globally dominant. Since the assessment of the influenza B virus lineage that may become globally dominant in the year ahead is difficult, more recently, quadrivalent vaccines have been produced. Thus, the emergence of the unexpected influenza B lineage or the antigenically drifted can occur at repeated intervals. Currently available vaccines against influenza do not induce long-lasting immunity and provide protection only against strains closely related to the vaccine strains. Thus new vaccines are required to be reformulated for every flu season, based on global human influenza surveillance data. The subtype of future outbreaks or pandemic influenza strains is also unpredictable. Hence, development of a successful "universal" vaccination strategy is urgently needed and a number of research teams are currently involved in such vaccine design. A systematic approach of interdisciplinary research, newer approaches of vaccination, increasing use of vaccines and rational usage of antiviral drugs to combat annual influenza outbreaks are essential to reduce the global toll of epidemic and pandemic influenza.

Influenza surveillance in Sri Lanka over the last decade

Human influenza

During the period July 2003 – August 2004, 300 nasopharyngeal aspirate (NPA) samples were obtained from patients reported to the Out Patients Department, Colombo North Teaching Hospital, Ragama, with ≤ 4 days history of acute respiratory tract infection (ARTI). Influenza virus was isolated in monolayers of Madin Darby Canine Kidney (MDCK) cells. Isolates were identified by influenza A and B FITC monoclonal antibodies and characterized by haemagglutination inhibition assay. RT-PCR was carried out on all human influenza A isolates. Genetic sequencing and phylogenetic analysis of the haemagglutinin gene of representative isolates were carried out and phylogenetic tree was constructed.

It is revealed that both serotypes of influenza viruses (A and B) circulate at different times of the year and was the aetiological agent causing 11% of all ARTI. The largest peak of influenza A virus activity detected during May-June 2004, corresponded to peak in rainfall in the country. However, the peaks of influenza A did not correlate with that of influenza B activities. Influenza B virus activity occurred from September 2003 to December 2003 at low levels without a major peak of virus activity. About sixty percent (60.6%) of influenza viruses (influenza A and B) were isolated from patients less than 15 years of age, suggesting that influenza virus infection is more prevalent among this age group, at different times of the year. It was revealed that RT-PCR was more sensitive for the detection of influenza A than culture and 14% of influenza A positives would have been missed if only virus culture was relied upon. As we did not perform RT-PCR for influenza B, we probably may have missed a proportion of influenza B viruses as well. Our data were based only

on a single year and data from several years are needed before conclusions can be drawn on the seasonality of influenza virus activity in Sri Lanka. Such information is important before the timing of influenza vaccination for Sri Lanka is determined. Identification of prototype influenza virus for the annual influenza vaccination programme heavily depends on systematic surveillance of these viruses. Thus it is important to strengthen the influenza surveillance programme in the country.

In 2006, a sporadic respiratory outbreak was reported in the remand prison Mahara. A/H1N1/2000/99-like influenza virus was isolated from NPA samples obtained from inmates by inoculating into MDCK cells. Early aetiological detection enabled the Epidemiology Unit to start rimantadine, which controlled the outbreak that affected more than 100 prisoners and reflects the importance of early detection of the aetiological agent in public health perspectives. However, two prisoners succumbed due to secondary pneumococcal pneumonia.

Nasopharyngeal aspirate or nasal swab samples that were tested positive for H1N1pdm09 by RT-qPCR were obtained from the Medical Research Institute, Borella, Colombo, Sri Lanka. These samples had been collected in 2009 (n=263), 2010 (n=100) and 2011 (n=100). Twenty six (26/463) human H1N Ipdm09 viruses were isolated on MDCK inoculations. These samples had been sourced from hospitals located in different administrative districts of the country. Poor cold-chain maintenance and repeated freeze-thaws may be responsible for the low success rate for virus isolation from RT-PCR positive specimens. In addition9 Sri Lankan human H1N1pdm09 viruses isolated in 2009-2012; three viruses in 2009, one virus in 2010, two viruses in 2011 and three viruses in 2012; were obtained from World Health Organization, Influenza Collaborating Centre, Melbourne, Australia. Except in two Sri Lanka human H1N Ipdm 2009 isolates, A/SLK/24206/2009 and A/SLK/24558/2009, which had D222E substitution in HA gene, the other Sri Lankan humans isolate had conserved the consensus D222 in HA gene. Both Sri Lankan patients detected with D222E mutant H1N1pdm 2009 viruses had fatal infections. A mutant with a D222G or D222E substitution (D225G or D225E in the H3 numbering system) in the receptor-binding site of the virus haemagglutinin of H1N1pdm09 virus has caused a substantial number of severe and fatal infections. Although detected sporadically, the D222G substitution has been observed to correlate with cases of severe or fatal disease.

The H275Y and N295S substitutions in the NA gene, which are associated with oseltamivir resistance, were not present in Sri Lankan human isolates. However, these findings do not reflect the broader molecular picture of the human H1N Ipdm 2009 viruses isolated in the country as only 35 human viruses were included in the molecular characterization.

Swine surveillance

H1N1, H3N2 and H1N2 subtypes of influenza A viruses have been widely reported in pigs. Influenza A infections in swine causes respiratory disease in pigs, can lead to significant economic impact and plays paramount importance in generation of pandemic influenza in humans. Endemic swine influenza viruses have derived either directly from avian or human influenza viruses or by reassortant between them. The concept of pigs as a "mixing vessel" for influenza A viruses was proposed. The presence of both Sia a2-6 and Sia α2-3 in the swine trachea enables it to play a unique role in the epidemiology of influenza A infection. Concurrent infection of a single porcine host cell by distinct subtypes of influenza A viruses could lead to reshuffling of the gene segments leading to the generation of an influenza virus strain with a novel gene constellation. It is believed that most human pandemic viruses of 1957 and 1968 were generated in this manner.

The H1N Ipdm09 virus probably emerged from swine into humans though reassortment between the recent North American triple reassortant H1N2 swine viruses and Eurasian avian-like swine viruses. The double (either avian and human or human and swine), and triple (human, avian and swine) reassortant influenza A viruses isolated in pigs in North America in the 1990's period provide good examples of the "mixing vessel" theory. Isolation of wholly avian influenza viruses in European swine in 1979, a novel wholly avian H1N1 influenza in pigs in China, wholly avian influenza viruses (H4N6, H3N3 and H1N1) in Canadian pigs, sero epidemiological evidence of infection of H4, H5 or H9 avian influenza viruses in Asian swine herds, H9N2 avian influenza viruses isolation from pigs in several provinces in China and Hong Kong and isolation of highly pathogenic avian influenza H5N1 in pigs in some Asian countries supports the concept that pigs are permissible to influenza viruses of avian origin. Experimental settings documented that pigs are susceptible to HI-HI3 subtypes of avian influenza viruses and may be susceptible to H14-H16 subtypes as well. These observations and experimental findings strongly support the fact that swine could serve as both direct and intermediate hosts to different subtypes of avian influenza viruses particularly highly pathogenic avian influenza of the H5 and H7 subtypes. However, more recent studies suggest that swine may not be as readily permissive to a wide range of avian influenza viruses. For example there is low susceptibility of highly pathogenic avian influenza H5N1 infection to domestic pigs in nature and under laboratory conditions.

The landscape of the epidemiology of swine influenza viruses has been dramatically altered by the isolation of H1N Ipdm09 in swine herds leading to emergence of a range of novel reassortants viruses containing one or more gene segments of the H1N1pdm09 virus. Therefore, susceptibility to both avian and human influenza A viruses in pigs will continue to provide opportunities for the introduction of new influenza viruses, some of which may have capacity for interspecies transmission. Such reassortments are facilitated by regular close contact of swine with humans or birds.

Since ancient times, Sri Lankans had engaged in hunting and consuming wild boars (Sus scrofa). Accurate data on the introduction of domestic pig rearing in Sri Lanka is not available. However, it is highly likely that during eighteenth century the British planters introduced the practice of pig farming into the country simply to meet with their own pork demand. Initially, swine husbandry was based on smallholder backyard farming. In more recent years, backyard swine farming had reduced and modern livestock practices have been incorporated into the pig production system. The livestock statistics show that the swine population in Sri Lanka is around 80,000 and pork contributes to 1% to the livestock component of the Gross Domestic Product (GDP) of the country. Swine farms are predominately (– 61%) located in the Western coastal belt spanning Puttalam, Gampaha, Colombo and Kalutara administrative districts of the country, identified as the "pig belt" of Sri Lanka.

Currently, several different pig production systems are in operation in the country. This is based on the herd size, breeds, feeding systems, and market channels. Sixty percent (60%) of the farms are of small scale (<50 animals), 25% are of medium scale (51-100 animals) while only 15% are of large-scale.

The small-scale farmers mainly raise indigenous animals and cross breeds deriving from them. The animals are mainly fed with swill, rice bran kitchen refuse and non-human edible chicken refuse. Exotic breeds such as Land Race, Large White are backcrossed with Duroc breed. These animals are descendants of two main nucleus herds from the state-owned National Development Board (NLDB), Sri Lanka swine breeding farms located in Welisara and Horekelle. Medium scale farmers practice semi intensive farming system and raise indigenous pigs and their crosses.

The Government Slaughterhouse located at Dematagoda, Colombo operates for 6 days per week and slaughters around 20 pigs per day or more depending on the available number of pigs on a given day. The Dematagoda swine abattoir is under the direct purview of a veterinary surgeon in-charge of the site, which comes under the authority of chief veterinary surgeon of the Colombo Municipal Council (Dr. Colin Perera, personal communication). More than 90% of the animals that are slaughtered at the Dematagoda Government Swine Slaughterhouse receive pigs from the swine farms located in the Puttalam, Gampaha, Colombo and Kalutara administrative districts.

The pig population densities in the Puttalam, Gampaha, Colombo and Kalutara districts in 2010 were 7, 15, 12, and 1 animal per km^2, respectively. In 2001 (latest available human census data) for these districts, the human population densities were 246, 1,539, 3,330, and 677 persons per km^2, respectively.

Pigs are not imported on a regular basis to the country. Only on three occasions during last three decades have such imported consignments been documented. It is recorded that 30 live pigs were imported from Australia in 1987, and 32 and 26 animals from USA in 1995 and 1998, respectively (Dr. Pushpa Wijewantha, personal communications). Hence, the breeding program of the country is heavily dependent on artificial insemination. However, the pig-breeding farm located at NLDB Welisara maintains Land Race and Large White animals and surplus piglings are sold to farmers for breeding.

Although epidemiological and virological studies on influenza in humans have been carried out there is only one other study on influenza virus circulating among pigs in the country. Therefore, this study was carried to determine the ecology and evolution of swine influenza viruses in Sri Lanka.

The information that is generated from this study is not only important for monitoring the emergence of new influenza pandemics but would provide the opportunity to understand genetic diversity of the influenza viruses circulating among pigs in a South Asian country.

2004-05 period

Paired tracheal and nasal swabs, and blood samples were collected from freshly slaughtered pigs (n=300) brought to Government slaughterhouse, Dematagoda, Colombo in 2004-05. Samples were inoculated into monolayers of Madin Darby Canine Kidney (MDCK) cells and embryonated chicken eggs.

One influenza A virus, A/swine/Colombo/48/2004(H3N2), was isolated in MDCK cells from a tracheal swab sample collected in 2004 – 2005. All genes of this virus were closely related to human influenza (H3N2) virus isolate A/Ragama/190/2003 from Sri Lanka and to other subtype H3N2 influenza viruses isolated worldwide at this time. During January 2004 – March 2005, a total of 185 (61.6%) of 300 serum samples tested were positive for A/swine/Colombo/48/2004(H3N2); indicating that this human-like influenza (H3N2) virus was widespread in the swine population.

2009-13 period

Recommencement of our systematic virological and serological surveillance in swine abattoirs in Sri Lanka, during 2009 – 2013 detected H1N1pdm09 like virus in local swine herds. Infection in pigs followed each of the H1N1pdm09 outbreaks in humans; October 2009 - January 2010, October 2010 – February 2011 and November 2012 – March 2013, respectively. Genetic, phylogenetic, and epidemiologic analysis of the human, and swine influenza viruses indicated spillover events of H1N1pdm09 from humans into pigs, with self-limited transmission and extinction within pig herds. The data also indicated that although H1N1pdm09 was able to spill over from humans to swine, it is not ideally adapted to establish sustained transmission among swine in the absence of further reassortment with other swine influenza virus lineages.

These findings might reflect characteristics of swine husbandry in Sri Lanka, which has a low density pig population and remains isolated from global swine influenza viruses because of the absence of regular cross-border and cross-continental movements of swine. In contrast to some other parts of the world, we failed to isolate established lineages of swine influenza viruses, viz. Classical, North American triple reassortant and European Avian lineages. Seroprevalence to these endemic swine viruses was largely absent in local swine herds. Serum samples collected from swine during 2009-2012 were also mostly seronegative to this and to more contemporary human influenza (H3N2) viruses. To clarify transmission patterns between affected swine farms in Sri Lanka, we obtained contact patterns by interviewing pig farmers using a structured questionnaire with approval from the Ethics Review Committee, Faculty of Medicine, Ragama, Sri Lanka. There was no evidence of movement of persons or fomites between farms. However, during the peak demand period (November-December) of each year that surveillance was performed, a common truck owned by one farm, and driven by a single driver and an assistant, provided transportation from multiple farms to the abattoir, including from affected farms. On some occasions, animals taken to the abattoir for slaughter were returned to the farm.

This extended swine influenza study demonstrates natural independent spillover events of H1N1pdm09 influenza viruses from humans to swine. H1N1pdm09 viruses appear to be spread by multiple, discrete introductions to swine, afterwhich clonal expansion occurs within the swine. The spread of such virus lineages across multiple farms is consistent with virus dispersal by breaches of external biosecurity measures, including the manner of swine transportation, although this remains unproven given the small sample size. Unlike classical swine influenza, North American triple reassortant, and European avian swine viruses that have persistently circulated among swine for several decades in other countries, H1N1pdm09 does not appear to establish long-term lineages in swine in the absence of further reassortment. This observation requires confirmation in other geographic settings.

Avian Surveillance

Avian influenza: 2003-05

We conducted surveillance of avian influenza viruses in the live poultry markets, backyard flocks and small-scale poultry farms in Western Province in the country during 2003-2005. In total, 750 birds were sampled, and a tracheal and cloacal swab and blood sample were collected from each bird. Tracheal and cloacal swab samples were inoculated into embryonated chicken eggs and serum samples were tested by haemagglutination inhibition assay.

Ninety six (96, 12.8%) birds were seroconverted for A/quail/HK 1721-30199 (H6N1) and 39 birds (5.2%) were seropositive for A/duck/ Hong Kong /Y280 (H9N2) – like viruses. A total of 28 out of 750 (3.7%) bird sera tested were seroconverted for both A/quail/HK 1721-30/99 (H6N1) and A/Duck/ Hong Kong /Y280 (H9N2) – like viruses indicating dual infection. Higher number of layers showed seroconversion for bath subtypes of viruses to that of broiler birds. However, we failed to isolate avian influenza virus.

Avian influenza: *2009-2013*

We sampled 3650 domesticated birds (chicken n=2500, duck *n*=600, turkey *n*=250, quails *n*=300) Like in the 2004-05 period, a tracheal and cloacal swab and blood sample were collected from each bird. Serological studies revealed that H6 subtype of avian viruses largely replaced by H9 in 2009-13. Interestingly, seroprevalence of both serotypes of avian influenza viruses; H9 and H6 have dropped to 4.3% and 6%, respectively. Over 1500 migratory birds droppings and 500 bat rectal swab samples tested negative for influenza virus by RT-PCR method.

We failed to detect avian influenza viruses by embryonated chicken eggs or molecular methods in both domesticated and wild birds. However, NDV virus was frequently isolated in avian swab samples that tested negative for avian influenza viruses. However, seroepidemiological studies indicates that prevalence of avian influenza viruses is low in comparison to neighboring countries.

Avian astrovirus

We extended our studies to identify novel avian astroviruses. From our surveillance of astroviruses in poultry, chicken astroviruses (9.6%, 271282) were detected from chicken samples collected in Sri Lanka, while Turkey Astroviruses 1 (TAstV1) was detected from 3 cloacal swab samples collected from apparently healthy chickens in poultry in Sri Lanka. This is the first report of the detection of TAstVl-like virus in chickens anywhere. The chicken farm where these chicken samples were collected did not house turkeys. The source from which chickens acquired infection of these viruses was unknown. No avian astrovirus were detected in cloacal swabs collected from quails, ducks, and geese. The sample sizes of the minor poultry were smaller.

Novel corona and astro viruses in bats

A project on identification of novel corona and astrovirus in bats was commenced in early 2012. We collected 290 droppings, 250 rectal swabs and 220 oral swabs from 15 different bat species from different locations in the country. A novel coronavirus linage was detected in Sri Lankan flying foxes (Pteropus giganteus). A species of bat viruses that related to BtCoV-HKU-3 and SARS corona virus was detected in Rouseftus *leschenaultia*. Another species of bat corona virus that is related to BtCoV-HKU5-1 was detected in Rhinolophus *rouxii*.

History has proven that influenza is and will remain as a grave medical threat to humans and many livestock. A

systematic approach to interdisciplinary research and efficient networks with regular close communication between infectious diseases, scientific and veterinary professionals is crucial in order to assess and early detection of novel influenza viruses, specifically subtypes which could potentially lead to future influenza pandemics.

Therefore, a systematic approach of interdisciplinary research, newer approaches of vaccination, increasing use of vaccines and rational usage of antiviral drugs to combat annual influenza outbreaks are essential to reduce the global toll of epidemic and pandemic influenza.

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