
Preface

Hemorrhagic fever viruses (HFV) include some of the most lethal agents of the microbial world. Disease onset can be rapid and fatal. HFV can be extraordinarily contagious and will spread in hospitals without sophisticated infection control. HVF disease is characterized by a rapid onset of fever, muscle ache, and “flu-like” symptoms, followed by liver necrosis, lymph node depletion, coagulation defects, and organ systems failure. Most people have subclinical HFV infections but the potential for severe disease distinguishes the HFV as a major public health threat. As high level threat agents, there will be unique rules for handling HFV in research settings [1, 2].

Most virology texts do not embrace the classification of “hemorrhagic fever viruses.” According to the rules for virus classification [3], viruses should not be classified according to the diseases they cause since a virus that makes one organism ill might not affect another. The most useful classifications should allude to the structure of the virion or the region where the virus was found. The appellation “hemorrhagic fever virus” is an operational name given to those viruses with unique potential to cause severe vascular leakage disease.

Hemorrhagic fever viruses frequently belong to the taxonomic families *Hantaviridae*, *Nairoviridae*, *Peribunyaviridae*, *Phenuiviridae*, *Arenaviridae*¹, *Flaviviridae*, *Filoviridae* and *Rhabdoviridae*. *Arenaviridae* include the largest number of viruses causing viral hemorrhagic fever (VHF) [4]. The importance of *Arenaviridae* is reflected in this book as 19 of 30 chapters focus on arenaviruses as experimental examples of HVF. This compilation of work from many laboratories makes reference to high-containment facilities and precautions for working with high risk-group agents, even though most of the protocols herein have broad utility and are applicable to less virulent Risk Group 2 agents, as well as to highly virulent Risk Group 4 Select Agents.

In May of 2015, during the height of the Ebolavirusdiseaseoutbreak in Western Africa, the world faced the ravages of Ebola virus disease and the toll it took on populations and health care workers who voluntarily entered the danger zones. While it is important to encourage appropriate responses to epidemic disease, this type of information blurs the lines between fearsome natural infections and the attenuated versions that are so necessary for effective biomedical research. For example, Reston virus, so named because it was discovered in a dying monkey colony in Reston Virginia, was later suspected to be an attenuated virus that had co-infected the monkeys with a simian hemorrhagic fever virus [5]. Because it was an ebolavirus, similar in form to the ebolaviruses that ravaged Zaire and Sudan, Reston virus was branded a Risk Group 4 Select Agent. A surveillance team traveled to the Philippines, the origin of the ill-fated monkey colony, to find the source of Reston virus. The virus was not found in bats or rodents, but was detected on farms where both pigs and farmers had been infected [6–8]. As a result, pigs were exterminated and the farmers were quarantined. Since the domestic pigs that became ill were also co-infected with porcine arteriviruses and/or circoviruses, it was not clear that clinical disease was due solely

¹As of June 2017, the former *Bunyaviridae* family is now the order *Bunyavirales* containing the virus families *Hantaviridae*, *Nairoviridae*, *Peribunyaviridae*, *Phenuiviridae* and *Arenaviridae*.

or even mainly to Reston virus infection. Fear instilled by the name “Ebola” may have caused scientists to overlook the potential benefits of having an attenuated virus for vaccine development and even overlooked the contribution of other infectious agents to the disease in Philippine pig farms. These challenges must be met by rigorous studies using commonly accepted practices and standards. This book is intended to help develop a common understanding of how best to approach the study of hemorrhagic fever viruses of many types and in many places.

This book has five parts. The first part on *Surveillance, diagnosis, and classification of hemorrhagic fever viruses* begins by discussing methods used to predict viral pandemics. Following this introductory chapter are two chapters on the methods and strategies for classifying viruses: one describes the open-source software available for classifying sequences obtained during surveillance, and the other describes Pairwise Sequence Comparison (PASC) to help determine genetic distances between taxa. Next, there is a chapter on viral diagnostics with specific methods for antibody capture using Lassa virus antigens. Three chapters address approaches for epidemiological surveillance: one on surveillance of clinical samples, one on field surveillance of arthropod-borne viruses, and the other on surveillance of rodent-borne viruses.

The second part of the book covers *Structural studies and reverse genetics of hemorrhagic fever viruses*. Three chapters describe studies on viral entry and on envelope membrane fusion. One chapter describes assays for glycoprotein function, another, assays for Z matrix protein functions, and two chapters cover the structure and functions of the arenavirus nucleocapsid protein (NP). A chapter on RNA fluorescence *in situ* hybridization (FISH) describes the subcellular localization of viral gene expression. The techniques successfully used to reveal universal budding mechanisms for filoviruses, arenaviruses, and rhabdoviruses are described in a chapter on using virus-like particles to study virus egress. One chapter describes polymerase function of the Crimean-Congo hemorrhagic fever virus, typical of the large multi-functional polymerases of the hemorrhagic fever viruses. Finally, there are two chapters devoted to reverse genetic systems: one for filoviruses and the other giving us two reverse genetic approaches for Pichindé virus.

The third part contains chapters on *In vivo models of hemorrhagic fever virus infection*. The chapter on murine models for VHF describes a quantitative measure for vascular leakage using Evan’s blue dye that could be applied to other animal models of VHF. The authors who gave us a chapter on the guinea pig model for VHF also used this model to test their antibody therapy for Ebola virus infection. A primate model for VHF summarizes the methods used to sample infected rhesus monkeys. Finally, we present a method to obtain a subset of primary human liver cells that can be cultured long term and used for HFV infections.

The fourth part contains *Immune assays and vaccine production for hemorrhagic fever viruses*. The first chapter in this part is a remarkable description of the facilities and procedures used to produce the live attenuated-Junín vaccine against Argentinian hemorrhagic fever. Next is a chapter on detecting virus-antibody immune complexes in secondary dengue infection. The last chapter in this part is on DNA vaccines from a laboratory renowned for promoting such vaccines for a number of hemorrhagic fever viruses.

The fifth and final part describes *Host responses to viral hemorrhagic fever*. First is a method for identifying host restriction factors controlling Junín or dengue virus infection. Then we present two chapters analyzing antivirals: one that determines the life cycle stage blocked by an antiviral, and another that uses high-throughput screening to find antivirals against a retroviral surrogate for a hemorrhagic fever virus. The last chapter is a cell culture method to assess coagulation after HFV infection.

Despite a tremendous amount of interest, there remains a gap between identifying viruses during surveillance and linking these agents to specific disease risks. Viruses that cause hepatitis, like the HFV, carry more risk for the malnourished, the immunosuppressed, or the aged [9] than for the young techies of Silicon Valley. Our goal here is to promote research on the disease mechanisms of HFV by offering detailed instructions on exploring structure/function of viral molecules and assessing virus effects in cell culture and in animal models. Armed with this type of information, we will eventually be able to do more than classify a virus' taxon but also find its actual risk group. Such research will move HFV from the category of bio-terrors to the category of manageable bio-threats.

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