

TITLE:

Emerging **Viral Infections in India**

ABSTRACT:

Despite an elaborate armamentarium to tackle microbes, emerging infectious diseases remain a crucial global challenge. Emerging infections can be defined as “infections that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range.” Several factors like increase in international travel and trade, human encroachment on wild-life habitats, changes in agricultural practices and wild-life trade have contributed to the emergence of pathogens. Emergence/re-emergence of several viral infections has been reported from India in the past few decades; some of the important emerging viral infections are discussed in this review. They include infection due to Nipah, Hantaviruses, Chikungunya, Human Enterovirus-71, Influenza, Chandipura, Crimean Congo, SARS Coronavirus, Buffalopox, Dengue and Japanese Encephalitis viruses. Creating increased awareness and training of clinical microbiologists/virologists for identification of new/emerging pathogens, and prompt reporting and management of outbreaks is essential to tackle the threat posed by emerging/re-emerging infections.

Introduction:

It is time to close the book on infectious diseases and pay more attention to chronic ailments such as cancer and heart disease.... the war against infectious disease has been won.” This was the remark, made in 1967, by the then US Surgeon General William H. Stewart, buoyed up by the accelerating pace of scientific and technological progress, and the conquest of infectious diseases accomplished by use of “miracle drugs”-antibiotics, and vaccines. To write about infectious diseases, in 1962, said Macfarlane Burnet, Australian virologist and Nobel laureate “is almost to write of something that has passed into history.”

This over-optimism was soon shattered by the recognition of a new disease of infectious origin in 1981-AIDS, which remains the global killer today, threatening to outdo the Black Death of the fourteenth century and the Spanish Flu pandemic of 1918 which killed at least 50 million people, each. Indeed, despite an elaborate armamentarium to tackle infectious bugs, the continual evolution of emerging and re-emerging diseases, due to the astonishingly diverse microbial world and its adaptive capacity will remain a crucial global challenge in the twenty-first century.

Emerging infections can be defined as “infections that have newly appeared in a population or have existed previously but are rapidly increasing in incidence or geographic range.”[1] Emerging infections can be further classified as ‘newly emerging’, ‘re-emerging/resurging’ or ‘deliberately emerging’, since the underlying causes of emergence and preventive strategies differ between these groups. Newly emerging infections are those that have not previously been recognized in man. Re-emerging and resurging infections are those that existed in the past but are now rapidly increasing in incidence or in geographical or human host range. Deliberately emerging infections are infections due to microbes (naturally occurring or bio-engineered), deliberately introduced by man, usually for nefarious use [2].

International Travel and Trade ::: Factors Contributing to Emergence of Viral Diseases:

Microbes know no geographical boundaries and their dissemination is accelerated by human mobility. Circumnavigation of the globe took more than a year a century ago; today it can be completed in a record 67 h! [3] At any given time, over a million people, each one a potential carrier of pathogens, are airborne around the planet. Transit times of people and other commercial goods which can be a potential source of pathogens and vectors are very short now, compared to the incubation times of diseases. Carriers of disease can reach their destination before the threat they harbour is detectable, reducing health quarantine to a near absurdity [4]. Rapid global spread of the recent 2009 H1N1 pandemic influenza, introduction of *Aedes albopictus* (Asian tiger mosquito) into the United States, Brazil and parts of Africa in shipments of used tyres from Asia [5] and the world-wide spread of hantaviruses by rats [6] can be attributed to international travel and commerce.

Human Behaviour and Demographics, and Advanced Medical Care ::: Factors Contributing to Emergence of Viral Diseases:

Mass migration of people due to war, natural calamities or economic hardships play a key role in disease emergence. Other aspects of modern human behaviour contribute to the emergence of novel infectious diseases, e.g., water shortage, leading to increased usage of open containers for water storage, creates a breeding ground for mosquito vectors (Dengue), social practices like tattooing in tribal areas in India (HBV, HCV and other blood borne viruses), rural urbanization, sexual behaviour (HIV), needle sharing among IV drug abusers (HIV, HCV), use of contaminated blood/tissue products (HIV, HBV, HCV, vCJD), contaminated vaccines (polio vaccines contaminated with SV40-linked to increased risk of non-Hodgkin's Lymphoma). Immune deficiency associated with AIDS, and advanced medical care including cancer chemotherapy and organ transplantation, has contributed to an enormous increase in an immunocompromised population world-wide. 'Opportunistic re-emerging Infections' are frequently seen in such individuals due to reactivation of latent viruses or unrestrained replication of endogenous viruses, leading to enhanced virulence or changes in host or tissue tropism (CMV, EBV, HHV-6, Parvovirus B-19, JC, BK etc.) [2, 7].

Bio-Terrorism ::: Factors Contributing to Emergence of Viral Diseases:

Subsequent to the anthrax attacks through the US mail system in 2001, the possible use of viruses as weapons of bioterrorism has been considered. Potential viruses that can be deliberately introduced into susceptible human population as weapons of bio-terrorism include smallpox, Ebola, Marburg, Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Lassa fever virus etc. The potential use of genetically engineered viruses with enhanced virulence is also a possibility.

Microbial Adaptation ::: Factors Contributing to Emergence of Viral Diseases:

Microbes are constantly evolving to adapt to their environment. Many viruses like Influenza show a high mutation rate and rapidly evolve into new variants against which the immune system fails to mount a response, and vaccines against existing strains are ineffective. In certain viruses evolution of a new variant may result in new manifestation of disease, change in host or tissue tropism or altered virulence. Emergence of drug-resistant microbes by natural selection confers an added advantage to them. The molecular evolution of Chikungunya virus (CHIKV, A226V mutation in glycoprotein envelope gene) leading to a higher efficiency of replication and dissemination in the vector species *Aedes albopictus*, is reported to have contributed to resurgence of Chikungunya fever (CHIK) in Kerala state in 2007 [8].

Human Encroachment on Wild-Life Habitats ::: Factors Contributing to Emergence of Viral Diseases:

"When humans get infected, it's not because the virus is 'wily' or 'devious,' adjectives applied to this complicated pathogen. Humans get infected, quite often, simply because they have put themselves in some virus's way." [9]

Deforestation, expansion of human habitat and mining activities have been suggested as risk factors associated with re-emergence of vampire bat rabies in the Amazon basin [10]. The dramatic increase of human cases of Kyasanur Forest Disease (KFD), a tick-borne flavivirus in south west India in 1983 can be attributed to deforestation and agricultural development, which increased exposure of humans to the virus and the vector (ticks). Usage of cleared areas in the forest for cattle grazing favoured the proliferation of the ticks (*Haemaphysalis spinigera*) which prefer to feed on bigger animals, including humans [11].

Changes in Agricultural Practices ::: Factors Contributing to Emergence of Viral Diseases:

The emergence of Argentine haemorrhagic fever and its expansion to more areas in Argentina, has been linked to agricultural activities like cultivation of corn, that sustain the corn mouse, the main reservoir of the Junin virus, causing the disease [12]. In India, Japanese encephalitis was introduced where dams and canals were built to bring in water for rice cultivation. A large pig population is conducive for outbreaks of JE, while a large cattle population may support mosquito feeding, but act as dampeners for spread of JE, since viraemia is not present [7]. The worst affected districts in Kerala during the 2007 Chikungunya outbreak, Kottayam and Pathanamthitta, are hilly and heavily forested, with vast rubber plantations. The rainwater that collects in the hemispherical containers fitted to the trunks of rubber trees for latex collection in the region

supported prolific breeding of *Aedes albopictus* mosquitoes contributing to resurgence of the disease [8].

Wild-Life Trade/Ecotourism ::: Factors Contributing to Emergence of Viral Diseases:

Wild-life constitutes a large and often unknown reservoir of diseases; an estimated 75% of emerging infectious diseases are zoonotic, mainly of viral origin, and likely to be vector-borne [13]. Several factors related to wild-life, like wild-life trade and translocation, live animal and bush-meat markets, consumption of exotic foods, ecotourism, access to petting zoos and ownership of exotic pets contribute to emergence of infectious diseases [11]. Outbreaks of Marburg and Ebola have been associated with transport of animals. The rapid economic growth in southern China leading to an increasing demand for animal proteins including those from exotic game food animals such as civets, and many varieties of these wild game mammals in overcrowded cages in wet markets allowed the jumping of the novel SARS coronavirus from animals to human [14]. Currently very few countries have active wild-life disease surveillance and it is the need of the hour to monitor the emergence of novel microbes.

Nipah Virus ::: The Emergence of Viral Diseases: Indian scenario:

Nipah virus, a zoonotic paramyxovirus, is an emerging virus endemic in Southeast Asia. Beginning in September 1998 a major outbreak of disease in pigs and human beings appeared in the northern part of peninsular Malaysia, resulting in 265 infected individuals and 105 deaths, most of whom were farmers or abattoir workers who had come in close contact with infected pigs [15]. Surprisingly, while respiratory symptoms predominated in pigs, infected humans presented mostly with encephalitic symptoms. In March 1999, a novel paramyxovirus was isolated from the CSF of an encephalitic patient from Sungai Nipah village. This virus which was later named as the Nipah virus was found to be closely related to Hendra virus, another paramyxovirus, identified in 1994, which caused severe respiratory illness in horses and humans in Australia [15–17]. These two geographically remote, yet phylogenetically close viruses were classified into a new genus-Henipavirus [18, 19]. Fruit bats belonging to the genus *Pteropus* were identified as the natural hosts for Nipah virus, while pigs were considered the intermediary hosts. The most likely explanation for introduction of the virus into pigs appears to be related to the practice of growing fruit orchards near piggeries to allow efficient use of pig waste as fertilizer for the trees. With some trees overhanging the pig-pens, it is likely that pigs were exposed to virus laden excreta and masticated fruit pellets dropped from feeding fruit bats also called as flying foxes, who are symptomless carriers of the virus [15]. Pigs infected with the virus showed less mortality; however, due to their predominant respiratory involvement, they spread the infection to humans in close contact.

Mass culling of more than a million pigs contained the outbreak in Malaysia. However, subsequently several Nipah virus associated encephalitic outbreaks with a high mortality occurred in Bangladesh between 2001 and 2007 [20]. A similar outbreak which was retrospectively identified to be due to Nipah virus was reported in 2001 from Siliguri, West Bengal, not far from Bangladesh. All of the patients were residents of Siliguri and the outbreak occurred among hospitalized patients, family contacts of the patients, and medical staff of 4 hospitals. Sixty-six cases of encephalitis were identified, and the case-fatality ratio was 74%. Analysis of the limited sequence data suggested that the Nipah virus strains associated with the outbreak were more closely related to the virus isolated in Bangladesh than to the virus isolated in Malaysia, suggesting that viruses circulating in different areas have unique genetic signatures and may have co-evolved within local natural reservoirs [21].

Many of the epidemiologic features of the outbreak in Siliguri were comparable to those of the recent Nipah virus outbreaks in Bangladesh. In Bangladesh, no intermediate animal host was identified and many infected individuals were thought to have had direct contact with fruits eaten by bats or consumed contaminated date palm sap [22]. In the Siliguri outbreak, studies to detect an intermediate host were not conducted. However, serological evidence suggested *Pteropus giganteus* fruit bats as reservoirs of infections in Bangladesh [23] as well as in India [24]. In both outbreaks, human-to-human transmission occurred in healthcare settings through contact with infected persons, supporting nosocomial mode of transmission. Initiating adequate barrier nursing techniques helped to curtail further spread of infection [21].

In April 2007, 30 cases of fever with acute respiratory distress and/or neurological symptoms, with 5 deaths were reported from Nadia district of West Bengal. The cases presented mainly with fever, headache and body ache with a few cases having episodes of vomiting, disorientation and

respiratory distress. Samples (including urine, CSF, brain and lung tissue) from the five fatal cases were found to be positive for Nipah virus by RT-PCR [25].

In northern India, as in Bangladesh, *P. giganteus* bats live in close association with the human population. The multiple outbreaks of Nipah encephalitis in Bangladesh, and the 2001/2007 outbreaks in West Bengal, show a perpetual risk for spillover infection between bats and humans in this region suggesting that more such outbreaks may continue to occur in future. Establishing appropriate surveillance systems in these areas will be necessary to initiate prompt infection control measures [21, 24].

Procedures for the laboratory diagnosis of Nipah virus include serology, histopathology, PCR and virus isolation. All work involving the propagation of Nipah virus must be done in containment level 4 facilities (BSL-4), considering the high virulence of virus, wide range of susceptible hosts and absence of therapeutic and prophylactic interventions. There is no effective treatment for Nipah virus disease, but ribavirin may alleviate the symptoms of nausea, vomiting, and convulsions [26]. Currently no vaccine is available for use; however, a few candidate vaccines include a recombinant sub-unit vaccine formulation which protects against lethal Nipah virus challenge in cats [27] and ALVAC Canarypox vectored Nipah F and G vaccine which appears to be a promising vaccine for swine and has potential as a vaccine for humans [28].

Hantaviruses ::: The Emergence of Viral Diseases: Indian scenario:

The occurrence of 'hantavirus pulmonary syndrome' in 1993 in the Southwestern United States due to the emergence of a disease caused by viruses known for decades, was attributed to the relatively abrupt and short-lived weather changes due to the El Nino effect. The El Nino effect provoked heavy rainfall across the southern states in US, leading to proliferation of vegetation and consequently the rodent population, the natural hantavirus hosts which are asymptomatic carriers of the virus. As the population of rodents, especially deer mice (*Peromyscus maniculatus*) exploded and they sought food and shelter in human dwellings, people were exposed to the aerosolized animal droppings, urine and saliva, which lead to the infection [29]. The outbreak has continued since and by December 1, 2009, a total of 534 cases of hantavirus pulmonary syndrome with 36% mortality have been reported in the United States [30].

Hantaviruses are the most widely distributed zoonotic rodent borne viruses known to cause two significant clinical syndromes-haemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS), also referred to as Hantavirus cardiopulmonary syndrome (HCPS). Hantaviruses belonging to family Bunyaviridae, genus Hantavirus are enveloped RNA viruses and currently up to 22 species and more than 30 genotypes have been described. Important species of hantaviruses include Hantaan virus, Seoul virus, Puumala virus, Sin Nombre virus and Dobrava-belgrade virus. HFRS and HPS are being increasingly reported from various geographical locations worldwide and are a cause of global concern.

Apart from inhalation of aerosols from virus containing rodent faeces, urine or saliva, transmission is reported to occur even through rodent bites [31]. Though considered unlikely, human-to-human transmission has been reported in an outbreak due to Andes virus in Argentina [32].

Thottapalayam virus, the first hantavirus to be isolated, is the only indigenous isolate from India, isolated from an insectivorous shrew, *Suncus murinus* captured in Vellore, South India during field studies of Japanese encephalitis in 1964 [33]. However, there is limited data to suggest that the Thottapalayam virus is pathogenic to humans [34]. Future studies on the epizootiology of the virus and exploration of new shrewborne hantaviruses will provide more insights into the evolutionary origin of hantaviruses in their rodent and insectivore reservoir hosts.

Clement et al. documented the first evidence of Seoul virus like and/or Puumala virus like antibodies in a serosurvey of leptospirosis-suspected cases from India in 1998-1999.

Subsequently, they also reported nephropathy due to hantaviruses, mimicking leptospirosis in a prospective study in the Chennai and Cochin area in southern India, providing clinical evidence of disease due to hantaviruses in India. Seven (12%) and three (5%) of the 60 patients with suspected leptospirosis screened for IgG antibodies were positive for Seoul virus and Puumala virus, respectively. Nine of these cases were also positive for specific IgM antibodies confirming for the first time recent hantavirus infections in these Indian cases. Two deaths due to acute renal failure were reported. Wild rats which have a documented presence world-wide, including India are the reservoir of Seoul virus. They can cause thrombocytopenia with concomitant renal and hepatic involvement, thus mimicking leptospirosis. Interestingly, the bank vole, which spreads the Puumala virus, is not present in India. Therefore, the positive serological results for Puumala virus in Indian patients may represent cross-reactions with the Thottapalayam virus or yet another unknown hantavirus in India [35-37].

Chandy et al. found 14.7% of 152 serum samples from patients with febrile illness positive for anti-hantavirus IgM and in 5.7% of healthy blood donors in South India, indicating the presence of symptomatic and asymptomatic hantavirus infections in the Indian population [38].

Few cases of ocular involvement due to hantavirus were reported in patients admitted with pyrexia of unknown origin or hemorrhagic fever following exposure to flood waters in Mumbai in 2005 [39].

There are no specific anti-viral drugs for treatment of all hantavirus infections. Ribavirin used in clinical trials in China for HFRS has been reported to cause significant reduction in fatality [40]. Prevention of exposure to rodent excreta is the best preventive strategy. There is no WHO-approved hantavirus vaccine available. However, at least three different inactivated vaccines, developed in Korea and China, are being used only locally. Many other vaccine approaches, have been investigated using both inactivated virus and recombinant DNA technology [41].

Chikungunya Virus :: The Emergence of Viral Diseases: Indian scenario:

Chikungunya fever (CHIK) caused by an arbovirus CHIKV a RNA virus belonging to family *Togaviridae*, genus *Alphavirus*, is a re-emerging viral disease. To date, a vertebrate reservoir or sylvan transmission cycle for CHIKV has only been identified in Africa; in Asia, the virus is presumed to exist in a human-mosquito-human cycle [42]. Re-emergence of CHIKV was recorded in India during 2005–2006 after a hiatus of 32 years, causing more than 1.38 million cases of CHIK in 14 states and Union Territories in India [43]. Several islands of the Indian Ocean reported similar outbreaks in the same period.

The illness is characterized by abrupt onset of fever with severe arthralgia followed by constitutional symptoms and rash lasting for 1–7 days. Most often the disease is a self-limiting febrile illness, however, atypical manifestations like cardiovascular, renal and neurological complications [44, 45], as well as mother to child transmission [46, 47] has been reported.

CHIKV is known to cause epidemics after a period of quiescence. The first recorded epidemic occurred in Tanzania in 1952–1953. In Asia, CHIKV activity was documented since its isolation in Bangkok, Thailand in 1958. In India, well-documented outbreaks occurred in 1963 and 1964 in Kolkata and southern India, respectively, and in Sholapur district, in Maharashtra in 1973. CHIKV emerged in the islands of South West Indian Ocean viz. French island of La Reunion, Mayotte, Mauritius and Seychelles in early 2005. After a hiatus of about three decades, CHIKV has re-emerged in India in the states of Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh and Tamil Nadu since December, 2005. Cases have also been reported from various other states including Rajasthan, Orissa, Gujarat and Kerala. The outbreak is still continuing.

CHIKV is transmitted by *Aedes* species mosquitoes, primarily *Aedes aegypti*. However, unusually, the vector responsible for transmission between humans in the 2005–2006 CHIK epidemic on Reunion island was apparently the Asian tiger mosquito, *Aedes albopictus*. Interestingly, the same epidemic was associated with a strain of CHIKV with a mutation in the envelope protein gene (E1-A226V), which was reported to play a role in the adaptation of CHIKV to *Aedes albopictus* mosquitoes, providing an insight into how a genetic change in the pathogen can increase the host range and geographical distribution [48].

The precise reasons for the re-emergence of CHIKV in the Indian subcontinent remain an enigma. Lack of immunity may have possibly facilitated rapid spread of the infection [49]. Other factors include lack of efficient vector control activities, globalization, mutation of the virus and emergence of *Aedes albopictus*, in addition to *Aedes aegypti* as an efficient vector for CHIKV [8]. Molecular characterization has demonstrated two distinct lineages of strains which cause epidemics in Africa and Asia. The recent outbreaks were attributed to the African genotype of CHIKV.

Laboratory diagnosis can be achieved by detection of IgM antibodies in serum and CSF (in neurological involvement), virus isolation and direct detection of nucleic acids in the samples using PCR based techniques. To tackle this re-emerging virus, laboratory research aimed at the development of vaccine candidates, antiviral strategies and commercially available diagnostic kits is needed [42].

Human Enterovirus-71 (EV-71) :: The Emergence of Viral Diseases: Indian scenario:

Even as we grapple with the challenge of eradicating poliomyelitis, another enterovirus EV-71, responsible for neurological disease has emerged [50].

Hand-Foot-and-Mouth Disease (HFMD) is a mild exanthematous illness seen worldwide, with characteristic lesions on the palms, soles and oral mucosa, affecting mainly children under 10 years of age. The causative agents were initially Coxsackie virus type A 16 (CA 16) and related

serotypes. However, in recent years, a new aetiological agent of HFMD, EV-71, has emerged, causing large outbreaks with severe complications and many deaths, especially in the Asia Pacific region [51]. This geographical predilection is unexplained but could be related to the frequency of intra- and inter-typic genetic recombinations of the virus, the genetic predisposition of host population, environmental hygiene, and standard of healthcare [52]. EV-71 is a member of the family Picornaviridae, and belongs to the genus Enterovirus (human enterovirus A). Faeco-oral route is the principal mode of transmission and humans are the only known natural host. A major epidemic of HFMD induced by EV-71 was reported in Sarawak, Malaysia in 1997 followed by smaller outbreaks in Peninsular Malaysia. More than 2600 children, mostly under 6 years of age, were affected. Most of them presented with characteristic febrile illness and skin lesions, but severe neurological complications like aseptic meningitis, acute flaccid paralysis, fatal encephalomyelitis, and cardiopulmonary complications were also reported in a small proportion of patients, unlike the benign type of HFMD caused by CA 16 and other aetiological agents. Subsequently, EV-71 associated HFMD has been reported from Taiwan in 1998, Perth in 1999, and Singapore, Korea, Malaysia, Japan and Taiwan in 2000, Vietnam in 2005 and China in 2008. Brainstem encephalitis, especially affecting the medulla, associated with cardiopulmonary dysfunction has become a notable feature in EV-71 epidemics in Asia, and is the primary cause of death.

In India, outbreaks of HFMD have been reported from Calicut, Kerala in 2003 and three districts of West Bengal in 2007, however, these outbreaks were not associated with neurological complications [53, 54]. Various case reports and hospital based or clinico-epidemiological studies from India have reported neurological complications caused by EV-71 [55–57]. Like all other enteroviruses, children are the most significant target as well as reservoir of EV-71. In a study of aetiology of viral encephalitis in children in Uttar Pradesh, the most common causative agent was EV-71, identified in 42% of the 87 patients enrolled in the study. Generalized convulsions with altered sensorium were a significant finding in these patients. The infection was associated with a significantly high morbidity and mortality; 50% of the children with EV-71 encephalitis died [57]. Nucleotide sequence analysis of an EV-71 isolate from a patient with acute flaccid paralysis (Guillain-Barré syndrome) from Haryana, revealed that the Indian isolate is genetically distinct from the EV-71 strains isolated from other outbreaks in the Asia Pacific region [56].

Diagnosis of HFMD can be made clinically with certainty, if a strong index of clinical suspicion exists. Laboratory confirmation is dependent upon direct isolation of the virus from skin vesicle fluid, throat and rectal swabs/stool specimens in cell cultures, using neutralization with a type-specific antiserum, indirect fluorescent assay (IFA) and reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of the viral RNA.

Prophylaxis and treatment with intravenous immunoglobulin (IVIG) has been used with some success in patients with complicated EV-71 infection and agammaglobulinaemic individuals prone to the development of chronic enteroviral meningoencephalitis. Vaccines being developed include inactivated whole-virus, live attenuated, subviral particle, and DNA vaccines.

Since the densely populated Asia Pacific region is the epicentre of emergence of this virus in the past decade, continuous monitoring of this disease and its epidemiology in India is warranted.

Pandemic 2009 H1N1 Influenza :: Influenza :: The Emergence of Viral Diseases: Indian scenario: Since the first outbreak of respiratory illness caused by a novel triple re-assortant, swine origin influenza A (2009 H1N1) was identified in Mexico in March 2009. Influenza A (2009 H1N1) cases have been documented throughout the world causing a tremendous impact on health and economy. The pandemic strain hit Indian shores on May 13, when a 23 year old passenger who traveled from USA to Hyderabad, Andhra Pradesh, tested positive for Influenza A (2009 H1N1). The first death due to 2009 H1N1 Influenza in India, of a 14 year old female student, was reported on August 3, 2009 from Pune, Maharashtra. As of November 30, 2009, samples from 87,417 persons were tested for Influenza A (2009 H1N1) in various laboratories designated by the government across the country and 18,198 (20.8%) of them were found positive and 575 (3.15%) deaths recorded [59]. In India, the state of Maharashtra was the worst affected, followed by Karnataka [58].

The mode of transmission is through droplets from coughing or sneezing, and through direct or indirect contact with the respiratory secretions of an infected person. The clinical spectrum of the illness varies from a mild respiratory tract illness to severe complications such as pneumonia, resulting in acute respiratory distress syndrome (ARDS), respiratory and multi-organ failure, and death. The risk of complications is higher among pregnant women and those with pre-existing diseases such as asthma, heart disease, obesity and kidney disease. Laboratory confirmation is

achieved by detection of viral RNA by RT-PCR on respiratory samples. The drug of choice is Oseltamivir; Zanamivir can be used as an alternative. Indigenously developed vaccines have been approved for use in India.

On 10 Aug 2010, the WHO Director-General declared that the pandemic was over; by then 18,449 deaths had been reported worldwide. In the SEA Region 76,302 cases and 2,054 deaths had been reported. However, in the post-pandemic period it is likely that the pandemic H1N1 2009 virus can continue to circulate as a seasonal virus for some years. Several countries including Sri Lanka, India and Thailand continue to report cases each week although the trend is on a downswing [60].

With the continual circulation and interspecies transmission of human, swine, and avian influenza viruses in countries around the world, there is an increased need for influenza surveillance in pigs, birds, and other animals to aid in monitoring and assessing the risk of future pandemic virus emergence involving different species. Vaccination in humans and continued surveillance can curb the occurrence of recurrent waves of the pandemic.

Avian Influenza (H5N1) ::: Influenza ::: The Emergence of Viral Diseases: Indian scenario:

Highly pathogenic H5N1 avian influenza emerged in Hong Kong in 1997. Infection was acquired by humans directly from chickens, without the involvement of an intermediate host. The outbreak was contained with effective culling of more than 1.5 million chickens [61]. Since December 2003, outbreaks have been reported in poultry from China, Vietnam and Thailand, and more than 70 countries across Asia, Europe, the Middle East, and Africa have reported the disease in poultry and wild birds. As of 11th April 2011, 549 confirmed human cases of Avian Influenza A/(H5N1) have been reported to WHO with 320 deaths [62].

Epidemics of the highly pathogenic Avian bird flu (H5N1) among Indian poultry birds were reported for the first time on a large scale during 2006 in the states of Maharashtra, Gujarat and Madhya Pradesh; subsequently several outbreaks in 2008–2009 were recorded in West Bengal, Sikkim, Assam and Tripura, in 2010. Recently, an outbreak of avian influenza has been reported from Tripura in February 2011 [63]. Mass culling of birds in affected farms led to the control of the disease spread. No suspected human cases of this deadly bird virus have appeared in India, where the mechanisms for spreading the disease and its potentially serious public health dangers in India are understood. However, as long as the avian influenza outbreaks due to H5N1 continue to occur from infected poultry in various parts of the world, the threat of an imminent pandemic looms large and hence a constant vigil is warranted.

Chandipura Virus ::: The Emergence of Viral Diseases: Indian scenario:

Chandipura virus is a member of the family Rhabdoviridae and genus Vesiculovirus that is associated with an encephalitic illness in humans. It was first identified in 1965 after isolation from the blood of two adult patients with febrile illness from Chandipura (Nagpur) in Maharashtra state, India [64] and subsequently from a child with acute encephalopathy syndrome in Raipur in central India [65].

However, the significance of Chandipura virus as a human pathogen was unresolved until 2003, when an outbreak of acute encephalitis in children in various districts of Andhra Pradesh with a case fatality of 55.6% was attributed to Chandipura virus [66]. Twenty-six suspected cases were investigated for the aetiological agent in another outbreak of encephalitis that affected children in tribal areas of Gujarat state in western India in 2004, with a case fatality rate of 78.3%.

Demonstration of Chandipura viral RNA in 9 (45%) of 20 cases, IgM antibodies in 3 (15%), and isolation of the virus from one patient provided strong evidence for Chandipura virus as the aetiological agent of the encephalitis outbreak. The virus sequences from the outbreak were closely related to prototype strain (1965) and isolates from the Andhra Pradesh, outbreak in 2003 [67].

Outbreaks of encephalitis due to Chandipura virus were reported in 2003 and 2005 from Maharashtra [68, 69] and in an hospital based surveillance of acute encephalitis among children from North Telangana in Andhra Pradesh [70]. Chandipura virus was confirmed as an aetiological agent of an acute encephalitis syndrome (AES) outbreak among children from Nagpur division in 2007, with a case fatality ratio of 43.6% [71].

Fever and neurological manifestations are the predominant features; bleeding tendencies have been reported in some cases of encephalitis due to Chandipura virus. The clinical course of Chandipura encephalitis is short with rapid progression to death, with 60% of the deaths occurring within 24 h [71].

Chandipura virus has been isolated from sandflies of *Phlebotomus* species in Andhra Pradesh and Maharashtra in India [71–73] and West Africa [74] and is probably spread through its bite. The

presence of the virus in Africa indicates a wide distribution although no human cases have been observed outside of India. Laboratory studies indicated venereal transmission of this virus from infected male to female sand flies while mating [75]; the virus has also been found to be transmissible through *Aedes aegypti* mosquitoes in laboratory studies [76]. These findings may have important epidemiologic implications in spread of the infection.

Evaluation of a combination of recombinant glycoprotein (rGp) and a commercially available DPT vaccine (CHP-DPT) has shown promising results in a murine model [77].

Chandipura virus has emerged as an important encephalitis-causing pathogen with high mortality among paediatric population in India. The need for immediate comprehensive analysis of the transmission mechanisms of this virus, including the role of vectors and other ecologic factors, for the development of appropriate control strategies is warranted [67].

Crimean Congo Haemorrhagic Fever :: The Emergence of Viral Diseases: Indian scenario:

In January 2011, the first ever report of an outbreak of Crimean Congo Haemorrhagic Fever (CCHF) has been confirmed from the village of Kolat, in Sanand Taluka, 30 kms south-west of Ahmedabad in Gujarat state in India. A 30 year old woman, and the doctor and nurse who treated her succumbed to the illness creating panic waves not only in the local population, but the country as well [78–80].

The RNA virus causing CCHF is a member of the genus *Nairovirus* of the family *Bunyaviridae*. Ixodid ticks of the genus *Hyalomma* specifically act as a vector and reservoir for the virus and numerous wild and domestic animals can serve as amplifying hosts. CCHF was first described as a clinical entity in 1944 when many soviet military personnel were infected in war affected Crimea. A similar illness was recognized in Congo and Uganda in 1967, resulting in the name Crimean Congo Haemorrhagic Fever. The virus is also distributed throughout the Mediterranean, northwestern China, central Asia, Africa, southern Europe, middle east and the Indian subcontinent [79, 80]. Outbreaks of CCHF have recently been reported from Iran in 2008 [81] Turkey in 2001–2003 [82] and Pakistan in 2010 [83].

Transmission of virus occurs through bite of an infected tick, contact with blood or tissues of infected animals or humans; nosocomial transmission also has been reported [79–81]. The virus causes predominantly haemorrhagic manifestations in humans, with high case fatality rates ranging from 15 to 70%. After an incubation period of 3–6 days, the onset is sudden, with high fever, chills, headache, joint pain, myalgia, vomiting and abdominal pain. The spectrum of haemorrhagic manifestations includes red eyes, flushing of face, throat congestion, petechiae over the palate, epistaxis, haematemesis, haematuria and intracerebral bleeds. The complications include jaundice, disseminated intravascular coagulation, hypovolumic shock and multi-organ failure.

Laboratory diagnosis of CCHF has to be performed in biological safety level-4 (BSL-4) facility. Immunohistochemical staining to demonstrate viral antigen in formalin fixed tissues, detection of viral antigen and antibodies by ELISA, virus isolation and detection of viral nucleic acids by PCR are used for laboratory diagnosis. Treatment of CCHF is mostly supportive, though Ribavirin has shown benefits in in vitro studies and is also recommended for post-exposure prophylaxis [79, 80].

In the recent outbreak in Gujarat, 20% of the samples collected from cattle around the affected area have tested positive for antibodies to CCHF. Earlier studies from India have also reported evidence of circulating antibodies both in humans and domestic animals, suggesting the presence of the virus in India since many years [84]. This necessitates constant surveillance for the virus and any new outbreaks to initiate immediate control measures. Animal handlers, abattoir workers and veterinarians are at high risk and preventive strategies include use of effective personal protective measures against tick bites.

Severe Acute Respiratory Syndrome (SARS) Coronavirus :: The Emergence of Viral Diseases: Indian scenario:

Severe acute respiratory syndrome (SARS) is a form of atypical pneumonia that apparently originated in Guangdong Province of the People's Republic of China in late 2002. A novel coronavirus, the SARS-associated coronavirus (SARS-CoV) was identified as the agent responsible for this first pandemic of the new millennium. After its recognition in early 2003, the infection was reported worldwide. As of July 31, 2003, 8,096 cases of 'probable SARS', have been reported in more than 30 countries world-wide with 774 (9.6%) deaths [85]. Human-to-human transmission of the virus, the lack of awareness in hospital infection control, and international air travel facilitated the rapid global dissemination of this agent. The findings that

horseshoe bats are the natural reservoir for SARS-CoV-like virus and that civets are the amplification host emphasizes the importance of wildlife and biosecurity in farms and wet markets, which can serve as the source and amplification centers for emerging infections [86]. The typical clinical presentation of SARS is that of viral pneumonia with rapid respiratory deterioration. After 3–7 days of prodromal symptoms, the illness is characterized by an onset of dry, non-productive cough or dyspnoea, which may be accompanied by or progress to hypoxaemia. About 10–20% of the patients may develop acute respiratory distress requiring intubation and mechanical ventilation.

Laboratory testing requires a biosafety level 3 facility. A positive viral culture from respiratory, faecal, and, occasionally, urine or tissue specimens or a fourfold rise in the neutralizing antibody titer in paired serum samples is the most definitive evidence of infection. Rapid detection by nucleic acid amplification such as RT-PCR or antigen detection by EIA is the alternative [86].

Though a few suspected cases of SARS were reported from India, none of them were confirmed, and in the official declaration by the Government (as reported on May 2, 2003 in the media), India was declared by WHO as a “SARS free” country [87]. Absence of HLA B*46 in Indian population has been postulated as one of reasons for protection from SARS epidemic [88].

Coronaviruses are known to undergo genetic recombination which may lead to new genotypes and outbreaks. The possibility of the re-emergence of SARS and other novel viruses from animals or laboratories, and therefore the need for preparedness should not be ignored [86].

Buffalopox :: The Emergence of Viral Diseases: Indian scenario:

Buffalopox is an important contagious viral infection of buffaloes of all ages, occurring in epidemic proportions in countries including India, where buffaloes are reared, causing considerable morbidity and reduction in milk yield. Buffalopox is an important emerging zoonosis affecting humans and in recent years several reports of infection in humans have been described from various states in India [89, 90]. Buffalopox virus is a member of the Orthopoxvirus, and is closely related to Vaccinia virus. Human beings, including smallpox-vaccines contract infection upon close contact with infected animals, but the extent of disease is greater in non-immunized individuals. Milking of affected animals is one of the major modes of spread; human to human transmission has not been reported. Milkers develop pox-like lesions on the shin of their fingers, arms, fore-head and face associated with fever and axillary lymphadenitis. Regular monitoring of buffalopox outbreaks in India in animals and humans is essential to curb economic losses as well as to reduce public health impact of the disease [90].

Dengue :: Flaviviruses: Re-Emergence and Changing Epidemiological Pattern of Disease :: The Emergence of Viral Diseases: Indian scenario:

Dengue is the most significant and widespread flavivirus disease to have emerged globally; the global epidemiology of dengue fever/dengue hemorrhagic fever (DF/DHF) is changing rapidly [91]. The Dengue virus belonging to family Flaviviridae, genus Flavivirus, consists of four serotypes (1–4). Despite extensive cross-reactivity among these viruses in serological tests, there is no cross protective immunity in humans; a person living in an endemic area can have as many as four infections, one with each serotype, during their life. The disease spectrum caused by Dengue virus varies from a subclinical infection to a mild self limiting disease-dengue fever (DF) and a severe disease that may be fatal, the dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The Dengue viruses are the only known arboviruses that have fully adapted to humans, having lost the need for an enzootic cycle for maintenance. Usually transmitted by *Aedes aegypti* mosquitoes, the virus has been reported to be transmitted in recent years by *Aedes albopictus*, which may have significance with respect to dengue re-emergence [91].

Dengue infection has been known to be endemic in India for over two centuries as a benign and self limited disease. However, in recent years, dengue which has been an urban disease has spread to rural areas of India as well [92, 93]. Dengue and the vector have been reported in the arid zones of Rajasthan [94, 95]. The disease has changed its course manifesting in the severe form as DHF and with increasing frequency of outbreaks, with all four Dengue serotypes co-circulating and the predominant serotypes ever changing [96–98]. These severe complications are thought to occur as a result of evolution of the Dengue virus to escape high population immunity manifesting as increased viral virulence and human immunopathogenesis due to antibody-dependent enhancement of viral infection [99]. The resurgence of Dengue can be linked to urbanization and unprecedented population growth, increased density of the mosquito vector, reinfestation with *Aedes aegypti* of a new geographical area, warm and humid climate, water storage pattern in houses, storage of junk in open spaces, including tyres, coconut shells etc. that

trap rain water, introduction of new serotype of the virus, lack of effective mosquito control and to the enormous increase in the international transport of people and commodities [100, 101]. Since vaccines or antiviral drugs are not available for Dengue viruses, the only effective way to prevent epidemic DF/DHF is to control the mosquito vector, *Aedes aegypti* and prevent its bite [101].

Japanese Encephalitis :: Flaviviruses: Re-Emergence and Changing Epidemiological Pattern of Disease :: The Emergence of Viral Diseases: Indian scenario:

Japanese encephalitis (JE) is one of the most important endemic encephalitis in the world especially in Eastern and Southeastern Asia. JE virus belonging to family Flaviviridae, is transmitted through a zoonotic cycle between *Culex* mosquitoes, pigs and water birds. Humans are accidentally infected and are a dead end host because of low level and transient viraemia. Japanese encephalitis kills more than 10,000 children in Asia and the Pacific every year, and it leaves one-third of its survivors with permanent neurological damage.

JE virus activity in India was first established by a serological survey in 1952 and clinical recognition of this disease was first made in 1955 at Vellore of North Arcot district in Tamilnadu state. Between 1955 and 2007, there had been an increase and spread of infection throughout the country, except in arid and high land regions. Outbreaks of JE occurred in West Bengal during 1973–1976 and in Andhra Pradesh, Bihar, Tamil Nadu and Uttar Pradesh during 1977–1979. Subsequently, outbreaks were reported in Assam, Karnataka and Pondicherry, and Uttar Pradesh in the 1980s [102]. In recent years, JEV has spread to new geographical locations within India-into Haryana and Kerala states in northwestern and southwestern India, respectively [103, 104]. A new focus of infection in Sangli district of Maharashtra was reported in 1999. Since 2000, JE occurrence has been reported from 16 States and Union Territories and among them Uttar Pradesh, Assam and Karnataka have accounted for more than 90% of the total incidence in India [102]. In 2005, Uttar Pradesh faced a devastating epidemic outbreak of JE, which surpassed in intensity and magnitude, all previous reported epidemic outbreaks in the country. The outbreak was mostly confined to Gorakhpur affecting 6,061 cases with 1,500 deaths followed by another outbreak in 2006 with 2,320 cases and 528 deaths [105].

Immunization in India was inspired by a pilot program that the government of Andhra Pradesh started with PATH (Program for Appropriate Technology in Health) with funding from the Bill and Melinda Gates Foundation. This partnership helped introduce the live, attenuated SA 14-14-2 JE vaccine that has been effectively and safely used in China for almost 20 years, to high-risk districts in the state at affordable costs, beginning in 2001. Subsequently, the government of India launched vaccination campaign in highly endemic states of Assam, Karnataka, West Bengal and Uttar Pradesh in 2006 and in Andhra Pradesh, Bihar, Haryana, Maharashtra, Tamil Nadu in 2007 and 2008, respectively, which has resulted in reduced incidence of JE in these states. By the end of 2009, more than 60 million Indian children have been immunized through these successful vaccination campaigns [106, 107].

Effective prevention and control strategies need to be continually implemented, since the number of JE epidemics has increased in the recent past and the disease has spread to new geographical areas. The occurrence of epidemic JE is attributed mainly to vagaries of climatic conditions and thereby warrants preparedness to implement an effective preventive strategy like JE immunization of target population in risk area. Resurgence of JE in endemic areas is largely determined by agricultural activities, irrigation and animal husbandry practices; an integrated approach using vector control and selective immunization of children has been recommended to tackle this problem [102].

Rotaviruses, Noroviruses, Bocaviruses, Parvovirus B-19 :: The Emergence of Viral Diseases: Indian scenario:

Rotaviruses cause acute viral gastroenteritis worldwide. The diversity of rotavirus strains reported and the high prevalence of mixed infections are unique features of rotavirus epidemiology in India and emphasize the need for surveillance of cocirculating strains, to follow the rapid changes in circulation and to detect novel strains. Group B rotaviruses have been reported from Kolkata and Pune, and human infections with strains G6, G8, G10, and G9P [19], which may occur as a result of zoonotic transmission of bovine and porcine rotaviruses, have been reported from western, southern, and eastern India. The occurrence of these unusual rotavirus strains which are natural reassortants of human and bovine rotaviruses, suggests that reassortment may be an important mechanism for generation of rotavirus strains of newborns. This is catalyzed by the age old traditions of calves and humans living in the same household and socio-economic conditions in India [108, 109]. The emergence of new variants/strains of Noroviruses, causative agents of acute

gastroenteritis has been described in various studies from India [110, 111]. Human bocavirus, a new human parvovirus identified in children with respiratory tract disease in 2005, has been reported from India as well [112]. Red Baby Syndrome, a new disease characterised by abrupt onset of high fever and generalized erythema involving the entire skin, seen in infants and young children associated with human parvovirus B-19 infection was recently reported from India [113].

Disease Surveillance in India: The Challenge of Emerging and Re-Emerging Infections:

With the adoption of the International Health Regulations [IHR(2005)] by the WHO, India has made considerable progress and continues to demonstrate a strong commitment for establishing and operating a disease surveillance programme. The Union Ministry of Health and Family Welfare launched an Integrated Disease Surveillance Project (IDSP) in 2004 to connect all district hospitals and state-run medical colleges to facilitate tele-education, training of health professionals and monitoring of disease trends. The project is implemented through the Central Surveillance Unit within the National Centre for Disease Control (NCDC) at New Delhi and aims to detect early warning signals of impending disease outbreaks and initiate effective and rapid health actions with the help of information and communication technology at the district, state and national levels. In addition to monitoring diseases like vector borne infections, diarrhoeal diseases, respiratory diseases, and vaccine preventable diseases, the IDSP also has a category called 'unusual clinical syndromes,' which would alert public health professionals to an intentional release of any biological-agent [114].

Laboratories in India played a major role in the management of the outbreak investigations during the recent 2009 H1N1 Influenza pandemic. Some laboratories geared their capacity by training manpower to carry out confirmation of index cases and test large number of samples. This activity may have helped build capacity of several state level laboratories to tackle similar emergencies, though, lessons learnt from managing one outbreak may not always suffice for another, as the agent, mode of transmission and susceptible population may differ. Two main factors for containment of an outbreak are isolation and contact tracing [115]. There is a need to move from conventional time consuming laboratory techniques to molecular technologies with rapid and sensitive detection capabilities and increased throughput for responding to the threats posed by emerging and re-emerging viruses in the recent past [115, 116]. There is also a need to create awareness among clinical microbiologists/virologists about existing outbreak reporting systems, laboratories designated for handling and processing samples for outbreak management, and for training and development of skills for testing and identifying new and emerging pathogens [115].