Preface

This book describes the seven different classification of viruses, including their effects on common human diseases (i.e., common cold, chicken pox) as well as the more serious diseases (AIDS, avian influenza and SARS). The nature of the human T-cell leukemia virus type I (HTLV-1), the first human retrovirus and the etiologic agent of neoplastic disease, adult T-cell leukemia (ATL) is explored, as well as its effect on several inflammatory diseases. Since 1997, great concern aroused that the Asian highly pathogenic avian influenza (HPAI) H5N1 virus might turn into a pandemic strain. Thus, the enigmatic nature of the HPAI H5N1 influenza virus is also discussed. An attempt is made to identify and to characterize, qualitatively, various concrete factors that may readily become or propel critical masses. Furthermore, giant viruses, which are ancient double-stranded DNA viruses that infect a wide range of host organisms are addressed. This book also highlights current information regarding the replication, transcription, and roles of proteins of coronaviruses, viruses which are known to infect a wide range of mammalian and bird species.

Chapter I - The genus *Flavivirus* of the family *Flaviviridae* is a diverse group of RNA viruses comprising more than 60 viruses, including many medically important viruses such as yellow fever virus, dengue virus, and West Nile virus. The members of the genus are phylogenetically and biologically classified into four subgroups, each with a clear demarcation of host range, including insects, vertebrates, and two vector-borne subgroups transmitted between vertebrates and ticks or between vertebrates and mosquitoes. Thus, this group is ideal for determining the history of and the mechanisms involved in adaptation of animal RNA viruses to two disparate phyla of hosts. In the past, in the absence of fossil records, evolutionary studies of nearly all RNA viruses have relied only on phylogenetic inference based on sequences of a limited number of genes. Ideally, in addition to phylogenetic inference based on sequence data, holistic approaches incorporating multiple viral traits including other useful genomic and phenotypic traits should be used to determine the evolutionary history of the viruses. Flaviviruses are exceptionally suitable for such studies because of distinct host range among subgroups within one genus. This phenotypic expression provides a unique opportunity to investigate the correlations among full-length genome sequence, other genomic and organizational traits, phylogenetic relationship, host range shift, and taxonomic relation. The recent rapid increase in the number of flaviviruses with known full-genome sequence and other recent progress in sequence analyses of flaviviruses representing the four subgroups, as well as in vitro host range study, made it

possible to undertake a holistic approach for the study of these viruses. The authors' most recent studies presented in this article reveal that open reading frame (ORF) length range increased in the four subgroups, exactly correlating to the branching order depicted in the NS5 gene tree. Furthermore, an incremental increase in the length range of several genes, increasing trend of the complexity of conserved sequence organization in the 3′ noncoding region, and host range specificity, are, in combination, more compatible with the branching order illustrated in the phylogeny based on the NS5 gene than with the phylogeny based on the NS3 gene or ORF. Also, host range specificities based on in vitro laboratory tests correlate very well with the data based on field observations and in vivo laboratory experiments. When in-depth analyses are focused on the mosquito-borne subgroup, the largest subgroup with the most diverse host range in the genus, similar correlations are again observed. Furthermore, in this subgroup, significant correlations are observed among branching order of the viral lineages characterized by unique arthropod and vertebrate hosts, gene length, and unique organization of the conserved sequences in the 3′ noncoding region to each viral lineage. Collectively, multiple correlations among viral traits corroborate the validity of the phylogenetic tree based on the NS5 gene. Accordingly, the authors' studies demonstrate the utility of a holistic approach to evaluate the soundness of phylogenetic tree proposed for flaviviruses and to better understand how two vector-borne subgroups with their host ranges in two phyla of animals evolved. Understanding this adaptive mechanism may provide insight into the molecular bases of the emergence of new zoonotic viral diseases of humans. Furthermore, the inconsistencies of the current taxonomy of flaviviruses revealed in this study should be seriously considered for taxonomic revision in the future.

Chapter II - Retroviruses and related transposable elements, termed LTR retrotransposons, reside in the majority of eukaryotic genomes. In particular, endogenous retroviruses (ERVs) and LTR retrotransposons (LRs) constitute approximately 8% of the human DNA and 10% of the mouse genome. In some species, these selfish elements are still transpositionally active, i.e., able to self-reproduce in the host genomes, whereas in the other cases, LR/ERV copies accumulate deleterious mutations and cannot proliferate any longer. Regardless of their transpositional capacities, once inserted into the host cell DNA, the copies of LR/ERVs remain there forever. It is clear now that such newcomers, having a functional promoter, enhancer, polyadenylation signal, splice sites and even protein-coding open reading frames should not be considered simply junk DNA. The effect of their presence in the genome can be deleterious, neutral or advantageous to the host. Co-evolution of the original DNA and fixed LR/ERV-derived sequences created new regulatory networks for numerous eukaryotic genes. In this chapter, the authors review recent experimental findings evidencing the importance of LR/ERV DNA domestication by the host eukaryotic organisms for the progressive genome evolution as a whole, for normal gene functioning and for speciation processes.

Chapter III - Alfamo- and ilarviruses are plant viruses with a positive strand tripartite RNA genome belonging to the family of Bromoviridae. Unlike the bromoviruses and cucumoviruses, the genomic RNAs of ilar- and alfamoviruses cannot be charged with an amino acid at their 3' end, although tRNA-like structures (TLS) have been proposed for *Alfalfa mosaic virus* (AMV) and *Prunus necrotic ringspot virus* (PNRSV) RNAs. Whether a TLS is also present in other ilarviruses has not been investigated in detail. Here the authors present secondary structure models for the 3' terminal 180 nucleotides of all sequenced alfamo- and ilarvirus RNAs. On the basis of these structure models the ilarviruses can be

divided into three distinct types. In all these types the 3' end can exist in two mutually exclusive conformations: one that consists of a "linear" array of hairpins and another "pseudoknotted" conformation that displays features of a TLS. One type, which includes PNRSV, shows remarkable structural resemblance with the AMV model. In one of the types, the pseudoknotted conformation shows low resemblance with tRNA and some viruses of this type have found an alternative solution. In most ilarvirus RNAs a hairpin that is similar to the AMV core promoter hairpin can be identified. The relationship with other members of the *Bromoviridae* is discussed on the basis of RNA structural features.

Chapter IV - Human T-cell leukemia virus type 1 (HTLV-1) is the first human retrovirus and is the etiologic agent of neoplastic disease, adult T-cell leukemia (ATL), and several inflammatory diseases. HTLV-1 is a complex retrovirus belonging to the Deltaretrovirus family, which also includes human T-cell leukemia virus type 2 (HTLV-2), the simian T-cell leukemia virus (STLV), and the bovine leukemia virus (BLV). The genome of HTLV-1 consists of a diploid plus-strand RNA, and like other retroviruses, integrates as a provirus into the cellular genome of the host. The integrated proviral genome contains long terminal repeat (LTR) regions flanking the genes coding for the major structural proteins, *gag*, *pol* and *env* (5′ to 3′ order). HTLV-1 genome also contains an additional region called *pX*, which resides between the *env* gene and the 3′ LTR. Tax encoded by the *pX* region is the transcriptional activator, which seems to play a central role in the pathogenesis of these diseases. Generating infectious viruses from cloned proviral DNA has led to a better understanding of the biology and has improved methods of disease control, including the development of vaccines. In this chapter, the authors describe the construction of an infectious molecular clone of HTLV-1 using an overlapping PCR methodology. This technique was both rapid and unproblematic and may be applicable to other genes (<10 kb) for which only partial sequences are known. The constructed molecular clone required the transactivator Tax to produce the virus efficiently.On the other hand, the field of retrovirus research has been stimulated by the discovery of an innate host defense factor, APOBEC3, against the retroviruses. HTLV-1 is relatively resistant to the antiviral effects of APOBEC3. This chapter will focus on HTLV-1, reviewing its genome, a novel technique for making an infectious molecular clone and its relationship to APOBEC3.

Chapter V - The enigmatic nature of the HPAI H5N1 influenza virus, particularly in the sense of possibly shaping into a pandemic strain, is largely intriguing, both scientifically and practically. Either way, since its appearance in 1997, this protean pathogen has been widely researched, so as to gauge and foresee its pandemic transforming. Such a viral transition has not been studied, however, in terms of critical masses that are likely to trigger the emergence of a pandemic strain. Fundamentally, the genesis of a pandemic strain requires human infectivity and transmissibility. Although infectivity constitutes a natural prerequisite for transmissibility, the occurrences of those two phases are temporally and spatially independent of each other, as herewith shown. This chapter examines a line of different factors that are regarded as bearing appropriate potential for becoming critical masses prone to materialize those two essential phases. It thereby pertains to certain virological, epidemiological, host-related and phylogeographically-based elements that may lead or significantly contribute—either separately or in conjunction—to the emergence under discussion. It sheds light, as well, on some paramount aspects so far inadequately incorporated within the prognostic paradigm of the pandemic strain in question.

Chapter VI - The giant viruses are ancient double-stranded DNA viruses that infect a wide range of host organisms and have genomes larger than 280 kbp. Mimivirus is the largest at an incredible1.2-million base pairs, larger than some of the smallest bacterial genomes. Most of the giant viruses are members of the NCLDV (nuclear cytoplasmic large DNA virus) family and include representatives of the *Phycodnaviridae*, *Mimiviridae*, and *Poxviridae* families, though *Herpesviridae*, *Nimaviridae*, *Myoviridae* and Polydnaviruses are also represented in this elite group. It is likely they are extremely abundant in aquatic environments.

Chapter VII - Coronaviruses are known to infect a wide range of mammalian and bird species. The coronavirus genome has a leader sequence at its 5′ end and a poly(A) tract at its 3′ end. When a coronavirus infects cells, negative-strand RNA is transcribed from the genome. The mRNAs are transcribed from the negative-strand RNA and have a leader sequence at the 5′ end. Severe acute respiratory syndrome (SARS) is a newly discovered infectious disease caused by a novel coronavirus, SARS coronavirus (SARS-CoV). Since the SARS outbreak in 2002–2003, new coronaviruses have been discovered in both humans and animals. To understand mechanisms of the pathogenesis of coronaviruses, including SARS-CoV, unique viral proteins have been characterized. This chapter highlights current information regarding the replication, transcription, and roles of proteins of coronaviruses.

Chapter VIII - Search of novel efficient approved anti-HCV drugs and drug targets is timely and appropriate because there is still no vaccine against HCV. The current approaches are based on the use of drugs specifically acting on virus targets. Mathematical modeling provides new opportunities for numerical analysis of the course of changes in the viral components (RNA, polyprotein, proteins, supramolecular complexes) in the presence of potential drugs. Many of these changes are difficult to study experimentally. These numerical data would be helpful in defining optimal targets for the action of potential anti-HCV drugs. The authors developed a mathematical model that describes replication of subgenomic HCV replicon in an Huh-7 cell and incorporates the mechanisms of the action of inhibitors leading to blockage of the steps of HCV replicon replication: translation, polyprotein processing, and replication of the HCV strand RNAs. The effect of inhibitors on HCV replicon replication was estimated from the calculated kinetics of the decrease in the steady-state concentration of HCV RNA, the active replicase complex, supramolecular complexes of the plus- or minus-strand RNA and the active replicase complex, and polyprotein. Based on comparisons of the kinetics of the reduction in the concentrations of viral components in the presence of drugs acting with the same affinity on various HCV targets, NS5B polymerase was identified as the optimal target. Under the effect of anti-HCV drugs on the NS5B target, the half-lives of the main HCV components were minimal and these half-lives were significantly shorter than the time needed for fixation of mutations conferring HCV resistance to drugs. Minimization of the half-lives of the main HCV components under drug effect may be a strategy for overcoming the acquirement of HCV resistance to drugs.

Chapter IX - Eastern equine encephalitis viruses (EEEVs) are members of the genus Alphavirus, family *Togaviridae* and are transmitted by arthropods. In North America, EEEV continues to be a significant public health concern due to the high mortality rates that occur in infected humans, equines and select game birds such as pheasants, emus and turkeys. On average, there are five human cases per year in the U.S. However during 2005 there were 21 confirmed human cases of EEE in the U.S. which resulted in several fatalities. The EEEV genomic sequences available at the time of this report were compared to determine significant

similarities and differences. Three of the five North American EEEV genomes previously sequenced had an unknown passage history and the many sequence differences from the FL91-4697 field isolate may be largely attributed to cell culture adaptations or sequencing artifacts. It has long been recognized that EEEVs from North America are antigenically distinct from those found in South and Central America; the latter appear to be less virulent due to the absence of recorded disease in humans and experimental animals. A comparison of the North American field isolate FL91-4697 genomic sequence to two recently sequenced South American EEEV field isolates revealed several interesting differences.

Chapter X - Despite obvious phylogenetic diversity within the *Lyssavirus* genus, generally, the viral genome has the same organization. Albeit 7 recognized and 4 putative genotypes have been proposed in lyssaviruses, more genotypes are seemingly being suggested. The Lagos bat virus (genotype 2) is being divided into novel or subgenotypes. Since viral full genome sequences provide rich information for various phylogenetic purposes, the partial single gene sequence once applied in phylogeny should be reevaluated. However, the available database for lyssavirus full genomes is very limited. After comparison of previous methodology in sequencing lyssavirus full genomes, a novel, simple and universal method was developed in their laboratory, which helped generate 4 full genome sequences for the putative lyssavirus genotypes: Aravan (ARAV), Khujand (KHUV), Irkut (IRV), and West Caucasian bat virus (WCBV). Lyssavirus is a single negative stranded RNA virus with a genome between 11 k and 12 k nucleotides (nts). Currently, the WCBV has the longest genome of 12278 nts in the genus. In gene organization, from the 3' to 5' extremity, the viral genome contains sequentially leader sequence, nucleoprotein (N), phosphoprotein (P), matrix (M), glycoprotein (G), RNA dependent RNA polymerase (L), ψ (or G-L 3' non-translated region), and trailer region. The ψ is between 400 and 700 nts long with no coding capacity or functionality. This redundancy is unique in the *Lyssavirus* genus. The relative conservativeness has revealed the gene order of N >L >M>G>P in the genome. No overall positive selection has been detected in lyssaviruses. The overwhelming evolutionary driving force is point mutation and purifying (deleterious) selection. Synonymous mutations are dominant while nonsynonymous sites are constrained. The few suggested recombinant events are probably due to subquality of the sequence database for the extrapolation. No solid biochemical model for explanation of recombination has been hypothesized in lyssaviruses. In contrast to the expanding diversity trend in genotypes, viral protein structures and functions are conserved in lyssaviruses. Extensive viral protein-protein, protein-RNA interactions have been investigated. Intensified co-variation sites have been detected within and among individual viral structural proteins. In addition, both *trans*- and *cis*- acting signals for viral transcription and replication are strictly conserved in lyssaviruses.

Chapter XI - Dengue is a major public health problem in many parts of the tropical developing world and is expanding geographically. The disease is caused by infection with one of four serotypes of dengue virus, which is belonging to the family *Flaviviridae*. Although most dengue virus (DENV) infections are asymptomatic, a proportion result in clinically apparent disease that varies in severity from mild undifferentiated dengue fever (DF) to more severe syndromes, primarily dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), all them called re-emerging infectious diseases. Since no protective vaccine or specific treatments are available for dengue, accurate and efficient detection of the infections is of great importance to the clinical care, surveillance support, pathogenesis studies and vaccine research. Despite all obstacles, there are many dengue diagnostic tools

available such as virus isolation, detection of RNA or virus antigen in plasma or tissues and presence of dengue virus specific antigen in serum and other body fluids. More recently, new techniques were developed or are in improvement such as ELISA assays to detect the DENV proteins in acute plasma, nucleic acid amplification by nested reverse transcriptase-polymerase chain reaction (RT-PCR), quantitative RT-PCR and real-time PCR, centrifugation amplification to enhance virus isolation rate, serology and the flow cytometry method for early detection of cultured virus. Although the numerous of dengue diagnosis tests developed, several problems are found. Cross-reactivity between epitopes shared by *Flaviviruses* represents a great difficulty for the correct diagnosis, epidemiological surveillance and preventing dengue. Furthermore, difficulty in detecting primary and secondary infections, time consuming, need of specific equipment and trained people, expensive costs and quality control of some techniques are also obstacles for the dengue laboratory diagnosis and research. Future studies must be performed to improve and extend currently used methods and to develop new ones. The use of modern techniques, nucleic acid chips, protein chips and biomarkers represents a new perspective to the development of new, cheap and useful laboratory tests. In the absence of a safe and effective mass immunization, the prevention and control of dengue outbreaks depend upon the surveillance of cases and mosquito vector. Given the world expansion of DF and DHF/DSS, there is evident need to intensify the studies and improvement of vector-control methods.

Chapter XII - Dengue is an endemic viral disease affecting human population in tropical and subtropical regions around the world, predominantly in urban and semiurban areas. The dengue virus has four antigenically related serotypes. It is transmitted by *Aedes aegypti* and *A. albopictus* mosquitoes. The virus is perpetuated in the gut epithelium of female mosquitoes and disseminated by transovarian transmission. Dengue fever (DF) and its more serious forms, the dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are becoming important public health problems. The global prevalence of dengue has grown dramatically in recent decades. An estimated 2.5 billion people in more than 100 countries are at risk of acquiring dengue viral infection with more than 50 million new infections being projected annually, and 20,000-25,000 deaths, mainly in children. The symptoms of dengue are similar to that of Leptospirosis, Typhoid, Malaria etc. thereby complicating the clinical diagnosis. Moreover, the other flaviviruses, namely Japanese encephalitis, West Nile fever, and Chikungunya produce cross reacting antibodies making diagnosis difficult. At present there is no effective therapeutic agent or licensed vaccine for humans against dengue fever, hence laboratory diagnosis of dengue infection is of paramount importance for early and timely patient management. This can be made by detection of the specific virus, viral antigen, genomic sequence, and/or antibodies. DF is characterized by fever for 3 to 5 days, headache, muscle and joint pain, rashes, which is self-limited, and the patients usually recover completely from primary infection. In case of dengue, viremia persists for 2-3 days of fever followed by appearance of IgM antibodies in primary infection and IgG in secondary infection. Due to presence of non-neutralizing antibodies against the implicated serotype, there occurs a phenomenon called Antibody Dependent Enhancement (ADE) leading to DHF/DSS during secondary infections. At present, the three basic methods which are being used by most of the laboratories are; viral isolation and characterization, detection of genomic sequence by nucleic acid amplification technology, and the detection of dengue virus specific antibodies.

Chapter XIII - Dengue virus (DENV) is a positive-sense, single-stranded RNA virus belonging to the *Flaviviridae* family. It causes dengue fever in humans and in some cases, progresses to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which result in mortality. The DENV comprises four antigenically distinct serotypes (1 to 4). The envelope (E) protein of the virus comprises three Domains - I, II and III. The Domain III (DIII) protein has been demonstrated to be involved in host recognition. More importantly, the DIII protein has been shown to be highly immunogenic, and is able to elicit the generation of neutralizing antibodies against the wild-type virus itself. For this reason, the DIII protein is believed to be a potential candidate as a protein subunit vaccine and as a diagnostic reagent for dengue serology.

The authors discuss the distinct biological properties of the DIII protein, issues relating to its production and the prospects for a DIII protein- based diagnostic assay.

Chapter XIV - *Dengue virus* (DENV), from the family *Flaviridae,* is a single-stranded RNA virus that is transmitted by the *Aedes aegypti* or *Aedes albopictus* mosquito and causes disease in 50-100 million people annually with dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) presenting in approximately half a million people a year. World-wide mortality associated with DHF/DSS surpasses the mortality of all other hemorrhagic fever viruses combined, which has led to increased attention as a global heath concern. Symptoms of DENV infections range from a mild febrile disease to severe forms of DHF/DSS. Differences in DENV strain virulence and host factors are believed to contribute to the diversity of disease symptoms. The lack of a hemorrhagic fever animal model has slowed the mechanistic description and characterization of DHF/DSS as well as vaccine and therapeutic development. Vascular plasma leakage is a characteristic of DHF/DSS; however, the mechanism of initiation remains unclear. Chapter XIV aims to review the possible molecular mechanisms that attribute to the variation of DENV disease and include a description of: DENV strain diversity; differences in DENV strain virulence; the effects of neutralizing antibodies; the possible mechanisms of vascular leakage; the role of the host immune system in DHF/DSS; and antibody-dependent enhancement of DENV infection. Additionally, a description of the animal models that have been developed to study DENV pathogenesis and to test potential vaccines and therapeutics will be reviewed. In all, this article will review the current understanding of DENV pathogenesis and the challenges confronting vaccine and therapeutic developers.

Chapter XV - Dengue virus (DENV) infection is considered the major human arbovirosis, which affects millions of people in tropical urban centers leading to thousands of deaths annually. The clinical presentations of DENV infection range from asymptomatic, or a mild self-limited illness, dengue fever (DF), to severe and potentially life-threatening diseases, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The symptoms of DHF and DSS comprise continuous fever lasting 2 to 7 days, hemorrhagic tendencies and thrombocytopenia with hemoconcentration, which result from a sudden increase in vascular permeability followed by loss of intravascular fluid volume. The alterations in the endothelium leading to fluid and protein leakage are attributed to a massive release of cytokines from T cells, monocytes, macrophages and endothelial cells. In addition, there are evidences showing that liver dysfunction is a characteristic of severe dengue infection. Several human cell types have been considered targets for DV infection, and upon infection, both a direct action of the virus in the cell function as well as the effect of the inflammatory mediators produced during infection are likely to be responsible for the tissue

damage and the systemic manifestations of the disease. In Chapter XV, the responses of the target cells, including dendritic cells, macrophages, endothelial cells, hepatocytes, T and B cells, to DV infection will be reviewed and their implications for pathogenesis and therapeutic intervention will be discussed.

Chapter XVI - The name dengue perhaps derives from Spanish *denguero* = dandy, because of the difficult to walk, as having tightness of clothes, or derives from Swahili "*Ki denga pepo*", that means: "*Sudden cramp sent by an evil ghost*". The infection spreads from 25° northern parallel to 25° southern, but can also overflow from these limits. The same, caused by 4 viral types, and various subtypes of Dengue viruses (DENV) is transmitted by mosquito vectors of *Aedes* genus, and sometimes by healthcare-related modalities, including blood products administration.

Two forms of illness are known: a classic primary form, more benign, and a severe one, characterized by hemorrhagic fever (DHF), possibly associated with shock syndrome (DSS). The last situations generally occur as secondary infection, or in the case of maternal sensitised children.

Pathogenesis of DHF/DSS is very complex and can involve different mechanisms affecting vessels and different organs and systems. In particular, if a before sensitised subject is successively infected with other antigenic type of DENV, different from that responsible of a previous infection, not only the same results unprotected, but on the contrary the viral interaction to monocytic-macrophagic cells is promoted by the presence of immunoglobulins receptors. In addition, a consequent T cells activation induces a great release of proinflammatory cytokines and of other biological mediators, that promote vessel permeability and high release of anti-platelets and anti endothelial cells auto-antibodies.

Examination of clinical manifestation, together with epidemiological knowledge, can suggest dengue diagnosis, that however needs laboratory confirmation. In particular, laboratory diagnostic methods of dengue infections include virus isolation, detection of virus antigens or nucleic acid, and specific antibodies detection. Besides, epidemiological studies can be performed also directly on arthropod vectors of dengue.

At time, specific therapies for dengue are not yet available, but only a supportive care can be performed. However, a correct employ of the same can significantly reduce the mortality, particularly in the case of children, that more easy are affected by severe forms of dengue.

Finally, development of valid and sure vaccines, in addition to contrast to vectors diffusion, are effective priorities to prevent dengue spread. About vaccines, other than safety and effectiveness, it is important that they have a good duration of protection, suitable schedules of administration, and possibility to boosters perform, especially in travellers. Finally, they must protect against all the viral types, in order to prevent facilitating phenomena of infection, and hemorrhagic forms onset.

The over reported points of interest are deeply analysed and discussed in Chapter XVI.

Chapter XVII - The control of dengue virus largely relies on the ability to quickly and reliably detect the virus. As such, numerous different assays have been developed. While not all of the available assays have the ability to distinguish between serotypes, this constantly evolving pathogen necessitates robust diagnostics capable of detecting emerging variants. In recent years, the majority of new pathogen identification and diagnosis assays have been developed using nucleic acid-based technologies. These assays are both quick and easy, offering the same advantages as immunological methods with the potential to provide the same specificity, sensitivity, and reliability of culture-based diagnoses depending upon the

quality and combination of the primers/probes in the assay. In Chapter XVII, recent developments in molecular diagnostics for dengue virus are reviewed, comparing and contrasting the individual assay's reliability and ability to distinguish between strains and/or serotypes.

Chapter XVIII, dengue viruses belong to the family Flaviviridae, that has more than 70 viruses that cross-react in serological tests as they share group antigens, thus complicating diagnosis. Diagnosis based on clinical syndromes is not reliable and should be confirmed by laboratory studies. For a diagnosis of "confirmed" dengue, dengue virus should be identified by isolation or there should be a four-fold rise in antibody titre. Isolation of viruses can take from 7-10 days and serological tests depend on the demonstration of the presence of IgM antibody or a rise in IgG antibody titre in paired acute and convalescent phase sera. Serological tests are generally the tests of choice to diagnose acute flavivirus infections with most utilizing IgM ELISA formats. More than 90% of patients are IgM positive by the 4th day of illness but the IgM antibody may be due to infection up to 3 months earlier. Commercial kits for the measurement of antibodies include the ELISA kits, a dipstick and a rapid dot-blot assay. These kits do not require specialized training but their sensitivity and specificity is very variable. The choice of a test, therefore depends on the availability of facilities, human resources and also time of sampling.

With the escalating incidence of dengue infections, and the absence of vaccines for the prevention of this disease, early diagnostic confirmation of dengue infections in patients is needed as it allows for timely clinical intervention, etiologic investigation and disease control. Hence diagnosis of dengue disease during the acute phase should be a priority goal for patients and public health reasons. In-house IgM capture ELISAs is still the main stay of dengue diagnosis in many laboratories throughout the world. However, serological diagnosis has several limitations. Firstly, it is detected only after day 3 of onset of symptoms making early diagnosis impossible as more than half of the patients present early to the clinician. Secondly, as flaviviruses share common group epitopes particularly on the envelope (E) protein, a high degree of cross reactivity is frequently observed. After the onset of illness, the virus is found in serum, plasma, circulating blood cells as well as other tissues for four to five days. As such during the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection. At the end of the acute phase of infection, serology is the method of choice for diagnosis. The use of polymerase chain reaction (PCR) shortens the detection time and two multiplex PCRs (Standard and Real Time) targeting two different regions of the virus (Capsid-preM and NS5) were developed in order to further improve diagnostics of dengue infections. Both assays have shown 100% specificity, are more than 98% sensitive and are able to detect/amplify virus from Day 1 of onset of fever. Viral antigens can be detected up to Day 7 of fever even in the presence of high titres of circulating antibodies. These assays have excellent performances in detection and confirmation of infection within 24 hours but require specific equipment, facilities and skilled technicians. The non structural antigen 1 (NS1) was recently evaluated as an early diagnostic marker for dengue infection. It is a useful assay in the first 4 days of fever but its detection rate decreases with increasing levels of antibody. However, these assays are still very new and careful evaluations in multiple settings still need to be done to validate the accuracy of the tests, their utilities and cost-effectiveness. To enhance diagnostics for dengue virus infections, an assay that diagnoses the infection independent of the date of onset of symptoms, thus encompassing all stages of the disease would be timely and certainly the best

alternative. Utilizing labeled suspension beads an assay that detects and quantitates dengue specific NS1 antigen, dengue-specific IgM and dengue-specific IgG in a single step was developed. This assay was shown to be 100% specific for dengue, had detected viral antigen and IgM in more specimens than existing assays. Using the quantitative measure, primary and secondary infections were also determined. With automation and integration of all the steps, this assay can ultimately replace the traditional methods as it is of similar sensitivity. This would have a positive impact on timely control and prevention interventions. However it is hoped that reference laboratories will still maintain the standard, traditional methods in order to ensure detection of new organisms, thus ensuring their discovery and characterization for research purposes. Thus the ideal diagnostic test would be one that detects infection at any time during the course of an acute infection, is rapid, sensitive, easy to perform, can be automated and if possible affordable.

Chapter XIX - Dengue infection is a mosquito-borne arboviral infection. Dengue fever viral infection includes a variable spectrum of illness that ranges from a simple fever to severe dengue hemorrhagic fever (HDF), a fatal disease. Mairuhu et al. said that dengue fever came to be more recognized as one of the more significant contagious illnesses of the world due to increased incidence and the spreading geographical distribution of dengue fever in the last 50 years. Nogueira said that dengue fever was one of the very frequent predominant acute contagious illnesses and might be extremely fatal if associated with complications. Halstead said that the antibodies played important roles in protection and virulence of infections of dengue fever; therefore, studies to determine which cells are infected in human dengue fever, along with comprehension of early antibody-accessible steps of the infection, should concentrate on the cellular level. Nogueira also noted that control of the vector was still the most effective measure. Although there have been many efforts during the last six decades to produce a vaccine to fight this infection, few have been capable of meeting the challenges placed by the exceptional interaction between this virus and its human host. Owing to present-day globalization, dengue fever has emerged as a rising contagious problem not only in tropical but also in nontropical countries. Wilson recently said that travelers could also be seen as messengers that transport pathogens and microbial genetic matter to other regions where investigators could carry out detailed analyses that could help map the location and movement of strains, genotypes and resistance patterns. Knowledge of dengue infection is, therefore, an interesting theme for general practitioners throughout the world.

Chapter XX - The authors present mathematical models on dengue transmission and control. In the first part of the chapter the authors discuss a compartmental model for the transmission of single strain virus of dengue via a set of ordinary differential equations. They show the existence and the stability of the disease-free and endemic equilibria for the system and their relation to the basic reproduction number of the disease. The basic reproduction number is a very important threshold in mathematical epidemiology measuring the numbers of secondary infection of the disease following the introduction of a single infection in a totally susceptible population. It is a function of demographical and epidemiological parameters. Controlling the transmission of the disease is basically controlling this basic reproduction number to have the value below one by giving certain treatment to the agent or the vector or the disease as well as to the population. Throughout the discussion the authors will assume that the control takes part as a vaccination to susceptible population. In this regards, the authors discuss the minimum level of vaccination which able to eradicate the disease for various vaccination strategies. To increase the realism of the model, in the second

part of the chapter they discuss the transmission of dengue by considering the existence of more than one strain of dengue viruses and also take into account the known ice-berg phenomenon by classifying infected human into asymptomatic/mild and severe infection. Some recommendations on the safe and scientifically-sound vaccination strategy and also the directions for further investigation on dengue transmission modeling are provided to conclude the chapter.

Chapter XXI - Interspecies transmission is a crucial feature in the ecology and epidemiology of influenza virus. Transmission of avian influenza virus to a new mammalian species is of great concern, because it potentially allows the virus to adapt to a new mammalian host, cross new species barriers, and acquire pandemic potential. Infection of an entire avian influenza virus to an unrelated mammalian species is a rare event. Until now, several outbreaks of avian influenza infection have occurred in mammals. Several cases of infection in mammals by avian origin influenza viruses (H7N7, H4N5, H5N1, H3N2) have been reported. Especially, avian influenza viruses are occasionally transmitted to other bird species, particularly poultry, and to aquatic (seals, dolphins, whales) or terrestrial mammals (horses, pigs, mink). Also in humans, cases of infection by a number of avian influenza viruses transmitted main from poultry have been documented.

Here, the authors provide a current advance in their knowledge of interspecies transmission of avian influenza virus to dogs at serological and molecular level, and give an overview of available data on the intra- and interspecies virus transmission and pathogenicity.

Chapter XXII - Avian influenza H5N1 virus, family Orthomyxoviridae, naturally persists in waterfowl and domestic bird reservoirs with sporadic outbreaks of highly pathogenic strains. Several human cases were reported during the 1997 H5N1 avian epidemic in Hong Kong, showing direct transmission from domestic poultry and the first occurrence of an H5 influenza subtype in humans. Highly pathogenic avian influenza (HPAI) H5N1 variants later re-emerged following years of circulation in wild bird reservoirs and new human cases were identified in Southeast Asia during 2003. Evidence suggests that the H5N1 virus is rapidly evolving and although HPAI H5N1 has not yet adapted for efficient human-to-human transmission, it is currently considered a major threat for a global influenza pandemic. The World Health Organization (WHO) and several nations have prioritized improving available inactivated or LAIV, and the development of alternative platforms against potential influenza outbreaks. While currently approved vaccines have been successful against influenza viruses of the same subtype, complete cross-protection has yet to be achieved. This chapter reviews different vaccine strategies against avian influenza H5N1, reflects on the requirements for effective vaccine development, and discusses the direction of future influenza vaccine research. The rapid development of several experimental platforms in recent years has enhanced protective efficacy and immunogenicity following immunization, additionally benefiting understanding of influenza virus pathogenesis. The most promising platforms have been evaluated successfully in ferrets and non-human primate models, with several candidates currently in human clinical trials. The objective of influenza vaccine research will be to develop a universal, single vaccine candidate capable of complete cross-protection against divergent influenza subtypes.

Chapter XXIII - Avian influenza or "bird flu" is causing increasing concern across the world as experts are preparing for the possible occurrence of the next human influenza pandemic. Countries worldwide are preparing for the arrival of the virus in wild birds and

poultry within their territories. All countries need to prepare for the possible arrival of human cases of influenza imported through foreign travel.

Preparedness for biological threats requires awareness, planning, organization, infrastructure and equipment stocking, education of personnel, and conducting drills as well as availability, willingness and perceived self efficacy of the staff to respond in due time. International collaboration has a key impact on successful medical preparedness. Cooperation and coordination between countries is needed in the verge of a pandemic.

Most health authorities initiated disease prevention and containment policies. The World Health Organization (WHO) is the basic coordinating and supervising force behind global preparedness. The WHO has described the preparedness measures needed to be taken in the pre-pandemic stage, during primary detection of highly pathogenic avian influenza (HPAI) and at the pandemic stages. Countries worldwide have prepared multi-factorial programs dealing with the subjects. The preparedness and contingency plans differ among different countries and regions due to different resources availability, local experience with the disease, specific local challenges and limitations. Many countries suffer from under-endorsed and untested planes. In those areas suffering from lack of effective pandemic control plans, the regional cooperation is also lacking.

This article reviews status of the worldwide preparedness to prevent eruption of pandemic flu and to control pandemic spread after its emergence.

Chapter XXIV - Small interfering RNA (siRNA) technology is now available to 'switch off' a target gene. Many studies reported promising results of siRNA in combating viral infection in animals, including avian influenza infection. This review will discuss the molecular pathogenesis and the prospect of siRNA for the therapy of avian influenza infection.

Chapter XXV - In an avian flu pandemic, which methods could be used to treat or prevent infection with influenza A (H5N1) virus? Foremost are antiviral drugs and vaccines, which have already been used to prevent and treat human influenza A and B virus infections. Although formally approved for other indications (i.e., treatment of hepatitis C), interferon might also be useful for controlling avian flu. As has been shown for other viral infections, RNA interference could be a powerful means with which to suppress the replication of avian H5N1. Combined use of the currently available methods should be taken into account and attempts should be made to develop new strategies directed at unexplored targets such as the viral proteins hemagglutinin and viral polymerase (and endonuclease) and non-structural protein.

Chapter XXVI - The re-emergence of avian influenza (H5N1) in Southeast Asia has heightened concern for a potential influenza pandemic. Global pandemic preparation for avian influenza (H5N1) has begun and it is imperative for healthcare workers (HCWs), who in most cases serve as first responders, to be part of preparedness training. As relevant to other transmissible agents, HCW preparedness training should include an understanding of the modes and risks of avian influenza (H5N1) transmission and how to implement appropriate infection control strategies to prevent and control of spread of avian influenza (H5N1). In this chapter, the authors review the evidence for avian influenza (H5N1) transmission, identified infection control strategies for both resource-adequate and resource-limited settings, and post-exposure management of avian influenza (H5N1) for HCWs. Healthcare epidemiology and infection control strategies include vaccination and chemoprophylaxis of exposed HCWs, access to stockpiled oseltamivir, surveillance for

unrecognized cases and coordinated preparedness response plans by interdisciplinary groups such as local and regional health departments, HCWs, healthcare administrators and epidemiologists. The preparedness plans must include rapid creation of temporary isolation facilities, restricted access to pre-identified HCWs, involvement of specialists for screening and early case identification and continuous monitoring for optimal infection control practices and regular feedback to involved HCWs. Although human-to-human transmission of avian influenza (H5N1) has rarely occurred, vigilant preparedness and implementation plans are essential in thwarting a potential avian influenza (H5N1) pandemic.

Chapter XXVII - One strain of avian influenza currently identified in Asia and Europe is known as Influenza A/H5N1. Although it is a bird flu, it has infected a relatively small number of people — killing around 50% of those infected. Scientists are unsure if H5N1 will cause the next influenza pandemic, but there is general consensus that one is overdue. Flu pandemics have occurred cyclically, roughly between every 30 and 50 years. Since 1997, when the first human contracted H5N1 in Hong Kong, the virus has resurfaced and spread to more than a dozen countries in Asia and Europe — infecting more than 140 people and killing approximately half. Britain and Taiwan both reported avian flu cases of H5N1 in 2005. In the latter cases, the infected birds were identified as imports, and died in quarantine.

A global influenza pandemic could have a number of consequences. Global competition for existing vaccines and treatments could ensue. Some governments might restrict the export of vaccines or other supplies in order to treat their own population. Some countries might face a shortage of vaccines, antiviral medication, or other medical equipment, because of limited global supply. Hospitality and airline industries, and international trade could be negatively impacted. If global travel and trade were to suddenly drop, there could be productivity losses and service disruptions. Essential workers might become ill or stay home out of fear of contracting the virus. Such workers could include law enforcement, medical personnel, mass transit drivers and engineers, and other crucial emergency personnel.

For FY2006, Congress has provided $25 million for global initiatives to prepare for pandemic influenza through Foreign Operations appropriations; directed $33.5 million to global disease detection through Labor, HHS, and Education appropriations; and reserved for international avian flu efforts a portion of $3.8 billion through Defense appropriations.

Bills introduced in the 109th Congress would increase U.S. resources allocated to the global fight against avian flu; develop a "Pandemic Fund" to augment ongoing U.S. and international avian flu and pandemic preparedness initiatives; increase funding for preventing the spread among animals of the H5N1 virus; and strengthen surveillance capacity within affected countries.

This chapter provides an up-to-date account of global H5N1-related human infections and deaths, outline U.S. government and international responses to the global spread of H5N1, discuss situations in various countries affected by H5N1, and present some foreign policy issues for Congress.

Chapter XXVIII - Since the fall of 2003, a strain of highly pathogenic avian influenza (H5N1) has spread throughout Asia, infecting mostly poultry but also a limited number of humans. In recent months, the virus has spread into parts of Europe. Controlling avian flu in poultry is seen as the best way to prevent a human pandemic from developing, by reducing the number of animal hosts in which the virus may evolve.

Avian flu can be highly contagious in domestic poultry. Strict biosecurity measures are practiced among commercial poultry farms and are encouraged by governments. The

economic effects of any avian influenza outbreak can be significant, especially given international trade restrictions. This report will be updated as events warrant.

Chapter XXIX - Highly pathogenic H5N1 influenza A viruses have spread relentlessly across the globe since 2003. They are associated with widespread death of poultry, substantial economic loss to farmers, and reported infections of more than 300 people with a mortality rate of 60%. Influenza prevention and containment strategies can be considered under the broad categories of antiviral, vaccine, and non-pharmaceutical measures. In particular, using vaccination to reduce the transmission rate might provide an alternative to mass culling by reducing both the susceptibility of healthy birds and the infectiousness of infected birds. However, although vaccination can be a useful tool for control of avian influenza epidemics, it might engender the emergence of a vaccine-resistant strain. Field and experimental studies show that some avian influenza strains acquire resistance against vaccination. The authors investigated, in the context of the emergence of a vaccine-resistant strain, whether a vaccination program can prevent the spread of infectious disease. The authors' main findings are that such a program might lead to an emergence and replacement of the vaccine-resistant strain over a large geographical region, and that a vaccination to prevent the spread of disease can instead spread the disease. Thus, if the vaccinations are not used appropriately, prevention and control will be negatively affected by the vaccination program. Further, from their theoretical studies, the authors propose how a vaccination against avian influenza should be used.

Chapter XXX - Herein, the authors presented a personal view regarding the recent advances and future perspectives on facilitating influenza virus isolation, vaccination efficiency, and monitoring of vaccine production. Hopefully, readers such as researchers and manufacturers involved in influenza vaccine production will be motivated by this personal commentary, obtain information for their own research, and be inspired by new ideas for future research on influenza vaccine.