A Case-study Approach to Investigate Transmission, Co-infection, and Clinical Sequelae During Epidemics of Dengue and Ebola Virus Disease

Jennifer Elizabeth Giovanni

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A CASE-STUDY APPROACH TO INVESTIGATE TRANSMISSION, CO-INFECTION, AND CLINICAL SEQUELAE DURING EPIDEMICS OF DENGUE AND EBOLA VIRUS DISEASE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Pathobiological Sciences

by

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August 2019
# TABLE OF CONTENTS

- **List of Tables** ................................................................................................................................. iv
- **List of Figures** ................................................................................................................................. vi
- **List of Acronyms and Abbreviations** ............................................................................................... vii
- **Abstract** ........................................................................................................................................ ix

**Chapter I. Introduction to the Research and Review of the Literature** ...................... 1

**Chapter II. Clinical Severity and Identification of Diagnostic Discriminators in Patients with Dengue Virus Multi-Serotype Infections and Dengue Virus-Leptospira Co-Infection, Norte de Santander, Colombia** .......... 25

**Chapter III. Individual Behaviors, Social Pressures, and Failures of Epidemic Control Measures that Contributed to Transmission of Zaire Ebolavirus among Geographically-Dispersed Family Members, Liberia** ...... 52

**Chapter IV. Clinical Sequelae and Functional Decline among Survivors of Ebola Virus Disease, Bomi County, Liberia** ................................................................. 73

**Chapter V. Summary of Results and Contributions to Public Health Practice** ........ 88

**Appendix A. History of Human Ebolavirus Outbreaks, 1976-2014** .................... 93

**Appendix B. Published Reports of Dengue Virus Multi-Serotype Infections, by Country and Author** ............................................................................................................. 94

**Appendix C. Patient-Specific Functional Scale** ................................................................. 102

**Appendix D. LSU IRB Approval—Determinant Factors of Viral Diversity and Transmission Ecology of Dengue Virus in Colombia** ................................................. 103

**Appendix E. Colombia Seroprevalence Study Adult Consent and Pediatric Assent Forms, English and Spanish** ................................................................................. 104

**Appendix F. Centers for Disease Control and Prevention Dengue Case Report Form** ................................................................. 112

**Appendix G. Adult and Pediatric Clinical Laboratory Reference Ranges** .................. 113
LIST OF TABLES

TABLE 1. SEROPREVALENCE SURVEY DESCRIPTIVE DATA FOR DENGUE-POSITIVE AND NEGATIVE PATIENTS ....................................................................................................................... 33

TABLE 2. COMPARATIVE FREQUENCIES OF PRESENTING SYMPTOMS, FOR ALL ENROLLEES AND BY DENGUE VIRUS INFECTION STATUS ........................................................................ 34

TABLE 3. COMPARATIVE FREQUENCIES OF PRESENTING SYMPTOMS, DENGUE VIRUS SSIs AND MSIs ..................................................................................................................................... 35

TABLE 4. MEAN HEMATOLOGIC INDICES, BY DENGUE VIRUS INFECTION STATUS ............................................................. 36

TABLE 5. BETWEEN-GROUP DIFFERENCES IN MEAN HEMOGLOBIN ............................................................................................. 37

TABLE 6. PREDICTIVE MODELS OF LEUKOPENIA AND DENGUE VIRUS SEROTYPE 1 ................................................................................... 38

TABLE 7. BETWEEN-GROUP DIFFERENCES IN MEAN PLATELET COUNTS ......................................................................................................... 38

TABLE 8. ASSOCIATION OF THROMBOCYTOPENIA WITH SEROTYPE-SPECIFIC INFECTION AND MULTI-SEROTYPE INFECTION .................................................................................. 43

TABLE 9. ADULT MULTI-SEROTYPE INFECTIONS .............................................................................................................................. 44

TABLE 10. PEDIATRIC DENGUE VIRUS MULTI-SEROTYPE INFECTIONS ................................................................................................. 45

TABLE 11. PEDIATRIC MULTI-SEROTYPE INFECTIONS WITH DENGUE VIRUS SEROTYPES 2 AND 4 ........................................................................................................ 46

TABLE 12. DENGUE VIRUS TRIPLE-SEROTYPE INFECTION ......................................................................................................................... 47

TABLE 13. DENGUE VIRUS-LEPTOSPIRA SPP. CO-INFECTIONS .................................................................................................................. 48

TABLE 14. DEMOGRAPHICS AND FREQUENCY OF CLINICAL SYMPTOMS, BY DRY AND WET PHASES OF ILLNESS ............................................................................................................. 60

TABLE 15. EPIDEMIC TRANSMISSION PARAMETER ESTIMATES, EBOLA VIRUS DISEASE FAMILIAL CLUSTER ......................................................................................................................... 61

TABLE 16. DEMOGRAPHIC CHARACTERISTICS, TRANSMISSION EVENTS, SYMPTOMS, AND OUTCOMES, EVD CLUSTER .............................................................................................................. 67

TABLE 17. ESTIMATIONS OF CASE-SPECIFIC INCUBATION AND INFECTIOUS PERIODS ................................................................. 68
TABLE 18. HISTORY AND PHYSICAL EXAMINATION SUMMARIES, EVD SURVIVORS ........................ 75

TABLE 19. FUNCTIONAL ASSESSMENTS USING THE PATIENT-SPECIFIC FUNCTIONAL SCALE ....... 78
LIST OF FIGURES

FIGURE 1. DENGUE VIRUS GENOME ............................................................................................... 4

FIGURE 2. NUMBER OF PUBLISHED REPORTS OF DENGUE VIRUS MULTI-SERO-TYPE INFECTIONS, BY COUNTRY, 1985-2018................................................................................................................ 6

FIGURE 3. PHASES OF CLINICAL DENGUE, WITH CLINICAL SYMPTOMS AND LABORATORY CHANGES ....................................................................................................................... 7

FIGURE 4. FILOVIRUS GENOME STRUCTURE ................................................................................... 9

FIGURE 5. GEO-DISTRIBUTION OF HUMAN EBOLAVIRUS OUTBREAKS AND PUTATIVE BAT-RESERVOIR SPECIES, SUB-SAHARAN AFRICA.................................................................................. 10

FIGURE 6. VIRAL LOAD KINETICS, DISTRIBUTION OF VIRUS IN BODY FLUIDS, AND ANTIBODY RESPONSE ..................................................................................................................... 13

FIGURE 7. REGIONAL MAP OF MEXICO, THE CARIBBEAN, AND NORTHERN LATIN AMERICA ...... 26

FIGURE 8. SEROPREVALENCE STUDY ENROLLMENT FLOWCHART, NORTE DE SANTANDER, COLOMBIA ............................................................................................................................... 27

FIGURE 9. PATIENTS WITH DENGUE AND LEPTOSPIROSIS, BY MUNICIPALITY OF RESIDENCE.... 32

FIGURE 10. MAP OF GUINEA, LIBERIA, AND SIERRA LEONE INTERNATIONAL BORDERS............. 53

FIGURE 11. LOCATION OF ROADSIDE DRUGGIST, BOMI COUNTY, LIBERIA................................. 56

FIGURE 12. EVOLUTION OF THE FAMILIAL CLUSTER OF EBOLA VIRUS DISEASE: DISEASE PROGRESSION AND EVENTS ........................................................................................ 58

FIGURE 13. COUNTY AMBULANCE AND CHLORINE SPRAYER, LIBERIA........................................ 63

FIGURE 14. EBOLA VIRUS DISEASE FAMILIAL CLUSTER: TRANSMISSION EVENTS, SYMPTOM ONSET, AND CLINICAL OUTCOMES ............................................................................. 66

FIGURE 15. EXAMINATION OF MUSCULOSKELETAL PAIN SITE IN AN EBOLA VIRUS DISEASE SURVIVOR .............................................................................................................................. 76
# LIST OF ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADE</td>
<td>Antibody-dependent enhancement</td>
</tr>
<tr>
<td><em>Aedes</em></td>
<td></td>
</tr>
<tr>
<td>AFI</td>
<td>Acute febrile illness</td>
</tr>
<tr>
<td>aOR</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>Baso</td>
<td>Basophils</td>
</tr>
<tr>
<td>BDBV</td>
<td><em>Bundibugyo ebolavirus</em></td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFR</td>
<td>Case fatality ratio</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>Cycle threshold</td>
</tr>
<tr>
<td>DENV</td>
<td>Dengue virus</td>
</tr>
<tr>
<td>DENV–1</td>
<td>Dengue virus serotype 1</td>
</tr>
<tr>
<td>DENV–2</td>
<td>Dengue virus serotype 2</td>
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<tr>
<td>DENV–3</td>
<td>Dengue virus serotype 3</td>
</tr>
<tr>
<td>DENV–4</td>
<td>Dengue virus serotype 4</td>
</tr>
<tr>
<td>DHC</td>
<td>District health clinic</td>
</tr>
<tr>
<td>DHN</td>
<td>District health nurse</td>
</tr>
<tr>
<td>DHO</td>
<td>District health officer</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribose nucleic acid</td>
</tr>
<tr>
<td>DPO</td>
<td>Days post-illness onset</td>
</tr>
<tr>
<td>DRC</td>
<td>Democratic Republic of the Congo</td>
</tr>
<tr>
<td>DSI</td>
<td>Dual-serotype infection</td>
</tr>
<tr>
<td>EBOV</td>
<td><em>Zaire ebolavirus</em></td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Eos</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>Eryth</td>
<td>Erythrocytes</td>
</tr>
<tr>
<td>ETU</td>
<td>Ebola treatment unit</td>
</tr>
<tr>
<td>EVD</td>
<td>Ebola virus disease</td>
</tr>
<tr>
<td>HA</td>
<td>Headache</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>Hct</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>Hg</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IPC</td>
<td>Infection prevention and control</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional review board</td>
</tr>
<tr>
<td>kb</td>
<td>Kilobases</td>
</tr>
</tbody>
</table>
kg  Kilogram
Leu  Leukocyte
LSU  Louisiana State University
MAC–ELISA  IgM antibody-capture enzyme-linked immunosorbent assay
Mono  Monocytes
mRNA  Messenger RNA
MSF  Médecins Sans Frontières
MSI  Multi-serotype infection
NHP  Non–human primate
NIH  National Institutes of Health
NP  Nucleoprotein
OD  Right eye
OR  Odds ratio
OS  Left eye
qPCR  Quantitative polymerase chain reaction
PEVDS  Post-Ebola virus disease syndrome
PLT  Platelets
PREVAIL  Partnership for Research on Ebola Virus in Liberia
RESTV  Reston ebolavirus
RNA  Ribonucleic acid
RT–qPCR  Reverse transcriptase–quantitative polymerase chain reaction
SUDV  Sudan ebolavirus
SSI  Single-serotype infection
TAFV  Taï Forest ebolavirus
US  United States
VP  Viral protein
WHO  World Health Organization
$x$  Mean value
$x$  Median value
ABSTRACT

From within their ecologic niches, zoonotic viruses emerge from animal reservoirs into the edges and centers of human habitation to exploit opportunities for unabated transmission within immunologically-naïve populations. Our understanding of where, in whom, and how these viruses emerge is under direct challenge, driving the evolution of modern infectious disease epidemiology within a rapidly-connected global community. The studies presented herein are based on analyses of both aggregate and case-level data, which, we argue, provide unique insight into the complexities of transmission, co-infection, and clinical sequelae occurring within, and arising from, epidemics of emerging zoonotic viruses. In Chapter II, we investigate differences in disease severity between single- and multi-serotype dengue virus infections, and examine the clinical presentation of patients with dengue versus patients with dengue virus-Leptospira co-infection. Our objective was to construct a diagnostic algorithm to aid clinicians in the early recognition and treatment of patients with multi-pathogen infections. In Chapter III, we reconstruct a six-person transmission chain of Ebola virus disease in Liberia. We analyze the individual behaviors and epidemic control measures that contributed to, and interrupted, transmission. Finally, in Chapter IV, we present a case series of pain profiles and functional disability among survivors of Ebola virus disease, a contribution to the newly-recognized post-Ebola virus disease syndrome.

Our research contributes three principal implications for clinical and public health practice. In Norte de Santander, Colombia, we provide the first prevalence estimates of dengue virus multi-serotype infection and dengue virus-Leptospira co-infection. Identifying the reservoirs of pathogenic Leptospira spp. in Los Patios, and initiating public and clinical education campaigns, will be important interventions to mitigate environmental persistence, prevent additional cases, and encourage early initiation of antibiotic therapy. From case investigations in Liberia, we identify sweat as an under-recognized driver of the Ebola virus disease epidemic, and discuss the importance of social distancing to interrupt transmission. Finally, we describe the clinical symptoms and longitudinal disability experience of three Ebola virus disease survivors to contribute to the growing evidence of a post-viral syndrome in survivors.
CHAPTER I
INTRODUCTION TO THE RESEARCH AND REVIEW OF THE LITERATURE

Approximately 75% of new diseases emerge from animals. At the human-animal interface, a complex set of eco-evolutionary pressures induces changes in a virus’ genome, and, therefore, the RNA or DNA architecture. These architectural modifications enable expansion of the viral host range, and confer transmission and replicative advantages within immunologically-naïve populations. Genomic changes are the result of interdependent evolutionary pressures from diverse ecosystems, physiologic processes, population changes, social dynamics, and human behavior. Understanding the confluence of these pressures informs changes in the global risk surface, that is geographies with conditions, pathogens, and ecologies rife for emergence events. The Institute of Medicine developed the Convergence Model to characterize four principle categories of evolutionary pressures that drive emergence events.

1) Genetic and Biological Factors
Genome mutation, recombination, and reassortment optimize routes of transmission and exploit pathways of suppression and/or avoidance of the host immune response. Particularly for error-prone RNA viruses, these changes may encode a new protein or receptor, expanding the host range of the pathogen, such as the human adaptation of HIV from non-human primates (NHPs) to human, pandemic influenza H1N1 from birds, and Plasmodium falciparum from gorillas.

2) Physical and Environmental Factors
Deforestation and road construction create threads for human travel through previously impervious forests, providing opportunities for spillover from animal reservoir to human host. The repurposing of land for large-scale agricultural production, particularly for palm oil production, gives rise to food insecurity by removing food-source species habitats, driving changes in hunting practices and consumption of NHPs, which share genetic similarities with humans.

3) Ecological Factors
Rising atmospheric temperatures increase the amount of rainfall, which provides pools of standing water for mosquitos breeding sites, and changes the density and diversity of vegetation and resident species. Warmer temperatures and higher humidity permit expansion of mosquito vectors into new regions; increase biting rates, which ultimately increases the risk to humans of mosquito-borne viral and parasitic infections.

4) Social, Political, and Economic Factors
Urbanization, mining, political instability, and environmental degradation create conditions of poverty, social inequality, and human susceptibility to emerging viruses. The absence, or decline, of public health infrastructure in the developing world has created regions in which the detection and control of an emergence event is weakened or nonexistent, such as the West Africa Ebola epidemic and the ongoing outbreak in war-torn countries of Central Africa.
INTRODUCTION TO THE RESEARCH AND RATIONALE FOR THE USE OF CASE-STUDY METHODOLOGY

Shoe leather epidemiology has been replaced by the use of mathematical models for large-scale predications for epidemic response. These models necessarily rely upon the accuracy and amount of data used to inform model parameters that attempt to identify and explain causal links and pathways. The ability to detect and predict emergence events requires targeted surveillance among susceptible populations, combined with the ability to rapidly deploy a robust, appropriate, and adaptive public health response. A multidisciplinary engagement of basic science, medicine, social science, and policy is required to address transnational health issues and mitigate the consequences of epidemics. Working within these complexities requires integration of empirical evidence, the underlying mechanisms of transmission, clinical management, and preventive strategies for global public health practice.

The case study, or naturalistic approach, has a long tradition in clinical practice, medical research, and infectious disease epidemiology. The central tenet of a case study is a need for in-depth, multi-faceted explorations of complex scientific and anthropologic phenomena within their natural context. In contrast to the experimental design, in which predictor variables are manipulated to explore associations with an outcome of interest, the case study approach is based in interrogation, an approach that submits to examination of the critical events, interventions, policies, and consequential human and pathogen responses. The case study approach lends itself, with limitations, to in-depth analysis of complex events and associations when human behavior is an essential—and under-appreciated— influence on pathogen behavior. The case study affords granular insight into gaps in control strategies, microscale drivers of transmission, and cultural factors that determine the success or failure of transmission control strategies.

ORGANIZATION, HYPOTHESIS, AND RESEARCH OBJECTIVES

DENV is the most common arbovirus infection, increasing in both its global distribution and profound burden of disease, primarily due to the co-circulation of the four serotypes. EBOV, once said to be a ‘bit player in the global drama of emerging diseases’, has demonstrated its ability to cause widespread morbidity and mortality, and its potential for regional destabilization and interruptions to global transportation and commerce. The foci of this research are dengue virus and EBOV, identified by the World Health Organization (WHO) as two of ten greatest threats to global health in 2019. We present investigations into the epidemic transmission, co-infection, severity, and clinical sequelae of hyper-endemic circulation of dengue viruses (DENV) in northeastern Colombia and the 2014 emergence of Zaire ebolavirus (EBOV) in West Africa.

The overarching hypothesis of these studies is that case-study methodology is uniquely able to:
1) Identify changes in clinical severity related to multi-pathogen infections;
2) Describe the contextual drivers of viral transmission and estimate key transmission parameters for use in predictive modeling; and
3) Characterize post-viral symptomology and disability after infection with an emerging zoonotic virus.

**Chapter II. Dengue Virus Multi-Serotype Infection and Leptospira Co-Infection**

We performed a hospital-based serosurvey of patients with acute febrile illness (AFI) in Norte de Santander, Colombia during the epidemic years of 2013 and 2014.

*Research Objectives*

1) To estimate the prevalence of DENV multi-serotype infection (MSI) and DENV-*Leptospira* co-infection in Norte de Santander.

2) To examine differences in clinical severity and identify indicators associated with differential clinical presentation among patients with DENV MSI compared to patients with DENV single-serotype infection (SSI), and patients with DENV-*Leptospira* co-infected compared to patients with DENV only.

3) To develop a diagnostic algorithm for use by clinicians in DENV-endemic areas lacking diagnostic capabilities.

**Chapter III. Familial Transmission of Zaire ebolavirus**

Using an instrumental case study approach, we investigated the transmission events, progression of EVD, and the clinical outcomes of a six-person cluster in Liberia.

*Research Objectives*

1) To describe the individual behaviors, settings, modes and routes of EBOV transmission, highlighting the circumstances of single-day exposure and transmission events.

2) To estimate, with greater precision, the key transmission parameters of EBOV using individual exposure-transmission data.

3) To illustrate protective measures used by the family and local health workers to prevent community transmission, whilst describing deficiencies in the international response.

**Chapter IV. Post-Ebola Virus Disease Syndrome**

To investigate physical complaints and associated disability among survivors of the EVD cluster described in Chapter III, we conducted serial physical examinations and measured changes in functional ability by applying intrinsic case study methodology specific to the exploration of unique phenomena.

*Research Objectives*

1) To perform a comprehensive history and physical examination.

2) To identify and examine the anatomical locations of pain.

3) To measure functional ability between pre-EVD and at two post-EVD timepoints.
REVIEW OF THE LITERATURE—DENGUE VIRUS

Dengue is a complex viral illness transmitted among humans by *Aedes* mosquitoes. It is one of 53 human-pathogenic flaviviruses, which include Japanese Encephalitis virus (JEV), West Nile virus (WNV), Yellow fever virus (YFV), and Zika virus (ZIKV), each of which have historical or emerging global distribution. First identified during WWII in Southeast Asia among military personnel, its emergence and persistence have made it one of the leading causes of tropical morbidities. There have been episodic outbreaks and epidemics since the 18th century. As climate change alters weather patterns and increases ambient temperatures, rainy seasons are lengthening, and the mosquito vector is expanding from its tropical regions to temperate climates with naïve populations. Between 1990 and 2013, the incidence of dengue doubled each decade, from 8.3 million apparent cases in 1990 to 58.4 million in 2013. In 2010 alone, the total global burden of dengue was estimated at 390 million cases, of which more than 75% (294 million) cases were undiagnosed and untreated. In 2019, nearly 40% of the world’s population is at risk of infection with a dengue virus, a disease with a mortality rate of 20%.

Virology

DENV (family *Flaviviridae*, genus *Flavivirus*) is a positive-sense, single-stranded RNA virus of 11-kilobases (kb). During translation, the genome is cleaved into ten proteins to yield three structural proteins and seven non-structural proteins (Figure 1). *E*, on the virion surface, is the principle inducer of antigenicity and thereby the primary target for the human antibody response. *E* also forms the basis of classification of the four DENV serotypes (DENV-1-4), which share ~70% sequence homology but differ at the *E* protein in sequence and structural translation. The homology of the sequences and similarity in protein structure form the basis of temporary cross-protection of antibodies against one DENV serotype to infection of another serotype and to other *Flaviviridae*.

**Figure 1. Dengue Virus Genome**

Epidemiology

DENV has both a sylvatic transmission cycle and a human transmission cycle. While considered to be of the same evolutionary origin, the two cycles are now maintained separately in different ecologies, with different strains, and with different mosquito species. Sylvatic dengue transmission occurs
among forest-dwelling non-human primates (NHPs) and *Aedes luteocephalis* and *furcifer* in Sub-Saharan Africa, and *Ae. niveus* spp. in Southeast Asia. The human transmission cycle is maintained primarily by the anthropophilic *Ae. aegypti*, which utilize water-storage containers for oviposition and remain inside dwellings for frequent blood meal acquisition. Their introduction to the Neotropics during the Age of Sail likely occurred by slave and commerce vessels traveling from West Africa to the Caribbean. During the construction of the Panama Canal from 1903-1914, the morbidity and mortality from YFV threatened progress of its construction. Public health efforts focused on eradicating the mosquito vector, and transmission of the virus ended in 1906. However, when eradication efforts failed in Cuba, Venezuela, and the Caribbean Community, the species returned. Today, *Ae. aegypti* are endemic throughout the tropics, with climate change driving their expansion into temperate regions.

**Multi-Serotype Infection**

A mosquito acquires infection with more than one DENV serotype by acquisition of a single blood meal from a human with an MSI, or multiple blood meals from differentially-infected humans. Human MSI results from one or more bites by a mosquito infected with multiple DENV serotypes, or by consecutive bites from differentially-infected mosquitoes. The first report of a DENV MSI occurred in Puerto Rico in 1982. Since then, MSIs are reported with increasingly frequency and of and expanding global distribution (Figure 2, Appendix B), with India and Brazil reporting the majority of cases. The scholarship of the clinical implication of DENV MSI is sparse; some studies report and describe the clinical severity of MSI patients, while others wholly exclude them from analyses and provide no clinical data.

Among case reports of MSIs and studies of MSIs as the primary outcome, the evidence is inconclusive. In one study of MSIs in a Malaysian cohort, DENV-1/2 and DENV–1/3 combinations were associated with at least one severe dengue manifestation. Patients with DENV-1/2 had severe thrombocytopenia (<50.0 x 10^9 platelets/L), pleural effusion, and fluid accumulation with respiratory distress. Hemorrhagic manifestations have been observed with greater frequency in patients with MSIs (Appendix B). MSIs will be detected with increasing frequency, and understanding how they modulate disease and contribute to the modern epidemiology of dengue will be important for clinical care and public health surveillance programs.
Figure 2. Number of Published Reports of Dengue Virus Multi-Serotype Infections, By Country, 1985-2018
Corresponding references are listed in Appendix B by country and author’s last name.
**Human Infection and Disease**

Dengue is a systemic and dynamic disease with a clinical spectrum encompassing asymptomatic infection to a critical illness. Its severity is influenced by host genetics, pre-existing immune repertoires, and nutritional factors. Following an incubation period of three to seven days, the course of illness follows three phases—febrile, critical, and recovery (Figure 3).

### Febrile Phase

The initial symptom of dengue is characterized by a temperature ≥38.5°C and then sudden onset of headache, myalgia, and arthralgia. The patient will develop thrombocytopenia, leukopenia, and elevations in hepatic transaminases.

### Critical Phase

Between days post-illness onset (DPO) 4-7, the stable patient can deteriorate suddenly to the critical phase is marked by evidence of endothelial compromise (i.e., increasing hematocrit, lethargy, abdominal pain, hypotension, rapid pulse), severe thrombocytopenia, and diffuse ecchymoses. These signs and symptoms of hemodynamic collapse precede cardiopulmonary shock and death.

Not all patients with dengue progress to the severe forms of disease; however, early diagnosis of DENV infection enables identification of at-risk patients, and is critically important for initiation of supportive therapies.
Molecular detection of DENV RNA is the gold standard for laboratory diagnosis of acute dengue, but, for the majority of affected countries, not available. Therefore, diagnosis is symptom-based, although rapid antibody-detection kits increasing available, largely through WHO initiatives to expand laboratory capacity in developing countries.18

**Recovery Phase**
Recovery begins when IgM and IgG concentrations approach their peak (Figure 3). Endothelial compromise is reversed, and symptoms quickly resolve. A profound fatigue lasting up to a month has been reported in up to 50% of patients,50 and has been associated with age and female sex.51,52

**Immune Response to DENV Infection**
Disease progression and outcome are determined by the balance of the protective and pathologic immune responses (i.e., suppression of viral invasion) (i.e., pro-inflammatory).53 In the primary immune response, the immune system has its first encounter with a DENV serotype. Production of IgM antibodies produced by plasma cells and memory B-cells neutralizes the virus, which is cleared by the lymphatic system.31,54 Serotype-specific IgG antibody confers life-long immunity to infection by the same serotype, with temporary cross-protection against infection by a heterologous serotype.55

A unique aspect of dengue is the potential to have up to four infections with each of the four serotypes during a lifetime. The primary DENV infection is the first encounter of the immune system with the virus, producing IgM and serotype-specific IgG antibodies.31,54 Recovery occurs when the virus is neutralized and removed by the lymphatic system. Secondary infection, however, has the potential to invoke a more complicated immune response, and, therefore, a critical clinical progression of disease. Sequential infection by one of the four serotypes is the immunopathology of the critical phase of dengue. Referred to as original antigenic sin,34 and antibody dependent enhancement (ADE),56 this critical state is characterized by systemic, excessive immune activation of pro-inflammatory cytokines produced in response to a second infecting serotype. Due to their sequence homology and conformational similarities at the E receptor site, pre-existing antibodies produced during the patient’s primary infection are stimulated by a secondary serotype.56 Rather than mount a new, energy-expensive, highly-specific immune response, expression of viral epitopes activates pre-existing memory B- and T-cells.56 Cross-protection leads to cross-induction, resulting in suboptimal binding avidity and only partial neutralization of the secondary, heterologous serotype. Incomplete neutralization of viral replication results in excessive cytokine release, systemic inflammation, endothelial disruption, and hemodynamic instability.34,57

**REVIEW OF THE LITERATURE—Zaire ebolavirus**
The ebolaviruses (Filoviridae) are enveloped, non–segmented, negative–stranded RNA viruses.58 Paleoviral dating places their existence on Earth since the early Miocene period,59 approximately 23.8 to 5.3 million years ago.60 There are six recognized species61: Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV), Reston ebolavirus (RESTV),
and *Bombali ebolavirus*.\textsuperscript{62,63} At present, EBOV, SUDV, TAFV, and BDBOV are known to be pathogenic in humans. In the Philippines, where RESTV has been found in bats colonies,\textsuperscript{64} the virus caused outbreaks in laboratory NHPs\textsuperscript{55,56} and domestic swine.\textsuperscript{57} In both outbreaks, animal handles developed antibody evidence of exposure to the virus, but none developed clinical symptoms.\textsuperscript{68,69} The newly-identified *Bombali ebolavirus* can mediate entry into human cells but has not been associated with human disease.\textsuperscript{63}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Filovirus Genome Structure}
\end{figure}


**Virology**

The EBOV genome is seven linearly-arranged genes of 16-19 kb (Figure 4).\textsuperscript{58} Nucleoprotein (*NP*) forms the helical filaments that encapsidate the viral RNA and confer protection from nucleases and products of the innate immune response.\textsuperscript{58} Viral proteins (*VP*) 35 and 30 are transcription activation factors and interferon antagonists and suppressors of innate immune-cell signaling.\textsuperscript{70} Soluble glycoprotein (*sGP*) circulates at high levels early in infection and is thought to impair the humoral immune response by binding and steric impedance of neutralizing antibodies.\textsuperscript{71} *VP40* is required for assembly and budding of viral particles from infected cells, and is the most abundant protein in the virion.\textsuperscript{72} *VP24* is an interferon antagonist, and, *L*, the RNA-directed RNA polymerase responsible for synthesis of positive-sense RNA and transcription of viral mRNA.\textsuperscript{58}

**Ecology**

The fruit bat *Rousettus aegyptiacus* is a recognized reservoir of Marburg virus, a filovirus progenitor of the ebolaviruses.\textsuperscript{73,74} Historically, Central African outbreaks of ebolavirus have been linked to human contact and consumption of bats and sick or dead NHPs (Appendix A) within remote, densely-forested regions in Central Africa (Figure 5) often associated with mining activity.\textsuperscript{75} In areas where NHPs are rare or absent, fruit bats serve as an alternative protein source.\textsuperscript{76,77}

In perhaps the germinal paper of the 2013-2016 West Africa EBOV outbreak, a two-year-old boy, known to play inside a large tree stump that hosted a bat roost, was identified as the index case of the West Africa epidemic.\textsuperscript{78} In 2018, a partial sequence of EBOV was identified from a Greater Long-fingered bat in Nimba County, Liberia,\textsuperscript{79} an area with mining activity.\textsuperscript{80} In the same year, the full genome of *Bombali ebolavirus* was isolated from an Angolan freetail bat colony in the Bombali District of Sierra Leone,\textsuperscript{63} also a mining region.\textsuperscript{80} Given these recent findings, the long-standing hypothesis implicating *Chiroptera* bat species as reservoirs of filoviruses is gaining traction.\textsuperscript{73,81,82}
Epidemiology

Emergence History of the Ebolaviruses

The first recognized spillover of an ebolavirus, SUDV, into humans occurred in June 1976 in present-day Republic of South Sudan in a cotton factory worker who had consumed bushmeat. The second spillover event, also of SUDV, occurred in August 1978, near the Ebola River in DRC, in a man who had consumed bushmeat. He attended a local health clinic and received an injection. The contaminated needle was subsequently used to administer injections to other patients at the clinic, which infected 318 people (CFR 88%). In 1979, an outbreak of SUDV among 34 people (CFR 65%) occurred in the same region of the Republic of South Sudan as the 1976 outbreak. Following a quiescence of 15 years, TFV was isolated in 1994 from an ethnologist working in the Tai Forest in Côte d’Ivoire who received a percutaneous needle stick while performing a necropsy of a chimpanzee. In 1994, EBOV was identified as the causative ebolavirus species of three separate outbreaks in Gabon (n=52, CFR 60%), each linked to alterations in the forest canopy by mining activity, gorilla mortality, and the butchering and consumption of a sick chimpanzee. From this same outbreak, a Gabonese
HCW became infected, was transferred to South Africa, and infected a South African HCW. In 1995, a charcoal maker in DRC was the index case of an outbreak of EBOV that infected 315 people (CFR 81%). Between 2000 and 2012, there were ten outbreaks in Uganda, Republic of Congo, DRC, and the Republic of South Sudan (Appendix A). During this period, the newest ebolavirus species, BDBV, emerged in Uganda in 2007 and DRC in 2012.

**Epidemic Parameters**

The incubation period for a pathogen, defined as the interval from exposure to onset of clinical symptoms, is a function of the intensity and nature of contact. Therefore, the incubation period is dependent on the infectious dose (e.g., viral load), route of infection (e.g., percutaneous, inhalational, contact), and the duration of exposure (i.e., single contact or prolonged exposure periods). For outbreaks of ebolavirus, the incubation period is a key epidemic parameter used for determining the timing and length of isolation and quarantine, length of enhanced syndromic surveillance, and for declaring the end of an outbreak. The current incubation interval used for epidemic monitoring is 2-21 days, which is derived from the first outbreak of SUDV in 1976. Across all species and modes of transmission, the mean incubation period is 3.4-12.7 days (range 1-21 days). By species, the incubation period for EBOV is 5.3-12.7 days (range 1-21 days), 3.35-12 days (range 1-16 days) for SUDV, and 6.3-7 days (range 2-20 days) for BDBV. For single-day exposures, which provide greater precision but are rarely documented, the mean incubation period is 6.22 days (SD ± 1.57 days) across all species. Needle-stick transmission had a mean incubation period of 6.3 days, compared to 9.5 days for contact with blood and bodily fluids.

**The Changing Epidemiology of Ebolavirus Outbreaks**

In December 2013, a two-year-old boy in Guinea set off an historic outbreak in West Africa that infected at least 28,616 people (CFR 39.5%). For the first time in history, an ebolavirus outbreak had broached a highly-populated urban center. Then, in 2017, the DRC experienced its eighth outbreak of EVD when the index case butchered the wild boar at the same market where an NHP was butchered and sold. In 2018, two separate outbreaks the DRC would herald a watershed event for outbreaks of ebolavirus: transmission in an active war zone. Today, transmission continues unabated, with no evidence of slowing transmission or decreases in cases.

**Human Ebola Virus Disease**

**Sources and Modes of Transmission**

Ebolavirus transmission to humans occurs by contact with the blood, body fluid, or infectious carcass of a NHP, bat, antelope, and other forest mammals. Human-to-human transmission occurs by contact with infectious body fluids of a living or deceased human. Historically, an index case is a male member of a remote forested community who works in mining or logging, or who recently hunted and butchered an infected animal (Appendix A). An index case sets in motion an outbreak through two primary transmission pathways. In the first transmission pathway, female family members, who serve as primary caregivers in traditional African cultures, provide bedside care for the index case. Stool and vomitus are the primary sources of transmission during outbreaks, as high viremia and viral
shedding through fluid loss coincide with the phase of illness when patients require care. Additional secondary cases arise among community members who travel to provide care, attend a religious ceremony, or participate in funereal rituals. In the second transmission pathway, HCWs who are infected when the index case seeks care at a local or regional facility. For smaller, localized outbreaks, an index case infects an HCW at a rural facility, who generates tertiary cases among family members and a smaller number of patients who reside in the surrounding village. For larger outbreaks, an index case infects an HCW at a larger health facility, which serves a large population center. Tertiary transmission occurs within the hospital among patients and family members, who return to distant villages and homes within larger cities. Percutaneous exposures (i.e., needle-stick, scalpel injury, injection using a contaminated needle) are associated with a high infectious dose and high mortality. Among patients exposed by use of contaminated needles during the 1976 outbreak, the case fatality rate was 100%, versus 80% among patients exposed to infectious body fluids.

EBOV has been detected in saliva, sweat, urine, breast milk, synovial fluid, spinal fluid, ocular fluid, stool, vomitus, and semen. The contribution of saliva, sweat, urine, breast milk, and products of conception to transmission during an outbreak is unknown. There are no documented reports of human transmission from aerosols, but the role of aerosols and virus persistence on fomites has been suggested.

Until the West Africa outbreak, human transmission was considered restricted to the symptomatic period of EVD, and the risk of transmission following recovery was, theoretically, zero. However, in July 2015, two separate chains of transmission, one in Liberia and one in Guinea, were linked by molecular analyses to sexual intercourse with male survivors whose semen was EBOV-positive. It is now recognized that harborage of virus in immune-privileged sites is an ongoing risk for potential outbreaks in the region.

Clinical Progression

The latent period, or dry phase, is symptomatic onset fever, headache, myalgia, arthralgia, sore throat, and a distinguishingly profound weakness. In the first three days post-illness onset (DPO), the viral load increases exponentially in blood, from undetectable to >10^5 viral particles/mL, and continues within the vascular system for another 3-7 days. The infectious period, or wet phase, begins with onset of vomiting and diarrhea and shedding of virus through fluid loss. It corresponds to peak viral loads, and, for some, progression to hemorrhage and fatal sequelae. Pregnancies will spontaneously abort during this phase. Diaphoresis is remarkable; extensive fluid loss from vomiting and per-rectum peristaltic expulsion is estimated at 3-10 L per day. If present, hemorrhage appears first at the gum line at intravenous catheter and injection sites, with progression to subconjunctival hemorrhage, melena, petechiae, and diffuse ecchymoses. Loss of electrolytes, most critically potassium and calcium, causes muscular rigidity, confusion, alterations in consciousness, cardiac dysrhythmias, hypovolemia, and vascular collapse. In the absence of timely, supportive care, organ failure and death occur within 10-16 DPO.
**Disease Outcomes**

Viral load is a predictor of clinical severity and outcome.\textsuperscript{121} The West Africa outbreak provided a large dataset from which analyses of prospective and retrospective associations could be investigated. From these data, a clearly-defined, divergent course for survivors and decedents emerged based on the differences in the amount and kinetics of their viral loads.\textsuperscript{121-128} Patients who survive EVD have slower viral replication, lower peak viral loads (Figure 6), and a shorter duration of less severe symptoms.\textsuperscript{117} In contrast, patients who succumb to EVD have higher viral loads and demonstrate rapid increases and a prolonged symptomatic period of increasing severity.\textsuperscript{117} In an analysis of viral loads in 65 patients in Sierra Leone, patients who presented with fewer than <100,000 EBOV copies/mL of serum had a CFR of 33\%, whereas those with a viral load of \( \geq 10 \) million EBOV copies/mL of serum had a CFR of 94\%.\textsuperscript{128} Importantly, patients with higher viral loads have rapid onset of severe, debilitating symptoms. As patients become bedridden, requiring total care by family and community members, prolonged contact with highly-infectious body fluids dramatically increases the risk of infection for caregivers.

![Figure 6. Viral Load Kinetics, Distribution of Virus in Body Fluids, and Antibody Response](image)


**Immune Response**

While surviving EVD is multifactorial, the governing principles is inactivation of replicating virus by the humoral immune response.\textsuperscript{118,123} The natural portals of virus entry are the mucosal surfaces and the skin, where macrophages and dendritic cells, the primary cellular targets of ebolaviruses, promote dissemination to multiple cell types throughout the body fluids and tissues,\textsuperscript{58} including sites previously considered immune-privileged (e.g., central nervous system [CNS],\textsuperscript{129} ocular chamber\textsuperscript{130}).
Severe EVD is characterized by an intense inflammatory response with high concentrations of pro-inflammatory mediators. Simultaneous immune suppression and overactivation results in global immunosuppression, a complex pathogenesis of insufficient and deranged innate and adaptive immune responses. Systemic and uncontrolled viral replication causes apoptosis, tissue necrosis, coagulopathies, and compromise of the vascular endothelia.

There is no licensed treatment for EVD. Survival is achieved by rapid and balanced humoral and cellular immune responses. Aggressive rehydration with concomitant electrolyte replacement can support cell and tissue function until development of the antiviral immune response. In Africa, treatment for EVD is limited to oral rehydration salts, oral nutrition, intravenous fluid reconstitution, paracetamol, and presumptive treatment for malaria. In the absence of such therapies, multiple organ failure and death occur within approximately 10 days of symptom onset in humans.

REFERENCES


CHAPTER II
CLINICAL SEVERITY AND IDENTIFICATION OF DIAGNOSTIC DISCRIMINATORS IN PATIENTS WITH DENGUE VIRUS MULTI-SEROTYPED INFECTIONS AND DENGUE VIRUS-LEPTOSPIRA CO-INFECTION, NORTE DE SANTANDER, COLOMBIA

The clinical presentation of dengue as a non-specific AFI is indistinguishable from other tropical etiologies, which constitute an already heavy burden of disease in endemic areas. In the tropics, viruses, bacteria, and parasites occupy similar ecological niches, and infection is not restricted to one pathogen. For regions of hyperendemic circulation of all four DENV serotypes, such as Colombia, concurrent infection with more than one serotype, or MSI, occurs from the bite of a single mosquito infected with multiple serotypes, sequential mosquito bites from differentially-infected mosquitoes, as the presence of two DENV serotypes in one mosquito has been demonstrated, as has simultaneous infection with two arboviruses. Since 2003, Colombia has been hyperendemic for co-circulation of all four serotypes, yet there are no published reports of MSIs in Colombia.

Leptospirosis is a bacterial disease caused by one or more of 250 pathogenic leptospire serovars. The burden of disease is known to be high but is undefined. Untreated disease can lead to kidney and liver failure, with death in 5-15% of cases. The bacteria are responsive to treatment with readily available, cost-effective antibiotics, including doxycycline; therefore, early diagnosis is critical to improving patient outcomes. Because of overlapping geographies and the similarity of acute presentation, leptospirosis is often misdiagnosed as dengue. In Colombia, the seroprevalence of anti-Leptospira antibodies is as high as 76%. Among 53 patients in Córdoba given the diagnosis of dengue, more than half were retrospectively reclassified as cases of leptospirosis. As a high-consequence pathogen, these findings highlight the global need for clinical education, laboratory diagnostic capacity, and the early for the accurately diagnose and treat disease.

Despite similarities in their clinical presentation, dengue, an intracellular virus, and the pathogenic leptospires, which are extracellular bacteria, differ in their underlying pathophysiology, such that divergent host responses, including clinical presentation, may have utility in developing clinical algorithms to discriminate differences in their laboratory profiles. We hypothesized that clinical and laboratory indicators exist to differentiate between these infections, and we therefore aimed to develop a diagnostic algorithm for use in clinical settings.

MATERIALS AND METHODS

Population Screening and Enrollment

The State of Norte de Santander (Figure 7) lies within the northeast region of Colombia along a common border with Venezuela. The region hosts the Pan American Highway, an artery of vehicular and commercial traffic connecting the Caribbean, Venezuela, and internal regions of northern Latin America. The capital city, Cúcuta, hosts a border-crossing of the Pan American Highway and was the 2003 location of DENV-3 reintroduction from Venezuela to Colombia.
As part of a larger study of serotype diversity and transmission ecology in Norte de Santander, we conducted a cross-sectional seroprevalence study of DENV and pathogenic *Leptospira* among patients with an AFI presenting for care at Hospital Erasmo Meoz, a 700-bed referral and teaching hospital in Cúcuta, and the Hospital de Los Patios, a multi-service referral center in the Cúcuta suburb of Los Patios. An AFI was defined as a fever ≥38.0°C of unknown etiology. Continuous enrollment occurred from January 2013 to October 2014, which coincided with an epidemic of dengue in Colombia.21 Adults (≥18 years) who agreed to participate in the study provided written informed consent for use of clinical and laboratory data, and use of a serum aliquot for research testing at Louisiana State University (LSU). Children (7-17 years of age) provided verbal informed assent, with written informed consent from a parent or guardian; informed consent was provided by a parent or guardian for children <7 years of age.

Patients were interviewed for history of present illness. Clinical and laboratory data were extracted from medical records. De-identified data were transmitted electronically to LSU for analysis. Serum aliquots were maintained at -80°C by study personnel. Temperature integrity was monitored and maintained during international shipping to LSU.
Ethics Statement
The study protocol was approved by the Instituto Departamental de Salud of Norte de Santander, and ethics review boards at the Hospital de Los Patios, the Hospital Erasmo Meoz, and the LSU Human Subjects Institutional Review Board (IRB) (Appendix D). Serum aliquots were obtained in compliance with US and Colombian regulations for the use of human subjects in clinical research.

Laboratory Methodology

Detection of Dengue Virus RNA
Molecular testing for DENV serotypes 1-4 RNA was performed at the LSU School of Veterinary Medicine, Department of Pathobiological Sciences, by multiplex RT-qPCR targeting the DENV-specific NS1 gene, as previously described.\textsuperscript{22} RNA was extracted using the MagMax\textsuperscript{TM}-96 Viral RNA Isolation Kit (Ambion/Life Technologies, Carlsbad, CA) and detected by RT-qPCR with the Superscripts III\textsuperscript{®} Platinum\textsuperscript{®} One-Step RT-qPCR system (Life Technologies, Carlsbad, CA) on the LightCycler 480 (Roche Diagnostics Corp., Indianapolis, IN). Extracted samples were tested using the following protocol: (1) reverse transcriptase step (1 cycle) at 48°C for 2 minutes and 95°C for 2
minutes; (2) amplification and data recording step (40 cycles) at 95°C for 15 seconds and 60°C for 30
seconds. Primers and probes specific to each DENV serotype were designed and obtained through
Integrated DNA Technologies, Inc. (Coralville, IA).

Detection of Pathogenic Leptospira spp. DNA
Detection of the pathogenic gene LipL32 was performed according to the protocol developed by
Stoddard, et al. Leptospira DNA was extracted using the MagMax™-96 DNA Multi-Sample Kit
(Ambion/Life Technologies, Carlsbad, CA). The reference standard for LipL32 was Leptospira
interrogans serovar icterohaemorrhagiae from culture-extracted DNA (Dr. Sreekumari Rajeev,
University of Georgia, College of Veterinary Medicine, Veterinary Diagnostic Laboratory). The
primers and the probe were obtained from Integrated DNA Technologies according to the sequences
used by Stoddard, et al. The amplification protocol (LightCycler 480, Roche Diagnostics
Corporation, Indianapolis, IN) consisted of 8 minutes at 95 °C, by 45 cycles of amplification (95 °C
for 10 seconds and 60 °C for 30 seconds), finishing with a cool cycle of 45 °C for 5 minutes. Serial
dilutions of cDNA, probes, and primers were performed to achieve a desired concentration
approximating the expected concentration of the target gene in human sera.

Measurement of Anti-Dengue Virus IgM and IgG Antibody Concentrations
Anti-DENV IgM and IgG was detected by MAC-ELISA (InBios International, Inc., Seattle, WA).
The assay was optimized for each DENV serotype as previously described. Briefly, 96-well plates
were coated with 1 µg/mL of Flavivirus capture antibody 4G2 at 4°C overnight, blocked for 1 hour
with 5% dry milk in PBS at 37°C, and incubated with each of the serotypes or uninfected cell culture
(media control plate) for 2 hours at 37°C. Diluted human serum (100 µL/well) was incubated for 2
hours at 37°C on a shaker. Plates were washed 3 times with PBS and 0.1% Tween, and then incubated
at 37°C for 1 hour with 100 µL/well of goat anti-human IgG antibody diluted 1:1,000 horseradish
peroxidase-conjugated antibodies (Caltag Laboratories, Burlingame, CA).
Colorimetric development was obtained as follows: 100 µl/well tetra-methyl-benzidine incubated for
10 minutes at room temperature. The reaction was stopped with 100 µL/well of 1 M phosphoric stop
solution, and absorbance was measured at 450 nanometers. Each sample was tested in triplicate with
three controls per plate: 1) control blank: 2 wells without 4G2 or DENV to control for nonspecific
induction of color for any of the reagents used in the assay; 2) negative control: 2 wells with 4G2 and
no DENV to control for any nonspecific color induction of the coating antigen; and 3) positive control
from human serum with a known concentration of anti-DENV IgG antibodies.

Clinical and Etiologic Definitions
A dengue case was defined as a patient with molecular detection of DENV RNA, of any serotype, by
RT-qPCR, without consideration of anti-DENV IgM or IgG antibody status. Patients were classified
according to their infection status in by the following PCR-outcome categories:

DENV-negative: Patients without molecular detection of DENV RNA
DENV-positive: Patients with molecular detection of DENV RNA
DENV SSIDENV-positive patients with infection by 1 serotype
DENV-1DENV-positive patients with infection due to DENV-1 only
DENV-2DENV-positive patients with infection due to DENV-2 only
DENV-3DENV-positive patients with infection due to DENV-3 only
DENV-4DENV-positive patients with infection due to DENV-4 only
DENV MSDENV-positive patients with infection by >1 serotype

Leptospira-positive Patients with molecular detection of gene LipL32

Clinical and laboratory data were collected using the information queried by the CDC Dengue Case Investigation Report (Appendix F). Due to the lack of population-standardized hematotologic indices for the Colombian adult or pediatric populations, we applied the clinical reference ranges published by the American Board of Internal Medicine for adults, and those used by Texas Children’s Hospital Department of Anesthesiology and Pediatrics (Appendix G) for pediatric patients.

**Research Objectives**

Hypothesis 1: The means of the identified hematologic indices will be different and indicative of greater clinical severity in DENV MSI versus DENV SSI, such that:

\[ H_0: \bar{x}_{\text{Group 5}} = \bar{x}_{\text{Group 4}} \]
\[ H_a: \bar{x}_{\text{Group 5}} < \bar{x}_{\text{Group 4}} \]

\[ H_0: \bar{y}_{\text{Group 5}} = \bar{y}_{\text{Group 4}} \]
\[ H_a: \bar{y}_{\text{Group 5}} < \bar{y}_{\text{Group 4}} \]

\[ H_0: \bar{z}_{\text{Group 5}} = \bar{z}_{\text{Group 4}} \]
\[ H_a: \bar{z}_{\text{Group 5}} > \bar{z}_{\text{Group 4}} \]

**Rationale**

Hematologic indices are clinical predictors of severity. We identified the following endpoints to define clinical severity:

1) Leukocytes \((x)\) = where \(\bar{x}\) is the mean leukocyte count;
2) Platelets \((y)\) = \(\bar{y}\) is the mean platelet count; and
3) Hematocrit \((z)\) = where \(\bar{z}\) is the mean hematocrit.

The central importance of these indices in DENV pathogenesis and clinical presentation is supported by the following observations:

1) *Leukopenia*, defined by WHO as \(\leq 5.0 \times 10^9\) leukocytes/L for adults,\(^{25}\) is a hallmark feature of the early phase (i.e., DPO 2-10) of dengue. It occurs in 76-100% of dengue cases and is associated with viral destruction or inhibition of mononuclear lineage cells.

2) *Thrombocytopenia*, defined by WHO as \(\leq 100.0 \times 10^9\) platelets/L for pediatric and adult patients,\(^{25}\) is a major clinical manifestation of moderate-severe dengue occurring DPO 3.5. The estimated prevalence of thrombocytopenia is 26-50%.\(^{25}\) It is associated with capillary permeability secondary to endothelial dysfunction in the progression of dengue from the febrile to the critical phase.

3) *Hemoconcentration*, defined by WHO as a change in hematocrit >20% above baseline for adults.\(^{25}\) It is a major clinical manifestation of moderate-severe dengue and an indicator of dysfunction of the endothelial barrier and plasma leakage into the perivascular space.\(^{25}\)
Hypothesis 2: One or more clinical characteristics or hematologic indices differentiating DENV-
*Leptospira* co-infected patients from DENV-positive patients can be incorporated into a clinical algorithm for use in under-resourced clinical settings.

H₀: Due to the identical clinical presentation of dengue and leptospirosis in the acute phase (i.e., DPO <7), no clinical characteristic or hematologic index is able to differentiate DENV-
*Leptospira* co-infection from DENV SSI and provide decisional support for clinicians without access to comprehensive diagnostic testing.

H₁: Despite the identical clinical presentation of dengue and leptospirosis in the acute phase (i.e., DPO <7), one or more clinical characteristic(s) or hematologic index(ices) is able to differentiate DENV-Leptospira co-infection from DENV SSI to provide decisional support for clinicians without access to comprehensive diagnostic testing.

*Rationale*
Leptospirosis is a differential diagnosis for tropical AFI, and leptospirosis is frequently misdiagnosed as dengue. A case-control study in Bucaramanga, Colombia, demonstrated significant differences in leukocyte and platelet counts between patients with dengue and patients with leptospirosis, supporting the utility of these indices as part of a diagnostic algorithm. The central importance of investigating DENV-Leptospira co-infections is supported by the following:

1) Dengue and leptospirosis have overlapping geographic regions of endemnicity;
2) Dengue and leptospirosis have identical acute clinical presentations;
3) Laboratory diagnosis of leptospires is highly technical, lengthy, and not readily available;
4) Leptospirosis is a disease of high consequence but is treatable by cost-effective, readily-available antibiotics.

**Statistical Analyses**

*Frequency of Demographic and Clinical Characteristics*
The prevalence of demographic characteristics was expressed as numbers and percentages. Categorical variables (i.e., clinical characteristics) were expressed as numbers and percentages. Group comparisons were performed using *χ²* test or Fisher’s exact tests, as appropriate for the sample sizes under comparison.

*Comparison of Means for Hematologic Indices*
Parametric One-way ANOVA was used to compare the means of continuous variables (i.e., hematologic indices). Between-group mean differences were compared using the Student’s *t* test and the Mann-Whitney U test (Wilcoxon rank sum test) where applicable.

*Logistic Regression*
Logistic regression was used to model the relationship between DENV infection status. Crude and adjusted odds ratios (OR) were used to assess measures of association.
Post-Hoc Analyses

Two types of post-hoc analyses were performed. First, restricting the analyzable cohort to only DENV-positive patients, we used a case-case approach with DENV-positive patients as the case-control and differentially-infected subgroups (i.e., SSIs and MSIs) as case-case groups. Due to both zero values and small samples sizes in the outcome subgroups, case-case analyses could not be performed or failed to approach statistical significance. A second approach was a principal component analysis aimed at detecting signals of association between variables, particularly the interrelated hematologic indices.

Statistical analyses were performed in SAS version 9.4 (Cary, NC). P values were based on 2-sided tests with statistical significance at $p < 0.05$. Confidence intervals were calculated at the 95% level.

RESULTS

**Participant Demographics**

Between January 2013 and October 2014, 216 patients with an AFI were enrolled and underwent molecular testing for DENV and *Leptospira* spp. Los Patios ($n=124, 57.4\%$) and Ocaña ($n=50, 23.4\%$) were overrepresented in the enrolled cohort; 64.1\% ($n=66$) of DENV-positive patients were from Los Patios (Table 1, Figure 8). Although recruitment was continuous, 50\% of patients were enrolled between October and December of 2013, presenting for care a mean of 3.6 DPO (range 2.0-8.0 days). Timing of presentation for care did not differ by infection status (DPO 3.6 for DENV-positive versus DPO 4.0 for DENV-negative, $p=0.1508$), nor did admission to hospital (16.8\% for DENV-positive versus 27.2\% for DENV-negative, $p=0.2003$). Twenty-eight (27.2\%) patients were hospitalized; 18 (64.3\%) were pediatric patients. There were no differences between SSI and MSI patients for timing of presentation for care or admission to hospital.

Female patients comprised 44.4\% ($n=96$) of the enrolled cohort; there were no pregnancies. Co-morbid conditions—trisomy 21 and hepatitis B virus (HBV)—were recorded for two patients (0.9\%). The mean age for the enrolled cohort was 20.0 years (range <1-92 years), compared to 19.8 years (range 1-87 years) for the DENV-positive cohort. Pediatric patients (<18 years) comprised 63.0\% ($n=136$) of enrollees; 34.9\% ($n=36$) were between the ages of 1-9 years (Table 1).

**Etiology**

One hundred and three (47.7\%) patients were positive for DENV (Figure 8). SSIs ($n=88$) accounted for 85.4\% of DENV-positive infections and MSIs ($n=15$) 14.6\% of all DENV-positive infections. Three patients were tested positive for infection with a pathogenic *Leptospira spp.*, providing a cohort infection prevalence of 1.4\%. Anti-DENV IgM and IgG antibody data were available for 91.2\% ($n=103$) of DENV-negative patients and 49.5\% ($n=51$) of DENV-positive patients. However, the data did not form the basis of classification of infection status, nor was it used to infer associations between acute etiology and clinical data.
Single- and Multi-Serotype Infection
DENV-2 was the predominant SSI ($n=34, 38.6\%$), followed by DENV-3 ($n=24, 27.3\%$), DENV-1 ($n=21, 23.9\%$), and DENV-4 ($n=9, 10.2\%$) (Figure 8). DENV-2/4 ($n=5$) accounted for $41.7\%$ of MSIs, followed by DENV-2/3 ($n=4, 33.3\%$), DENV-3/4 ($n=2, 16.7\%$), and DENV-1/2 ($n=1, 8.3\%$). Triple-serotype combinations were DENV-1/2/3 ($n=1$), DENV-1/3/4, ($n=1$), and DENV-2/3/4 ($n=1$).

Clinical Presentation
Headache ($52.3\%$) and myalgia ($49.1\%$) were the most frequently reported symptoms for all enrolled patients, similar to DENV-negative patients (headache $79.3\%$, myalgia $75.0\%$) (Table 2). DENV-positive patients reported headache ($52.4\%$), myalgia ($49.5\%$), and vomiting ($27.2\%$). Abdominal pain was reported by $46.2\%$ ($n=6$) of patients with DENV-1 SSI, and DENV-2 SSI patients reported retro-orbital pain ($n=11, 52.6\%$). Signs of bleeding were observed in seven ($6.8\%$) patients (Table 3).
Table 1. Seroprevalence Survey Descriptive Data for Dengue-Positive and Negative Patients

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>DENV-negative (N = 113)</th>
<th>DENV-positive (N = 103)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>&lt; 1</td>
<td>5 (4.4)</td>
<td>3 (2.9)</td>
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<tr>
<td>1-9</td>
<td>32 (28.3)</td>
<td>33 (32.0)</td>
</tr>
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<td>10-17</td>
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<td>29 (28.2)</td>
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</tr>
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<td>30-39</td>
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<td>13 (12.6)</td>
</tr>
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<td>40-49</td>
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<td>3 (2.9)</td>
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<td>8 (7.1)</td>
<td>4 (3.9)</td>
</tr>
<tr>
<td>65+</td>
<td>5 (4.4)</td>
<td>4 (3.9)</td>
</tr>
</tbody>
</table>

| Sex (Female) | 51 (45.1) | 45 (43.7) |

<table>
<thead>
<tr>
<th>Department of Residence</th>
<th>DENV-negative</th>
<th>DENV-positive</th>
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<tbody>
<tr>
<td>Bogota, DC</td>
<td>1 (0.9)</td>
<td>0 (0)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Norte de Santander</td>
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<td>103 (100)</td>
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<table>
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<tr>
<td>3</td>
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<td>30 (29.1)</td>
</tr>
<tr>
<td>4</td>
<td>12 (10.6)</td>
<td>15 (14.6)</td>
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<td>4 (3.9)</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
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<tr>
<td></td>
<td>N (%)</td>
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</tr>
<tr>
<td>Abdominal pain</td>
<td>33 (15.3)</td>
<td>17 (16.5)</td>
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<tr>
<td>Arthralgia</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Epistaxis</td>
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<tr>
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</tr>
<tr>
<td>Headache</td>
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<tr>
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</tr>
<tr>
<td>Hypotension‡</td>
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<td>1 (1.0)</td>
</tr>
<tr>
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<tr>
<td>Rash</td>
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<td>12 (11.7)</td>
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<tr>
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<td>Tachycardia§</td>
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</tr>
<tr>
<td>Vomiting</td>
<td>46 (21.3)</td>
<td>28 (27.2)</td>
</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus.

* Fisher’s exact $\chi^2$ $p$-value significant at < 0.05.
† Not applicable due to zero values.
‡ Blood pressure <90/60 mm Hg.
§ Heart rate >100 beats per minute.
<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>DENV-1 only</th>
<th>DENV-2 only</th>
<th>DENV-3 only</th>
<th>DENV-4 only</th>
<th>MSI only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
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<tr>
<td>Abdominal pain</td>
<td>6 (46.2)</td>
<td>4 (21.1)</td>
<td>4 (23.5)</td>
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<td>1 (6.7)</td>
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<tr>
<td>Arthralgia</td>
<td>2 (15.4)</td>
<td>5 (26.3)</td>
<td>4 (23.5)</td>
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<td>1 (6.7)</td>
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<tr>
<td>Diarrhea</td>
<td>1 (7.7)</td>
<td>1 (10.5)</td>
<td>1 (5.9)</td>
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<td>0 (0)</td>
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<tr>
<td>Epistaxis</td>
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<td>3 (15.8)</td>
<td>0 (0)</td>
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<td>Headache</td>
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<td>1 (5.9)</td>
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<td>0 (0)</td>
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<td>Hospitalized</td>
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<td>Myalgia</td>
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<td>7 (46.7)</td>
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<tr>
<td>Oliguria</td>
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<td>1 (5.3)</td>
<td>1 (5.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Petechiae</td>
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<td>1 (5.3)</td>
<td>2 (11.8)</td>
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<td>0 (0)</td>
</tr>
<tr>
<td>Rash</td>
<td>2 (15.4)</td>
<td>3 (15.8)</td>
<td>2 (11.8)</td>
<td>1 (12.5)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Retro-orbital pain</td>
<td>4 (30.8)</td>
<td>10 (52.6)</td>
<td>6 (35.3)</td>
<td>2 (25.0)</td>
<td>2 (13.3)</td>
</tr>
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<td>Tachycardia†</td>
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<td>0 (0)</td>
<td>1 (5.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vomiting</td>
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<td>7 (36.8)</td>
<td>7 (41.2)</td>
<td>3 (37.5)</td>
<td>5 (33.3)</td>
</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus; MSI, multi-serotype infection; SSI, single-serotype infection.

* Heart rate >100 beats per minute.
† Blood pressure <90/60 mm Hg.
### Table 4. Mean Hematologic Indices, by Dengue Virus Infection Status

<table>
<thead>
<tr>
<th>Index</th>
<th>DENV-negative</th>
<th>DENV-positive</th>
<th>DENV-1</th>
<th>DENV-2</th>
<th>DENV-3</th>
<th>DENV-4</th>
<th>DENV MSI</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>P value</td>
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<td></td>
<td>(Range)</td>
<td>(Range)</td>
<td>(Range)</td>
<td>(Range)</td>
<td>(Range)</td>
<td>(Range)</td>
<td>(Range)</td>
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</tr>
<tr>
<td>Erythrocytes (10^12/L)</td>
<td>4.9 ± 0.5</td>
<td>5.2 ± 0.6</td>
<td>4.96 ± 0.6</td>
<td>5.3 ± 0.6</td>
<td>4.8 ± 0.3</td>
<td>5.34*</td>
<td>5.4 ± 0.5</td>
<td>0.7123</td>
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<tr>
<td></td>
<td>(3.8-6.1)</td>
<td>(4.1-6.5)</td>
<td>(4.1-6.0)</td>
<td>(4.4-6.5)</td>
<td>(4.5-5.3)</td>
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<td>(4.8-5.9)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.6 ± 5.6</td>
<td>40.8 ± 4.7</td>
<td>40.4 ± 5.5</td>
<td>40.1 ± 9.0</td>
<td>40.1 ± 4.0</td>
<td>40.8 ± 3.6</td>
<td>40.8 ± 4.7</td>
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<td>(5.0-52.9)</td>
<td>(12.0-55.6)</td>
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<td>(33.0-48.8)</td>
<td>(36.7-45.2)</td>
<td>(33.0-50.8)</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.9 ± 1.7</td>
<td>13.1 ± 1.9</td>
<td>13.0 ± 2.0</td>
<td>15.0 ± 6.4</td>
<td>12.7 ± 1.5</td>
<td>13.1 ± 1.4</td>
<td>13.1 ± 1.9</td>
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</tr>
<tr>
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<td>(8.7-17.9)</td>
<td>(8.8-39.4)</td>
<td>(10.0-18.1)</td>
<td>(8.8-39.4)</td>
<td>(10.0-15.6)</td>
<td>(11.6-15.3)</td>
<td>(10.2-16.3)</td>
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<tr>
<td>Leukocytes (10^9/L)</td>
<td>4.4 ± 2.3</td>
<td>3.9 ± 2.0</td>
<td>3.7 ± 2.4</td>
<td>4.4 ± 2.1</td>
<td>3.8 ± 1.7</td>
<td>3.4 ± 1.1</td>
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<tr>
<td>Lymphocytes (%)</td>
<td>42.4 ± 24.6</td>
<td>44.0 ± 17.6</td>
<td>42.9 ± 22.0</td>
<td>39.8 ± 22.2</td>
<td>43.3 ± 22.9</td>
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<td>36.5 ± 11.8</td>
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<td>(2.0-90.0)</td>
<td>(2.5-89.0)</td>
<td>(7.2-84.0)</td>
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<td>(4.9-86.0)</td>
<td>(15.8-56.0)</td>
<td>(2.5-79.0)</td>
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<tr>
<td>Neutrophils (%)</td>
<td>53.3 ± 24.2</td>
<td>53.3 ± 17.2</td>
<td>52.8 ± 20.5</td>
<td>54.8 ± 23.1</td>
<td>54.6 ± 23.0</td>
<td>60.4 ± 11.2</td>
<td>53.3 ± 17.2</td>
<td>0.9649</td>
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<tr>
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<td>(10.0-94.5)</td>
<td>(10.0-94.1)</td>
<td>(14.0-89.6)</td>
<td>(10.0-91.0)</td>
<td>(14.0-92.1)</td>
<td>(44.0-76.9)</td>
<td>(21.0-94.1)</td>
<td></td>
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<tr>
<td>Platelets (10^3/L)</td>
<td>99.9 ± 38.6</td>
<td>102.3 ± 43.5</td>
<td>101.2 ± 38.3</td>
<td>81.8 ± 47.3</td>
<td>110.1 ± 40.8</td>
<td>103.1 ± 36.5</td>
<td>102.3 ± 43.6</td>
<td>0.1559</td>
</tr>
<tr>
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<td>(15.0-191.0)</td>
<td>(9.0-184.0)</td>
<td>(29.0-184.0)</td>
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<td>(23.0-161.0)</td>
<td>(32.0-135.0)</td>
<td>(16.0-174.0)</td>
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</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus; L, liter; g/dL, grams per deciliter; L, liter; MSI, multi-serotype infection’ SD, standard deviation.

* N=1.

Significant at p<0.05.
**Hematology**

*Hemoglobin and Hematocrit*

Despite the correlation between hemoglobin and hematocrit, and their dual-dependence on whole blood and plasma volume, this biological correlation did not hold in our analyses. DENV-2 had the highest mean hemoglobin (15.0 ± 16.4 g/dL), consistent with hemoconcentration, but there were no significant between-group differences (Table 5).

### Table 5. Between-Group Differences in Mean Hemoglobin

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>$\bar{x}_1 - \bar{x}_2$ (g/dL)</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
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<td>DENV-2</td>
<td>DENV-negative</td>
<td>2.1</td>
<td>0.90-3.22</td>
</tr>
<tr>
<td>DENV-2</td>
<td>DENV-1</td>
<td>2</td>
<td>0.33-3.63</td>
</tr>
<tr>
<td>DENV-2</td>
<td>DENV-3</td>
<td>2.2</td>
<td>0.61-3.86</td>
</tr>
<tr>
<td>DENV-2</td>
<td>DENV-4</td>
<td>1.81</td>
<td>-0.42-4.03</td>
</tr>
<tr>
<td>DENV-2</td>
<td>DENV MSI</td>
<td>1.9</td>
<td>0.09-3.70</td>
</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus; g/dL, grams per deciliter.

$\bar{x}_1 - \bar{x}_2$ Difference between mean values of group 1 and group 2.

* $t$-test significant at $p<0.05.$

The mean hematocrit for the enrolled cohort (40.8%) was statistically equivalent across all groups ($p=0.9616$, data not shown), while the mean hemoglobin was significantly different across all groups ($p=0.0232$) (Table 4). In the principle component analysis, no significant associations emerged, including between hemoglobin and hematocrit. The extent of missing data (Appendix I), particularly for DENV-negative patients, likely contributed to this counterintuitive finding.

*Leukocytes*

The mean leukocyte count for all enrollees was 3.9 x $10^9$ cells/L, which falls just below the lower limit of the adult clinical reference range of 4.0 x $10^9$ leukocytes/L (Appendix G). The DENV-2 SSI group did not have leukopenia ($\bar{x}_{\text{leukocyte count}} = 4.4 \pm 2.1 x 10^9$ cells/L) and was the only infection group with a mean leukocyte count exceeding the cohort mean ($4.4 \pm 2.3 x 10^9$ cells/L), although the difference was not statistically significant ($p=0.5382$) (Table 4). In regression analyses (Table 6), only DENV-1 was marginally associated with leukopenia (OR 2.85; 95% CI 1.05-7.74), which, when adjusted for age (aOR 2.82; 95% CI 1.02-7.79) and DPO (aOR 2.84; 95% CI 1.05-7.72) weakened but remained significant.
Platelets

All enrolled patients met the clinical criteria for thrombocytopenia (<150.0 x 10^9 platelets/L) with a cohort mean of 102.3 ± 43.5 x 10^9 platelets/L. We observed no overall association between platelet count and DENV infection status (p=0.1559). DENV-2 SSI patients had the lowest mean platelet count (81.8 ± 47.3 x 10^9/L), and DENV-3, the highest (110.1 ± 40.8 x 10^9/L). However, there were between-group differences for DENV-2 SSI patients. Compared to DENV-negative patients, DENV-2 SSI patients had a significantly lower platelet count (\( \bar{x} = 28.3 \) platelets, CI 6.70-49.91), as did DENV-3 SSI patients when compared to DENV-2 SSI patients (\( \bar{x} = 18.1 \) platelets, CI 2.42-33.75) (Tables 4 and 7). Neither SSI nor MSI predicted severity of thrombocytopenia.

<table>
<thead>
<tr>
<th>Table 6. Predictive Models of Leukopenia and Dengue Virus Serotype 1</th>
</tr>
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<tbody>
<tr>
<td><strong>Model</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>DENV infection status x leukocytes</td>
</tr>
<tr>
<td>DENV infection status x leukocytes Adjusted for Age</td>
</tr>
<tr>
<td>DENV infection status x leukocytes Adjusted for DPO</td>
</tr>
<tr>
<td>DENV infection status x leukocytes Adjusted for Sex</td>
</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus; L, liter.
\( \chi^2 \) p-value significant at <0.05.

<table>
<thead>
<tr>
<th>Table 7. Between-Group Differences in Mean Platelet Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>DENV-2</td>
</tr>
<tr>
<td>DENV-2</td>
</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus; g/dL, grams per deciliter.
\( \bar{x}_1 - \bar{x}_2 \) Difference between mean values of group 1 and group 2.
* t-test significant at p<0.05.
Multi-Serotype Infection and Clinical Severity

**Adult Patients**
The three adult patients were infected with a serotype combination that included DENV-2, and each patient had thrombocytopenia and monocytosis (Table 9). Clinical symptoms at presentation were documented for only one patient, a 62-year-old female (DENV-2/3) from Los Patios who presented for care on DPO 4 complaining of headache and myalgia.

**Pediatric Patients**
Twelve of 15 (80.0%) MSIs were in patients ≤15 years of age (median 8.5 years, range 1-15 years) (Tables 10-12). Males accounted for 75.0% (n=9) of pediatric MSIs. Ten (83.3%) resided in Los Patios, followed by Teorama (n=3, 25.3%) and Ocaña (n=2, 16.7%) (Figure 9). They presented for care a median of DPO 3 (range 3-4 days). DENV-2/4 was the most frequent serotype combination (n=4, 33.3%) (Table 11). Three patients <16 years of age were simultaneously infected with three serotypes (Table 12). Headache (n=6, 50.0%) and myalgia (n=6, 50.0%) were recorded for half of the pediatric MSIs (Tables 10-12). All 12 had thrombocytopenia; nine (75%) were classified as mild, two (16.7%) moderate, and one (8.3%), a three-year-old male, with borderline severe thrombocytopenia. Gingival bleeding was documented in a two-year old male with a platelet count of 75.0 x 10⁹ cells/L. Leukopenia (n=10, 83.3%) was the second most frequently observed condition, followed by anemia (n=4, 33.3%).

**Leptospira-Positive Patients**
Table 13 provides demographic, clinical, and hematologic data for Leptospira-positive patients. Each of the three patients was a resident of Los Patios, and two patients were co-infected with DENV. Arthralgia, myalgia, thrombocytopenia were common to the three patients; the DENV-negative and DENV-2 co-infected patients had moderately severe leukopenia. Based on the hematologic indices, there are indicators of additional etiologies other than those for which we conducted molecular testing. The DENV-negative patient, who presented with eosinophilia, could have a parasitic infection or other systemic inflammatory process. The patient co-infected DENV-2, who was the only patient admitted to hospital, had a clinical presentation consistent with a hematologic malignancy. Finally, the patient with DENV-3 and HBV had a clinical picture consistent with HIV infection, liver cirrhosis, and a neutrophil-to-lymphocyte ratio associated with a poor prognosis in patients with HBV.32

**DISCUSSION**
Little is known about the processes and consequences of DENV MSI, including their frequency and impact on regional transmission dynamics. In Norte de Santander, which includes the Colombian-Venezuelan border, neither the prevalence of MSIs nor the prevalence of leptospirosis have been determined. Employing hospital-based surveillance methodology, we tested the sera of 216 AFI
patients presenting for care in at two referral centers. We confirmed 103 cases of dengue, of which 14.6% were MSIs, and three cases of leptospirosis, of which two were in patients with dengue. To our knowledge, this is the first study to report DENV MSIs and dengue-leptospirosis co-infection in this region of Colombia.

DENV-2 and -3 were detected with greater frequency than the other serotypes during our study period from 2013-2014. A study conducted one year later in Norte de Santander, from 2015-2016, identified DENV-2 and -1 as the dominate serotypes in circulation for that period.33 As an aside, this study, which used hospital-based serosampling, also detected DENV-CHIKV and DENV-ZIKV co-infections in 40% of serum samples. Three patients were acutely infected with all three arboviruses.

**Indicators of Clinical Severity**

As a clinical syndrome, AFIs present both a diagnostic and treatment challenge for clinicians, particularly in developing countries. Dengue has long been identified as a clinical syndrome identified initially presenting with fever, myalgia, and headache.29 The difficulty, therefore, in diagnosing dengue has been the non-specific presentation of symptoms that mirror other AFIs. Thrombocytopenia and leukopenia were prevalent in this study population, including among the DENV-negative patients. Further, headache and myalgia, the most frequently reported symptoms by DENV-positive patients, did not differ by individual serotype of between SSIs and MSIs. Our findings underscore the challenge of dengue diagnosis: clinical presentation alone is insufficient.

Overall, no patterns emerged within the MSI cohort to indicate differences in either magnitude or severity. Clinically, the pediatric population is distinct from the adult population, and we were underpowered to detect differences between adult and pediatric patients. Due to small sample sizes, which limited statistical power, we grouped patients by infection status, ignoring sex-linked physiologic differences that contribute to clinical outcomes for infection diseases. In case-level examination, however, pediatric thrombocytopenia did not appear to differ by SSI or MSI, nor did the other hematologic indices. A larger study, powered to detect differences by age-sex groups, will be required to further explore the association of clinical severity and MSI.

**Leptospirosis**

The National Public Health Surveillance Center for Colombia has one documented case of leptospirosis in Norte de Santander, which was in 2007.34 Ours is the second report of leptospirosis in Norte de Santander, and the first to identify co-infections with DENV. We estimate the prevalence of leptospirosis to be 1.4%, which is almost certainly an underestimation of the true burden of disease in the region.

Arthralgia, myalgia, and mild thrombocytopenia were the common characteristics of *Leptospira*-positive patients. Leukopenia was observed in the one patient with DENV co-infection and in the DENV-negative patient. Leukopenia has been suggested as a potential clinical discriminator between
dengue and leptospirosis, although our findings do not reflect this relationship. Conversely, the patient with dengue, leptospirosis, and HBV did not have leukopenia, but he did have neutrophilia. In a comprehensive case report of fatal DENV-\textit{Leptospira} co-infection in Puerto Rico, thrombocytopenia, leukocytosis, with neutrophilia were each observed. Neutrophilia is a common finding in patients with HBV and, together with the neutrophil-to-lymphocyte ratio, is a prognosticator of HBV-associated mortality. This patient highlights the difficulty for clinicians in interpreting clinical data and determining differential diagnosis. This unexpected finding further emphasizes the ability of case-level data to examine how co-infection can alter the clinical presentation of disease.

Our results are subject to a number of limitations and should be considered within the context of logistical limitations inherent in global health partnerships. The main challenge to data analysis was the amount of missing data (Appendix I). Although initially powered for detecting differences among the study groups, enrollment fell short of the planned samples sizes, for reasons not related to enrollment or study-site participation. Our use of hospital-based convenience sampling in Norte de Santander limits the generalizability of the results to the general population of Colombia, as patients seeking treatment may have increased severity, including underlying comorbidities, greater that the experience of the general population. Finally, our study was not designed for a pediatric population, nor did we use pediatric specialists to evaluate these patients. For example, the finding of retro-orbital pain in a pre-verbal pediatric patient should be considered in this context.

A major strength of this study was the examination of hematologic values to evaluate disease severity. This enabled identification of clinical indicators that would be of utility at presentation for care, and thereby mediate a shorter interval between onset of illness and initiation of supportive or antibiotic therapies. Further, our use of PCR platforms for diagnosing dengue and leptospirosis avoided the ambiguity of antibody response and increased the specificity of inferences about pathogen-specific presentation. However, we did not perform additional testing for other etiologies, and therefore we cannot rule out confounding by other chronic and infectious conditions.

**Implications for Clinical and Public Health Practice**

Our objectives for this study were twofold: 1) to establish the first prevalence estimates of DENV MSIs and \textit{Leptospira} co-infections in Norte de Santander; 2) to design an algorithm to discriminate between the clinical presentations of dengue SSI and MSI, and dengue and dengue-leptospirosis. Our rationale for these objectives is the increasing frequency of co-infections and the need to explore how these infections alter disease severity. Although dengue has no pharmaceutical therapy, leptospirosis is treatable with readily-available antibiotics. Because of the small numbers of both types of infection, we identified case-level data as a source of granularity not achieved by the use of aggregate data.

We provided estimates, with limitations, of the prevalence of DENV MSI and DENV-\textit{Leptospira} co-infections, but we were unable to conclude that a difference in clinical severity between the acute presentation of dengue SSI is different than that of dengue MSI. While we detected serotype-specific
clinical features that invariably present across the spectrum of dengue, including leukopenia and thrombocytopenia, we failed to identify clinical features or hematologic measures of clinical severity in the MSI patient, although assessment of this relationship using a larger cohort of patients is warranted. The clinical data presented herein will be of utility for background information for pooled analyses for future studies of infection severity.

Under-diagnosis and under-reporting of leptospirosis may be due to a lack of clinician awareness of the prevalence of disease and environmental reservoirs, and consideration should be given to public campaigns and medical education throughout endemic regions. In Colombia, diagnosis of leptospirosis requires submission of acute and convalescent sera to private or referent laboratories for testing, a cost-prohibitive option for the patient and one too lengthy for delaying treatment. Increasing the availability of rapid diagnostics will be essential for defining the prevalence of leptospirosis, decreasing its incidence, and preventing the severe sequelae associated with untreated disease.

CONCLUSION

Multi-pathogen infections will continue to occur as emerging tropical viruses move into population centers where other diseases of poverty and urban density constitute an already high burden of disease. The broadening application of molecular technologies for clinical diagnosis, including the use of next-generation sequencing for describing the intra-host pathogen landscape, will detect these infections with increased frequency, requiring clinical interpretation for the treating clinician. In Norte de Santander, Colombia, we estimated the prevalence of multi-serotype dengue disease to be 14.6% and the prevalence of leptospirosis to be 1.4%. We identified co-infections of DENV with pathogenic *Leptospira* spp. and HBV. While we were unable to identify clinical or laboratory predictors of severity associated these co-infections, the implication of multi-pathogen infection on disease pathogenesis, clinical manifestation of disease, and pathogen-pathogen interaction should be the subject of targeted surveillance and investigation.
<table>
<thead>
<tr>
<th>Hematologic Index</th>
<th>Single Serotype</th>
<th>Multi-Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DENV-1 N (%)</td>
<td>DENV-2 N (%)</td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 100 x 10^9/L</td>
<td>10 (47.6)</td>
<td>18 (52.9)</td>
</tr>
<tr>
<td>≥ 100 x 10^9/L</td>
<td>11 (52.4)</td>
<td>16 (47.1)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.01 (0.42-2.47)</td>
<td>1.32 (0.62-2.81)</td>
</tr>
</tbody>
</table>

Abbreviations: DENV, dengue virus; L, litre; g/dL, grams per deciliter; MSI, multi-serotype infection.

Red font indicates the abnormal clinical range (predictor variable) of clinical severity, when divided at the World Health Organization cut point for dengue-associated leukopenia.
## Table 9. Adult Multi-Serotype Infections

<table>
<thead>
<tr>
<th>Sex Age City</th>
<th>DENV Serotype (Ct)</th>
<th>IgM IgG</th>
<th>Clinical Presentation</th>
<th>Hematology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 37 years San Pablo</td>
<td>2 (36.68) 4 (17.84)</td>
<td>IgG+</td>
<td>† † †</td>
<td>Eryth (x10^9/L) 4.8</td>
</tr>
<tr>
<td>Male 37 years San Pablo</td>
<td>2 (36.68) 3 (17.84)</td>
<td>ND</td>
<td>† † †</td>
<td>Eryth (x10^9/L) 5.9</td>
</tr>
<tr>
<td>Female 62 years Los Patios</td>
<td>2 (36.29) 3 (37.86)</td>
<td>† 4</td>
<td>Headache Myalgia</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: Admit, admission to hospital; Baso, basophil; Ct, cycle threshold; DENV, dengue virus; DPO, days post-illness onset; Eos, eosinophil; Eryth, erythrocytes; Hb, hemoglobin; Hct, hematocrit; IgG, anti-DENV IgG antibody; IgM, anti-DENV IgM antibody; Leu, leukocytes; Lymph, lymphocytes; Mono, monocytes; ND, none detected; Neut, neutrophils; Plt, platelets; and Yr, years.

Above (†) or below (↓) laboratory reference for age and sex. See Appendix 8 for adult and pediatric reference ranges.

† Missing value
<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>City</th>
<th>DENV Serotype (Ct)</th>
<th>IgM IgG</th>
<th>DPO</th>
<th>Symptoms</th>
<th>Hospitalized</th>
<th>Hematology</th>
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<td></td>
<td></td>
<td></td>
<td>Eryth (x10^9/L)</td>
</tr>
<tr>
<td>Female</td>
<td>8 years</td>
<td>Los Patios</td>
<td>1 (29.00)</td>
<td>IgG+</td>
<td>3</td>
<td>Headache, Myalgia, Rash, Retro-orbital pain</td>
<td>No</td>
<td>↑ 2.3</td>
</tr>
<tr>
<td>Male</td>
<td>2 years</td>
<td>Los Patios</td>
<td>2 (21.68)</td>
<td>IgM+ IgG+</td>
<td>3</td>
<td>Gingival bleeding, Headache, Vomiting</td>
<td>No</td>
<td>↑ 5.0</td>
</tr>
<tr>
<td>Male</td>
<td>13 years</td>
<td>Los Patios</td>
<td>3 (36.29)</td>
<td>IgG+</td>
<td>3</td>
<td>Myalgia</td>
<td>No</td>
<td>↑ 3.6</td>
</tr>
<tr>
<td>Male</td>
<td>3 years</td>
<td>San Pablo</td>
<td>3 (33.40)</td>
<td>ND</td>
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<td>↑</td>
<td>5.3</td>
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<td>Male</td>
<td>10 years</td>
<td>Los Patios</td>
<td>4 (35.47)</td>
<td>IgG+</td>
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<td>Yes</td>
<td>Yes</td>
<td>↑ 2.7</td>
</tr>
</tbody>
</table>

Abbreviations: Admit, admission to hospital; Baso, basophils; Ct, cycle threshold; DENV, dengue virus; DPO, days post-illness onset; Eos, eosinophils; Eryth, erythrocytes; Hb, hemoglobin; Hct, hematocrit; IgG, anti-DENV IgG antibody; IgM, anti-DENV IgM antibody; Leu, leukocytes; Lymph, lymphocytes; Mono, monocytes; ND, none detected; Neut, neutrophils; Plt, platelets; and Yr, years.

Above (↑) or below (↓) laboratory reference for age and sex. See Appendix # for adult and pediatric reference ranges.

† Missing value
## Table 11. Pediatric Multi-Serotype Infections with Dengue Virus Serotypes 2 and 4

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>City</th>
<th>DENV Serotype (C_t)</th>
<th>IgM IgG</th>
<th>Clinical Presentation</th>
<th>DPO</th>
<th>Symptoms</th>
<th>Hospitalized</th>
<th>Hematology</th>
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<td></td>
<td></td>
<td>Eryth (x10^9/L)</td>
<td>Leu (x10^9/L)</td>
<td>Hb (g/dL)</td>
<td>Hct (%)</td>
<td>Plt (x10^9/L)</td>
<td>Neut (%)</td>
<td>Lymph (%)</td>
<td>Eos (%)</td>
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</tr>
<tr>
<td>Male</td>
<td>1 year</td>
<td>Los Patios</td>
<td>2 (30.24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
<td>6.1</td>
<td>10.2</td>
<td>33.0</td>
<td>132.0</td>
<td>27.0</td>
<td>72.0</td>
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<td>4 (29.80)</td>
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<td></td>
<td>†</td>
<td>3.0</td>
<td>11.4</td>
<td>37.0</td>
<td>131.0</td>
<td>67.0</td>
<td>33.0</td>
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<tr>
<td>Female</td>
<td>4 years</td>
<td>Los Patios</td>
<td>2 (24.82)</td>
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<td></td>
<td></td>
<td>3.0</td>
<td>3.8</td>
<td>10.6</td>
<td>36.0</td>
<td>174.0</td>
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<td>4 (18.41)</td>
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<td>55.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Female</td>
<td>8 years</td>
<td>Los Patios</td>
<td>2 (27.10)</td>
<td>IgG+</td>
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<td>4 (22.65)</td>
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<tr>
<td>Male</td>
<td>9 years</td>
<td>Los Patios</td>
<td>2 (27.29)</td>
<td>IgG+</td>
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Abbreviations: Admit, admission to hospital; Baso, basophils; C_t, cycle threshold; DENV, dengue virus; DPO, days post-illness onset; Eos, eosinophils; Eryth, erythrocytes; Hb, hemoglobin; Hct, hematocrit; IgG, anti-dengue virus IgG antibody; IgM, anti-dengue virus IgM antibody; Leu, leukocytes; Lymph, lymphocytes; Mono, monocytes; ND, none detected; Neut, neutrophils; Plt, platelets; and Yr, years.

Above (↑) or below (↓) laboratory reference for age and sex. See Appendix # for adult and pediatric reference ranges.

† Missing value
### Table 12. Dengue Virus Triple-Serotype Infection

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>City</th>
<th>DENV Serotype (Ct)</th>
<th>IgM IgG</th>
<th>Clinical Presentation</th>
<th>Hematology</th>
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<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>DPO Symptoms</td>
<td>Eryth</td>
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<td>Hospitalized</td>
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<td>Baso</td>
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<table>
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<tr>
<th>Sex</th>
<th>Age</th>
<th>City</th>
<th>DENV Serotype (Ct)</th>
<th>IgM IgG</th>
<th>Clinical Presentation</th>
<th>Hematology</th>
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<th>IgM IgG</th>
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<th>City</th>
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</tbody>
</table>

| Abbreviations: | Admit, admission to hospital; Baso, basophils; C, cycle threshold; DENV, dengue virus; DPO, days post-illness onset; Eos, eosinophils; Eryth, erythrocytes; Hb, hemoglobin; Hct, hematocrit; IgG, anti-dengue virus IgG antibody; IgM, anti-dengue virus IgM antibody; Leu, leukocytes; Lymph, lymphocytes; Mono, monocytes; ND, none detected; Neut, neutrophils; Plt, platelets; and Yr, years. |

Above (↑) or below (↓) laboratory reference for age and sex. See Appendix # for adult and pediatric reference ranges.

† Missing value
# Table 13. Dengue Virus-Leptospira spp. Co-Infections

<table>
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<tr>
<th>Sex, Age</th>
<th>City</th>
<th>DENV Serotype</th>
<th>DENV Antibody</th>
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<th>Hospitalized</th>
<th>Symptoms</th>
<th>Leu (x10^9/L)</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>Plt (x10^9/L)</th>
<th>Neut (%)</th>
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<td>Negative</td>
<td>IgG+</td>
<td>3</td>
<td>No</td>
<td>Arthralgia Myalgia</td>
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<td>13.8</td>
<td>43.9</td>
<td>106.0↓</td>
<td>63.0↓</td>
<td>29.0↓</td>
<td>8.0↑</td>
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<td>IgG+</td>
<td>6</td>
<td>Yes</td>
<td>Arthralgia Myalgia Vomiting</td>
<td>2.8↓</td>
<td>13.4↓</td>
<td>43.0</td>
<td>112.0↓</td>
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<tr>
<td>M, 33 yr</td>
<td>Los Patios</td>
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<td>IgG+</td>
<td>3</td>
<td>No</td>
<td>Arthralgia Diarrhea Headache Hepatitis B virus Myalgia Retro-orbital pain</td>
<td>4.4</td>
<td>12.6↓</td>
<td>38.8↓</td>
<td>117.0↓</td>
<td>85.0↑</td>
<td>15.0↑</td>
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</table>

Abbreviations: DENV, dengue virus; DPO, days post-illness onset; Eos, eosinophils; Hb, hemoglobin; Hct, hematocrit; IgG, anti-DENV IgG antibody; Leu, leukocytes; Lymph, lymphocytes; Neut, neutrophils; Plt, platelets; and Yr, years.

Above (↑) or below (↓) laboratory reference range for age and sex (Appendix 8).
† Missing value
REFERENCES


CHAPTER III
INDIVIDUAL BEHAVIORS, SOCIAL PRESSURES, AND FAILURES OF EPIDEMIC CONTROL MEASURES THAT CONTRIBUTED TO TRANSMISSION OF ZAIRE EBOLVIRUS AMONG GEOGRAPHICALLY-DISPERSED FAMILY MEMBERS, LIBERIA

A single introduction of EBOV can set off a chain of rapid transmission that accelerates through interconnected populations through unregulated border crossings, and all undetected due to a lack of public health infrastructure. In December 2013, EBOV emerged for the first time in West Africa. The index case, a two-year-old child in a rural village near the international borders of Guinea, Sierra Leone, and Liberia, was that single introduction (Figure 10). In March 2014, the first cases of EVD were detected in Lofa County, Liberia, with onward transmission to rural villages and the capital city, Monrovia. On 23 March 2014, MSF notified WHO of the outbreak, which had overwhelmed humanitarian efforts to isolate and care for cases, and sent affected communities into social turmoil. Not until 139 days later did the WHO respond, on 8 August 2014, with an issuance of a ‘public health emergency of international concern’. The most extensive and protracted outbreak of EBOV unfolded, infecting at least 28,616 people. Community resistance, the collapse of an already inadequate public health system, and a grossly inadequate supply of isolation beds contributed to unabated transmission through urban centers and rural areas, in a location and on a magnitude previously inconceivable.

We reconstructed a chain of transmission of EBOV that originated in a single source and spread to five, geographically-dispersed family members. Through the use of case-level data, we describe the physical contacts with the source patient, the circumstances of those contacts, and rarely-documented single-event contact between an infected source and secondary cases. We provide estimates of epidemiologic parameters of transmission to inform outbreak dynamics, providing granular knowledge applicable to current and future outbreaks of ebolaviruses. Coupled with case-level data, we explain how an inadequate capacity of isolation beds early in the outbreak, at the most crucial intervals, perpetuated transmission and contributed to community mistrust and resistance, violence, and refusals to accept control measures intended to interrupt outbreak progression. Finally, we identify underrecognized modes of transmission, which may account for EVD in persons with no identifiable risk factors, that should be the subject of community education efforts in future outbreaks of EVD.

METHODS

Ethical Considerations

This study was conducted during the international Ebola emergency response, in which the investigators participated in outbreak response efforts, direct patient care, clinical diagnostics, and infection prevention and control. The cases described herein were known to the investigators as part of a survivor cohort for which the authors assisted with community reintegration efforts and clinical
Figure 10. Map of Guinea, Liberia, and Sierra Leone International Borders
Yellow arrowhead indicates the home village of the West Africa epidemic index case. Lofa County, where the first cases appeared in Liberia, is encircled in orange. Red arrows indicate spatial transmission into and through Liberia to the capital city, Monrovia. Bomi County is indicated by the blue circle and within the inset. The yellow circles identify the Klay checkpoint, where the source patient died at the roadside, and Tubmanburg, the location of the isolation and treatment centers, respectively.
Map reproduced from the University of Texas Perry-Castañeda Library Map Collection. https://legacy.lib.utexas.edu/maps/liberia.html
evaluations of post-EVD sequelae. Human subjects approval was granted by the LSU IRB (Appendix J), and data collection conformed to all relevant General Principles of the Declaration of Helsinki. Cases and family members were informed of the purpose of the study, that their privacy would be protected, and that the findings would be shared within the international community to aid in understanding the events and behaviors that contribute to the transmission of EBOV. Written informed consent was obtained and witnessed by a third party. Cases were interviewed in their homes, alone or in the presence of family and community members, as per choice.

**Constructing the Chain of Transmission**

Detailed information was collected by standardized questionnaire; open interviews with cases, family and community members; and by interviews with staff and review of medical records at the district health clinic (DHC). We investigated, and cross-checked among varied data sources, exposure to the source patient, history of local and regional travel, contacts and types of physical interaction, dates of symptom onset, progression of clinical illness, and treatments. To create the fullest possible resolution of the chain of transmission, these data were supplemented by information collected from anecdotal reports, medications, and receipts obtained during health-seeking events by the cases. An exposure was defined as any physical contact with the source patient during the course of her illness, including contact with her body fluids and soiled linens.

**Clinical Descriptors and Definitions of Phases of Ebola Virus Disease**

We describe the evolution of EVD by exposure, onset of clinical symptoms, evolution of clinical disease, and outcome. We categorized EVD phase as dry and wet, according to the guidelines and 43-year history of use by Médecins Sans Frontières, the global humanitarian experts on viral hemorrhagic fever response and clinical management. Dry-phase symptoms include fever, myalgia, arthralgia, weakness, malaise, headache, and conjunctivitis. Wet-phase symptoms, including vomiting, diarrhea, abdominal pain, hemorrhage, profuse diaphoresis, subconjunctival hemorrhage, and mucosal bleeding.

**Definition of Key Epidemiologic Parameters**

To describe the transmission dynamics of EBOV in our cohort, we calculated the key intervals that correspond to transmission risk associated with the presence of virus in body fluids and tissues, and describe the clinical progression and outcomes of the six cases. The incubation period was calculated as the number of days between exposure to the source patient and symptom onset. For multi-day exposures, we used the first date of contact with the source patient during the wet or infectious phase of her disease. This was an intentional restriction to reflect the biological risk (i.e., probability) of virus transmission when virus is contained within the vascular system versus when virus is present in the tissues and extravascular fluids. The clinical onset serial interval (COSI) is the time between contact with the source patient in her wet phase and onset of dry-phase symptoms in a secondary case. The infectious period was determined by the date of wet-symptom onset to clinical outcome (i.e.,
recovery or death). Under epidemic conditions, recovery is necessarily defined as the date of discharge from the ETU, as real-time testing for the resolution of viremia was not, and could not, be performed in West Africa. Onset to clinical assessment for an epidemic of EVD is intended to define the number of days, and therefore approximate risk of ongoing transmission, that a symptomatic person spends in the community before recognition by a health care provider. We selected the onset of wet symptoms to more precisely describe the risk period of transmission using the same rationale for determination of the incubation period. Hospitalization to recovery/death was determined as the number of days between admission to the isolation unit and clinical outcome. Finally, the CFR was determined by dividing the number of deaths by six, corresponding to the number of cases in our chain of transmission.

Data Analysis

Due to our sample size of six persons, we calculated the median value where appropriate. The median for even-numbered data points was calculated by ranking the values in ascending order, averaging the two middle values, and dividing the sum by 2. For odd-numbered data points, the values were ranked in ascending order, with selection of the middle value.

RESULTS

Evolution of the West Africa Outbreak in Liberia

The first case of EVD in Liberia was reported in March 2014 in Lofa County, where five of six districts reported transmission. The virus reached the capital city of Monrovia in Montserrado County, seeding intense transmission and multiple focal outbreaks in rural and remote areas within and around Monrovia. By August 2014, Monrovia reported more than 200 incident cases of confirmed EVD per week, and Liberia recorded 2,080 suspected, probable, and confirmed cases for the month of September. By November, incident cases were concentrated in Monrovia and in smaller clusters in rural locations. In Bomi County, the markets and health centers were closed, and clinical staff was assigned to operate surveillance checkpoints. At the time of this transmission chain, there were no known sick persons in Bomi County. Only one source of exposure was reported by each of the surviving cases and by the family proxies for the decedent cases. Our investigation revealed the following sequence of events.

The Source Patient (Index Case)

On 27 September 2014 (Figure 12), the source patient, a 42-year-old female trader originally from Lofa County, traveled 15 km by taxi from her village north of Monrovia to the Red Light District in Monrovia to attend the weekly market of traders traveling from Lofa County to sell their highly-valued palm oil. Four days later, on 1 October, she complained of feeling weak while selling goods in a nearby village. On 3 October, she was examined by the district health nurse (DHN), who recorded fever, weakness, and myalgia in the clinical dossier. She reported travel to Monrovia the week prior, but
denied contact with sick persons or attending a burial. The DHN suspected EVD because of her connection to Lofa County. No isolation beds were available in Monrovia or in Bomi County, and there were no options for testing. She was prescribed paracetamol and artesunate-amodiaquine in case of malaria. She was instructed to remain inside her home and contact the DHN in three days if her symptoms had not improved.

On 6 October, the source patient grew increasingly weak and developed a persistent, severe headache. She walked one km to visit the local roadside druggist in a neighboring village (Figure 11), where she purchased additional paracetamol and drugs indicated for malaria. Her symptoms continued to worsen, and her children left her house to stay with the grandmother, approximately 10 feet from the home of the index case. On 8 October, she began to have uncontrolled vomiting and diarrhea and was unable to walk. Her husband remained the sole care provider, and he did not allow anyone to enter the home. Family placed food and water on the porch, but there was no physical contact between the source patient and husband with outside persons.

![Figure 11. Location of Roadside Druggist, Bomi County, Liberia](image)

On 13 October, the source-patient’s mother arrived to assist in her care. On October 15, the husband and mother took the source patient by taxi to a private medical clinic at Point Four Junction in the West Point area of Monrovia. The husband slept at a family home in Monrovia where there were no sick persons, and the mother slept on the clinic floor next to the source patient. The index case was administered three bags of IV fluids, doxycycline 200 mg daily, cimetidine 800 mg daily, and iron 400 mg daily for her diagnoses of typhoid fever, malaria, and a bleeding ulcer. The husband and mother reported that the clinic staff told them that the source patient tested negative for EBOV.

On 17 October, the source patient, husband, and mother traveled by taxi approximately 20 km from Monrovia to the DHC. Learning of her arrival, the DHN and district health officer (DHO) brought an ambulance to transport her to the isolation center in Tubmanburg. The family explained that the patient has tested negative for EBOV at the Monrovia clinic, and they ardently refused the ambulance because they ‘make people disappear.’ The index case remained at the mother’s home. Believing the
source patient to be EBOV-negative, her niece provided bedside care by wiping saliva and vomitus from her face, and her sister cleaned buckets of vomitus and hand-washed soiled linens in the creek. The niece reported washing her face with a cup of water used by the source patient and receiving paper money from her to purchase soap.

On 18 October, a brother-in-law arrived to assist the source patient and husband to travel by taxi to the isolation center in Tubmanburg. The brother-in-law assisted the index case into the back of the taxi, next to the husband, and then seated himself in the front passenger’s seat. The DHN spotted the source patient in a taxi and stopped the car. He noted no vomiting or diarrhea by the source patient, but noted she was profusely diaphoretic and had bright-red eyes that confirmed she was in the hemorrhagic phase. They again refused transport by ambulance and continued by taxi to the CCC in Tubmanburg. At Klay checkpoint five km from Tubmanburg, the source patient was denied passage and instructed to wait on the roadside for an ambulance. The brother-in-law assisted the source patient out of the taxi and hired a motorbike to return to his home in Tubmanburg. He did not wear a helmet, and no information is available about the motorbike operator. The source patient and husband waited separately from the crowd of people gathered at the Klay checkpoint. The source patient experienced intractable vomiting and died four hours later. The husband carried her body behind the checkpoint station and waited one hour for the burial team. The body of the source patient was placed in a body bag, the grounds were sprayed by the attendants, and the husband and body were transported in their vehicle to the mother’s home. A safe burial was performed immediately, followed by a wake inside the mother’s home. Postmortem oral swabbing was not performed.

**Husband**

On 22 October, four days after the death of the source patient, the husband (aged 40 years) experienced onset of fever, weakness, and myalgia. He purchased gentamicin from a village trader and took the remainder of his wife’s medications for malaria and typhoid fever. On 26 October, the husband began to have vomiting and diarrhea. He remained self-sequestered in his home with no contact with family members, but he ran into the palm forest directly behind his home when the DHN made surveillance visits. On 29 October, due to a worsening of his condition, the husband agreed to be transported by ambulance to the isolation unit in Tubmanburg. He was confirmed EBOV-positive on 6 November and discharged on 21 November.

**Mother**

On 26 October, 8 days after the death of the source patient, the mother (aged 53 years) had onset of both diarrhea and ‘heavy weakness’ without fever. She denied having symptoms during surveillance visits by the DHN. She remained alone in her home until her admission to the Tubmanburg isolation unit 7 November. She was confirmed to be EBOV-positive and discharged on 23 November.
Figure 12. Evolution of the Familial Cluster of Ebola Virus Disease: Disease Progression and Events
Abbreviations: CCC, community care center; DHC, district health clinic; sx, symptomatic.
**Sister and Niece**

On 5 November, the sister (aged 38 years) presented to the DHC (Figure 12) with fever, abdominal pain, vomiting, diarrhea, subconjunctival hemorrhage, and inability to walk. She refused ambulance transport to the isolation center. On 6 November, the DHN and the DHO brought an ambulance to her home, where the niece (aged 23 years) reported a headache, anorexia, chills, and weakness. Both were transported to the isolation center. The sister died on 8 November. The niece progressed to wet symptoms, recovered, and was discharged on 23 November.

**Brother-in-Law**

On 23 October, five days after the death of the source patient, the brother-in-law (aged 33 years), had onset of fever, headache, and weakness. He remained in the presence of his family until 27 October, when he experienced vomiting and diarrhea. He isolated himself within a bedroom without contact with his family. On 30 October, he self-presented to the isolation unit and died on 3 November.

**Clinical Course**

We resolved the chain of transmission of one source patient and five secondary cases with dates of symptom onset and clinical outcome (Figure 15). The source patient died in the community and is classified as a probable case; the five secondary cases, who were each admitted to the isolation unit, were laboratory-confirmed as positive for EBOV. Contact tracing identified no additional cases of secondary transmission or tertiary cases, including the taxi driver from the day the source patient died.

The median age was 39 years (range 23-53); three (50%) were female (Tables 14 and 16). All six cases had direct physical contact with the source patient or her body fluids. The most common mechanism of contact was through bedside care, which accounted for transmission in three cases (50%). One case (17%) had contact only with soiled linens and body fluids, and one case (17%) had only skin-to-skin contact.

Clinical features (Table 14 and 16) at presentation were nonspecific and included weakness or profound weakness in six cases (100%), fever and headache in four cases (67%), myalgia in three cases (50%), anorexia in two cases (33%), and one case each had chills (17%) and conjunctivitis (17%). In the wet phase of disease, all six cases (100%) had diarrhea, five (83%) had vomiting, three (50%) had subconjunctival hemorrhage, two (33%) had abdominal/epigastric pain, and one case each had diaphoresis (17%), melena (17%), and muscular rigidity (17%). Evidence for hemorrhage was available for three cases (50%), of which two were decedents. The clinical course of the brother-in-law in the isolation unit was relayed by the husband without dates of onset.

**Epidemic Transmission Parameters**

The incubation period is used for monitoring contacts, isolation and quarantine, and for determining a region to be free of transmission following an outbreak.17 The median incubation period for the six cases was 6.5 days (range 4-14 days). For survivors, the median incubation period was 8 days (range
8-14 days), possibly suggesting an association between slower disease progression and survival. For the three decedents, the incubation period was 4 days (range 4-5 days) (Table 14). For single- and multi-day exposures, the median incubation periods were 5 days (range 4-8 days) and 11 days (range 8-14 days), respectively. The COSI was 21 days (range 20-24 days), which highlights the importance of the 42-day interval used by WHO prior to declaring an end to transmission.17

| Parameter                  |     |  
|----------------------------|-----|---
| Median age                 |     |  
| Years (range)              | 40  | (23-53) |
| Female sex                 | 3   | (50) |
| Occupation                 |     |  
| Farmer                     | 3   | (50) |
| Trader                     | 2   | (33) |
| School teacher             | 1   | (17) |
| Symptoms, dry              |     |  
| Anorexia                   | 2   | (33) |
| Chills                     | 1   | (17) |
| Conjunctivitis             | 1   | (17) |
| Fever                      | 4   | (67) |
| Headache                   | 4   | (67) |
| Myalgia                    | 3   | (50) |
| Weakness                   | 6   | (100) |
| Symptoms, wet              |     |  
| Diaphoresis                | 1   | (17) |
| Diarrhea                   | 1   | (17) |
| Abdominal pain             | 2   | (33) |
| Melena                     | 1   | (17) |
| Muscular rigidity          | 1   | (17) |
| Subconjunctival hemorrhage | 3   | (50) |
| Vomiting                   | 5   | (83) |

**Table 14. Demographics and Frequency of Clinical Symptoms, by Dry and Wet Phases of Illness**

Abbreviation: $n$ = number of observations.
The infectious period is a risk measure of secondary transmission. We calculated the infectious period from onset of wet symptoms to survival or death. The median infectious period was 11 days (range 7-32 days) (Tables 15 and 17). The infectious period could not be determined for the niece, because we could not determine the date of wet-symptom onset.

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<td>Single-day exposures</td>
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<td>Multi-day exposures</td>
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<td>Clinical Onset Serial Interval</td>
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<td>Infectious Period*</td>
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<tr>
<td>Onset to Clinical Assessment</td>
<td>17 (3-22)</td>
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<tr>
<td>Hospitalization to Discharge</td>
<td>18 (17-24)</td>
</tr>
</tbody>
</table>

*Calculated for the five secondary cases.

Onset to clinical assessment (Tables 15 and 17) is an indicator of how early in disease (i.e., time interval following symptom-onset) a potentially infectious person is identified in the community for intervention. For the five cases assessed by the DHN, the median onset to clinical assessment was 17 days (range 3-22 days). The source patient was the first to seek care, which occurred on her third day of symptoms (3 October). Active case monitoring by the DHN was initiated on 17 October, when the source patient returned to the area from Monro via. The mother, niece, and sister were initially assessed at 20, 21, and 22 days, respectively, post-symptom onset. For the three survivors, the median number of days from hospitalization to discharge was 18 (range 17-24 days). Three patients died (CFR 50%). The source patient died in the community after an 18-day infectious period. The median number of days from hospitalization to death was 15 (range 12-18 days) for the two secondary cases that died in isolation.

**DISCUSSION**

**Epidemic Response in Liberia**

With the WHO declaration of a public health emergency of 8 August 2014, the Armed Forces of Liberia established checkpoints to restrict the movement into and out of affected regions. Coincident
with the arrival of WHO to the region, the Ebola Response Incident Management System identified isolation of persons with EVD as the ‘immediate and overriding objective’ of all response activities in Liberia\textsuperscript{19}. From there, the international response was structured to address the Four Pillars\textsuperscript{19}: (1) early detection, isolation, and treatment; (2) safe patient transport; (3) safe and dignified burial; and (4) IPC. Mobilization of district-level surveillance, both within the community and at road checkpoints, proved essential for identifying sick persons and maintaining awareness of travel into and out of the Bomi County. The source patient made three separate trips to Monrovia during this time period, each of which was by public taxi. First, on 27 September, she traveled to attend the Lofa market in the Red Light District, followed by the second trip on 15 October to seek care at a private clinic. Her third trip was to seek admission to the isolation center in Tubmanburg. The husband recounted their travel southwest from their village, back to and through Monrovia, and then northeast, because they wanted to avoid temperature checkpoints stationed along the main larger roads.

\textbf{Isolation and Bed Capacity}

In March 2014, a holding unit was erected adjacent to the county hospital in Tubmanburg with the purpose of isolating symptomatic patients and then transporting infected patients to the MSF ETU in Monrovia\textsuperscript{20}. In July, however, concern over HCW infections and the lack of IPC within the holding unit led to its closure, and closed of the county hospital and all 23 clinics in the Bomi County Community Health Department\textsuperscript{20}. Then, on August 18 and October 9, two new isolation units opened again in Tubmanburg adjacent to the county hospital. Treatment was limited to oral hydration solution, and nurses entered only twice daily to carry in the food dropped off by family members. The only diagnostic laboratory was in Monrovia at the MSF ETU, and transport of blood samples and results required a motorcycle courier to make the two-hour drive to the laboratory. Patients often remained inside the holding unit for days without knowing their infection status, while being exposed to patients with EVD.

Between July and December, 2014, 93\% of secondary cases were generated by patients who died in the community, compared to only 7\% from patients who died inside an ETU\textsuperscript{14}. The failure of the WHO to respond rapidly to the epidemic allowed unchecked transmission throughout the affected countries\textsuperscript{21}. While the bed capacity expanded greatly in latter 2014 and into 2015, many treated only one or two patients, while others were fully constructed but never commissioned for use\textsuperscript{19,22}.

\textbf{Surveillance and Community Resistance}

Initial recognition by the DHF of illness in the source patient would prove critical, because the time spent in the community beyond the fourth day of illness presents the greatest—and increasing—risk for community transmission\textsuperscript{23}. The source patient sought care early in her illness (onset to clinical assessment=3 days). Had an isolation bed been available, the five cases of secondary transmission could have been prevented. However, repeated attempts to isolate cases, however, were frustrated not only by the lack of isolation beds, but later by the abject refusal of the family to ride in an ambulance. Despite decades of response to smaller VHF outbreaks in Central Africa, and the identification of key elements of a response, the West Africa epidemic again highlighted a gap in operationalizing a large-scale response: the fundamental importance of establishing a relationship with the communities.
concerned and the public at large. Missteps early in the epidemic, in which teams were forcibly removing children and family members from their homes, contravened closely-held cultural beliefs in honoring the death with burial rites. Among impoverished populations scarred by histories of colonialization and civil war, a disbelief in the existence of 'Ebola' thwarted both local and international efforts to control the outbreak (Brown H). Notably, the DHN and DHO, trusted members of the community, ended the chain of transmission by bringing the ambulance directly to the homes of the husband, mother, sister, and niece (Figure 13). A flattening of hierarchy was not understood by leaders of the Ebola response, who had encouraged enforcement rather than cooperation to control transmission.

Social Distancing
Children were not among the secondary cases in this cluster, due to cultural practices of not including them in the care of sick family members and self-sequestration by the cases, noted by Faye et al. as an effective strategy that contributed to interrupting transmission in Guinea. Social distancing (e.g., 'no-touch' policies), curfews, and prohibitions on large gatherings were announced by radio. This influenced the source patient, husband, and brother-in-law to self-sequester and have no physical contact with family. Of note, the inability to find treatment was a risk factor for transmission in our study, particularly the travel and three-day stay of the source patient and mother at the private clinic in Monrovia. PPE was not widely available in Liberia prior to the epidemic, and clinic staff,
desperately under-resourced, used the same pair of gloves to treat multiple patients, likely an attempt to maintain a barrier between themselves and the many patients under their care. We cannot rule out the clinic as the source of infection for the husband and mother, nor could we determine if the source patient infected other patients or family members in the clinic. If they were exposed to virus in the clinic, however, it likely superimposed latent infections from the care provided to the source patient once she progressed to the wet phase of EVD.

Exposures and Transmission

Although we cannot exclude the possibility of EBOV transmission from sources not disclosed or discovered, our extensive efforts to precisely identify the time and place of transmission failed to uncover other sources of transmission for the source patient or secondary cases than those proposed. As a schoolteacher, the brother-in-law was at home because of school closures, and there were no known sick persons in his hometown of Tubmanburg. We were unable to obtain information on his travel history. The index case and husband could not identify a known exposure event to which they could attribute her illness. Without a risk history, and in combination with the negative diagnosis received at the Monrovia clinic, they attributed her illness to another etiology. Interestingly, the husband stated that people in their village did not believe the virus could reach their community, despite its close proximity to Monrovia. It is important to note that at this time, ‘Ebola’ was synonymous with bleeding, as it was in the West. Contact with a bleeding person would be subject to recall, but contact with a sick person who appeared weak and sweating would not be an unusual event during the Liberian rainy season, particularly given the background burden of disease from malaria and other mosquito-borne diseases.

We considered the epidemiologic link of the source patient to Lofa County, known for their prized production of palm oil. Palm oil and the fruits were regularly purchased by the source patient. Palm plantations have been the setting of previous outbreaks in the DRC, as some bat species roost in the trees for fruit. The possibility of fallen fruits contaminated with bat saliva or guano was explored by Leroy et al. (2009), and has been proposed as a potential source of spillover of ebolaviruses from frugivorous bats to NHPs and humans. Recently, EBOV RNA was isolated from a bat (Miniopterus inflatus) caught in Nimba County, Liberia, a straight-line distance of 50 miles from Guéckédou, Guinea.

Our findings raise an important question about the role of sweat in EBOV transmission. Zaki et al. demonstrated the presence of EBOV and antigen within and around sweat glands in 14 of 14 axillae and neck biopsies. Based on those findings, they proposed that skin may service as a site of viral replication, not merely viral secretion. Sweat was identified as the mode of transmission between an infected father with only mild illness to his infant during a three-hour walk bush walk. Dowell et al. (1999) concluded sweat-based transmission was an ‘intriguing explanation’ for transmission events for which no risk factor could be identified. Based on our findings following exhaustive efforts to identify other modes of transmission or exposures not previously accounted for, we hypothesize that the source patient and brother-in-law were infected by skin-to-skin contact with the source patient, in
which sweat was transferred from an infected person to a susceptible person. We support the proposal of Zaki et al.\textsuperscript{30} to establish a skin surveillance system to explore the role of direct physical contact in transmission of the virus.

Infection of expatriate humanitarian workers provided the opportunity for real-time monitoring of EBOV viral loads in tissues and fluids in patients undergoing treatment in high-containment biofacilities in the US and Europe. In two patients, EBOV was detected in axillary sweat on day 24 and from axillary, inguinal, and forehead sweat from during days 20-40 of EVD.\textsuperscript{32} Moreover, anecdotal reports of skin-to-skin contact also implicate sweat as the mode of transmission, including as the source of the virus for the Liberian man who died of EVD in Dallas.\textsuperscript{33} Prior to his death, he recalled that his only contact with a sick person was when he assisted a sick pregnant woman into a taxi the week before leaving for the US. In 2015 in the Red Light District in Monrovia, two cases of EVD were diagnosed in two people who helped a sick person into a taxi.\textsuperscript{34}

The growing evidence of sweat in the epidemiology of EVD warrants further examination as an under-recognized mode of transmission, particularly for outbreaks in densely-populated urban environments of tropical Africa. Risk communication, and the promotion of social distancing, should include an awareness of skin-to-skin contact—not just contact with blood, stool, and vomitus—as opportunities for transmission.

**CONCLUSION**

The time, setting, and mechanism of EBOV transmission events are key observations upon which an epidemic response is operationalized. Reconstruction of chains of transmission provides the opportunity to examine person-level transmission and assess the effectiveness of control measures. We described the key transmission events that contributed to secondary transmission, and determined that the lack of isolation beds, coupled with fear and mistrust, contributed to secondary transmission to five family members of the source patient. Importantly, self-sequestration by three of the cases prevented additional secondary prevention and generated no cases of tertiary transmission. This was particularly important given the household setting and the presence of children.

After exhaustive efforts to identify a source of EBOV transmission to the source patient and the brother-in-law, we excluded all modes and sources of transmission except sweat. This is an important finding with implications for driving ongoing and unrecognized virus transmission. Risk communications for social distancing should be instituted immediately in future outbreaks. Our data join a limited number of descriptive studies in which single-day transmission events, and the precise dates of clinical onset, are documented. Together, our data provide estimates of epidemic transmission parameters with the greatest precision possible from an epidemic context. Our informed estimates will better characterize the epidemiology of EVD, improve spatiotemporal modelling of EBOV transmission and infection dynamics, and guide resource allocation during future VHF epidemics.
Figure 14. Ebola Virus Disease Familial Cluster: Transmission Events, Symptom Onset, and Clinical Outcomes
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Years)</th>
<th>Occupation</th>
<th>Date(s) of Exposure</th>
<th>Transmission Event(s)</th>
<th>Dry Symptoms</th>
<th>Wet Symptoms</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| Source Patient | 42          | Trader     | 27 Oct              | Public taxi to and from Monrovia  
Attend market in Red Light District, Monrovia, to purchase items from Lofa County | Fever  
Headache  
Myalgia  
Profound weakness | Diaphoresis  
Diarrhea  
Melena  
Subconjunctival hemorrhage  
Vomiting | Deceased    |
| Husband      | 42          | Farmer     | 8-18 Oct            | Household contact  
Bedside care  
Visit to clinic in West Point, Monrovia                                           | Fever  
Headache  
Myalgia  
Weakness | Diarrhea  
Epigastric pain  
Subconjunctival hemorrhage  
Vomiting | Survived     |
| Mother       | 53          | Farmer     | 14-18 Oct           | Bedside care  
Visit to clinic in West Point, Monrovia                                            | Anorexia  
Profound weakness | Diarrhea | Survived       |
| Sister       | 38          | Farmer     | 17-18 Oct*          | Washed soiled linens with bare hands  
Cleaned room where patient slept                                                   | Fever  
Myalgia  
Weakness | Abdominal pain  
Diarrhea  
Subconjunctival hemorrhage  
Vomiting | Deceased     |
| Niecec       | 23          | Trader     | 17-18 Oct*          | Bedside care  
Washed face with same cup of water used by source patient  
Received money               | Anorexia  
Chills  
Headache  
Conjunctivitis  
Weakness | Diarrhea  
Vomiting | Survived     |
| Brother-in-Law | 33         | School teacher | 18 Oct             | Assisted source-patient into and out of taxi                                     | Fever  
Headache  
Weakness | Diarrhea  
Muscular rigidity  
Vomiting | Deceased     |

*Less than 24 hours of exposure to the source patient.
Table 17. Estimations of Case-Specific Incubation and Infectious Periods

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Occupation</th>
<th>Date(s) of Exposure</th>
<th>Transmission Event(s)</th>
<th>Dry Symptoms</th>
<th>Wet Symptoms</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source Patient</td>
<td>42</td>
<td>F</td>
<td>Trader</td>
<td>27 Oct</td>
<td>Public taxi to and from Monrovia</td>
<td>Fever</td>
<td>Diaphoresis</td>
<td>Deceased</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Attended market in Red Light District, Monrovia, to purchase items from Lofa County</td>
<td>Headache</td>
<td>Diarrhea</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Myalgia</td>
<td>Melena</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Profound weakness</td>
<td>Subconjunctival hemorrhage</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>Husband</td>
<td>42</td>
<td>M</td>
<td>Farmer</td>
<td>8-18 Oct</td>
<td>Household contact</td>
<td>Fever</td>
<td>Diarrhea</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bedside care</td>
<td>Headache</td>
<td>Epigastric pain</td>
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<td></td>
<td>Myalgia</td>
<td>Subconjunctival hemorrhage</td>
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<td></td>
<td></td>
<td></td>
<td>Weakness</td>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>53</td>
<td>F</td>
<td>Farmer</td>
<td>14-18 Oct</td>
<td>Bedside care</td>
<td>Anorexia</td>
<td>Diarrhea</td>
<td>Survived</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Visit to clinic in West Point, Monrovia</td>
<td>Profound weakness</td>
<td></td>
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<tr>
<td>Sister</td>
<td>38</td>
<td>M</td>
<td>Farmer</td>
<td>17-18 Oct*</td>
<td>Washed soiled linens with bare hands</td>
<td>Fever</td>
<td>Abdominal pain</td>
<td>Deceased</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cleaned room where patient slept</td>
<td>Myalgia</td>
<td>Diarrhea</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td>Weakness</td>
<td>Subconjunctival hemorrhage</td>
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<td></td>
<td></td>
<td></td>
<td>Vomiting</td>
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</tr>
<tr>
<td>Niece</td>
<td>23</td>
<td>F</td>
<td>Trader</td>
<td>17-18 Oct*</td>
<td>Bedside care</td>
<td>Anorexia</td>
<td>Diarrhea</td>
<td>Survived</td>
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<td></td>
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<td></td>
<td></td>
<td>Washed face with same cup of water used by source patient</td>
<td>Chills</td>
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<td></td>
<td>Headache</td>
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<td></td>
<td></td>
<td>Conjunctivitis</td>
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<td></td>
<td></td>
<td>Weakness</td>
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</tr>
<tr>
<td>Brother-in-Law</td>
<td>33</td>
<td>M</td>
<td>School teacher</td>
<td>18 Oct</td>
<td>Assisted source-patient into and out of taxi</td>
<td>Fever</td>
<td>Diarrhea</td>
<td>Deceased</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Headache</td>
<td>Muscular rigidity</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Weakness</td>
<td>Vomiting</td>
<td></td>
</tr>
</tbody>
</table>

*Less than 24 hours of exposure to the source patient.
REFERENCES


CHAPTER IV
CLINICAL SEQUELAE AND FUNCTIONAL DECLINE AMONG SURVIVORS OF EBOLA VIRUS DISEASE, BOMI COUNTY, LIBERIA

The West Africa EVD epidemic, unexpected and protracted, heralded a new era of ebolavirus epidemiology. No longer a disease of hemorrhage and death, more than 17,000 people survived infection with a virus once considered to be nearly always fatal.¹ Post-Ebola sequelae were first described following the 1976 SUDV outbreaks.² Among survivors, a constellation of consistent, frequent, and debilitating symptoms, which encompass a spectrum of non-descript clinical sequelae of poorly understood pathogenesis without prescriptive treatments. Designated as post-Ebola virus disease syndrome (PEVDS),³ arthralgias, disabling fatigue, ocular complications, hearing loss, and neuropsychiatric symptoms are among the most commonly-reported sequelae, some persisting more than 20 years after infection.⁴⁻⁶ PEVDS presents a challenge to individual and national recovery, with a continued threat to global health security. The consequences of lost productivity, food insecurity, and the breakdown of cohesive community structures has the potential to maintain the enfeebled state of the affected countries, creating and maintaining large regions of susceptibility to future emergence events.

We conducted physical examinations and evaluations of functional status to investigate the clinical symptoms reported by three survivors from the transmission chain described in Chapter III. Our aim was to characterize, as fully as possible, and within the limitations of ongoing EBOV transmission, the physical and psychiatric symptoms, patterns of distribution by body system, and impact on longitudinal regain-of-function or increasing disability. We further present proposed mechanisms of pathophysiology with results of advancing studies conducted in the wake of the epidemic.

MATERIALS AND METHODS

Ethics Approval

All interviews and physical examination were conducted in the home village of each participant while active EBOV transmission was occurring in Liberia, and therefore occurred during the international Ebola emergency response. Data collection conformed to all relevant General Principles of the Declaration of Helsinki.⁷ Human subjects approval was granted by the LSU IRB (Appendix J).

Case Ascertainment and Informed Consent

The survivors presented in this case series were patients discharged from the ETU where the investigators provided direct patient care for EBOV-infected persons, and then participated health-system strengthening and community reintegration efforts. During June and July 2015, the
investigators resided in a rural village approximately 15 km northeast of Monrovia, the capital city of Liberia, to investigate the events of EBOV transmission and post-Ebola sequelae. Survivors and family members were informed of the purpose of the study, that their privacy would be protected, and that the findings would be shared with the international community to aid in understanding how EBOV is transmitted among family and community members, and to understand the symptoms associated with PEVDS. Written informed consent was obtained and witnessed by a third party. Cases were interviewed in their homes alone or in the presence of family and community members, as per choice.

**Data Collection and Analyses**

**Definitions**
A survivor was defined as a laboratory-confirmed case of EVD who was admitted to the Bomi County ETU, recovered from EVD, and discharged to their home community. Discharge from the ETU was based on two criteria: (1) serial laboratory-confirmed EBOV-negative blood samples, and (2) resolution of EVD-associated symptoms (e.g., afebrile, cessation of diarrhea). An additional criterion was the ability to perform self-care within their homes.

**Interviews**
Following informed consent, each survivor was interviewed and examined in their homes in English using an open-ended format (Appendix L). Survivors were asked about their activities, exposures and knowledge of EVD prior to disease onset and throughout the course of illness into the convalescent period. Initial ($t_1$) interviews and physical exams occurred in February 2015 (90 days post-discharge). Secondary interviews ($t_2$) and physical exams took place in June 2015 (120 days post-discharge). We dichotomized the convalescent period as acute (i.e., discharge to ≤90 days) and subacute (i.e., >90 days after discharge).

**Physical Examination**
Data collection was performed by one investigator, who is a clinician and infectious disease epidemiologist with experience in West African cultures. Vital signs (i.e., blood pressure, heart rate, pulse oximetry, temperature) were obtained using portable durable medical equipment. The physical examination conformed to the methodology outlined by Campbell et al., including 18-tender point exam, utilized by the American College of Rheumatology for assessment of symmetrical pain distribution. Direct physical examination of the urogenital and reproductive systems was deferred but remained part of the oral interrogation (Appendix M).

**Patient-Specific Functional Scale**
We selected the Patient-Specific Functional Scale (Appendix C), used previously in a Guinean survivor cohort, to obtain longitudinal global functional changes. The instrument requires participants to identify high-priority activities essential for daily living, and to rate their ability to perform the activities over a specified internal of time, based on a scale of 0 to 10 (i.e., '0' corresponds to wholly unable to perform the activity, '10' corresponds to being fully able to perform the activity. At 90 days post-discharge ($t_1$), survivors were instructed to rate their loss or gain in functional ability
based on their pre-EVD ability \((t_0)\). At 120 days post-discharge \((t_2)\), survivors were instructed to rate gains or losses in their functional ability based on their abilities at \(t_1\). An incremental change, from \(2\rightarrow3\), for example, represents a 10% gain of function; a \(3\rightarrow1\) rating represents a 20% loss of function. Final scores were calculated as percentages of recovery based. For example, a premorbid score of ‘10’ for four activities corresponds to a total possible score of 40. The functional ratings of each activity for \(t_1\) were added together, divided by 40, and multiplied by 100 to score the functional ability in the acute and subacute phases.\(^{11}\)

**RESULTS**

A full history and physical examination for each survivor is detailed in Table 18. The mother is the grandmother of the niece, and the husband is the surviving spouse of the mother's daughter, the source patient for their infections. The median age of the survivors was 40 years (range 23-53 years). None of the survivors had a history of chronic disease. The mother was born with bilateral exotropia that had not affected her vision. All three survivors experienced weight loss as a result of their disease. This was least extreme in the 23-year-old and most profound in the 53-year-old, who, despite a normal BMI, appeared cachectic and weak.

**Categories of Disease Severity**

We classified the severity of EVD as mild, moderate, and severe, based on their clinical manifestations during their admission to the ETU. Mild disease was unexpectedly observed in the 53-year-old mother, who was afebrile, had simultaneous onset of diarrhea and profound weakness, and did not manifest signs or symptoms of hemorrhage. The niece was classified as having moderate disease, based on her fluid losses from diarrhea and vomiting and conjunctivitis. The husband was admitted with signs of hemorrhage and was classified as having severe disease.

**Musculoskeletal Pain**

Each of the three survivors identified pain as the greatest obstacle to performing activities of daily living, reintegrating with the community, earning an income, and farming for food. In all cases, pain was symmetrical and mechanical, defined by: (1) pain with activity and improvement with rest, (2) no pain during the night, and (3) morning stiffness and pain <30 minutes duration.\(^{13}\) Arthralgias were persistent and polyarticular of both large and small joints. Direct physical examination of the joints and skin were without evidence of an inflammatory process.

Despite having mild disease, the mother demonstrated the most extensive profile of severe pain. Results of the 18 tender-point assessment\(^{10}\) demonstrated severe, symmetrical pain at each of the 18 sites (Figure 15). She had marked guarding and withdrawal to palpation of the joints. She unable to rotate her head (i.e., horizontal cervical rotation) >45 degrees in either direction. She could not form a closed first with either hand.
Table 18. History and Physical Examination Summaries, EVD Survivors

<table>
<thead>
<tr>
<th>Sex, Age (years)</th>
<th>Husband</th>
<th>Mother</th>
<th>Niece</th>
</tr>
</thead>
<tbody>
<tr>
<td>M, 40</td>
<td></td>
<td>F, 53</td>
<td>F, 23</td>
</tr>
<tr>
<td><strong>Vitals Signs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR 62</td>
<td>BP 161/93</td>
<td>HR 84</td>
<td>BP 127/94</td>
</tr>
<tr>
<td>O₂ sat 97%</td>
<td>T 99.3 C</td>
<td>O₂ sat 98%</td>
<td>T 99.7 C</td>
</tr>
<tr>
<td>H 180 cm</td>
<td>W 69 kg</td>
<td>H 175 cm</td>
<td>W 55 kg</td>
</tr>
<tr>
<td>BMI 22.4</td>
<td></td>
<td>BMI 19.0</td>
<td></td>
</tr>
<tr>
<td><strong>Past Medical History</strong></td>
<td></td>
<td>Bilateral exotropia</td>
<td>Appendectomy (date unknown)</td>
</tr>
<tr>
<td>Blood transfusion (2009)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical repair bisected anterior tibialis, civil war trauma (2003)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical Symptoms during EVD illness</strong></td>
<td></td>
<td>Diarrhea</td>
<td>Anorexia</td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td>Fever</td>
<td>Chills</td>
</tr>
<tr>
<td>Myalgia</td>
<td></td>
<td>Myalgia</td>
<td>Conjunctivitis</td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td>Vomiting</td>
<td>Headache</td>
</tr>
<tr>
<td>Weakness</td>
<td></td>
<td>Weakness</td>
<td>Weakness</td>
</tr>
<tr>
<td>Hyperlacrimation, OS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subconjunctival hemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical Findings</strong></td>
<td></td>
<td>Photophobia at peak sunlight hours</td>
<td>Bilateral myalgia, severe:</td>
</tr>
<tr>
<td>Full ROM of bilateral tibiofemoral joints; pain at extreme flexion, extension</td>
<td></td>
<td></td>
<td>- Rectus femoris</td>
</tr>
<tr>
<td><em>Arthralgias</em></td>
<td></td>
<td></td>
<td>- Vastus lateralis</td>
</tr>
<tr>
<td>Cervical spine</td>
<td></td>
<td></td>
<td>- Vastus medius</td>
</tr>
<tr>
<td>- Horizontal plane WNL</td>
<td></td>
<td></td>
<td>Persistent headaches</td>
</tr>
<tr>
<td>- Sagittal plane LROM</td>
<td></td>
<td></td>
<td>- &gt;2 days/week</td>
</tr>
<tr>
<td>Glenohumeral LROM, pain at extension superior to shoulders</td>
<td></td>
<td></td>
<td>- Must remain in bed</td>
</tr>
<tr>
<td>Arthralgia in phalanges and metatarsal joints, bilateral hands and feet</td>
<td></td>
<td></td>
<td>- Unable to care for children or walk to sell</td>
</tr>
<tr>
<td><em>Peripheral fields</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OD WNL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS decreased confrontation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Marked distal acuity deficit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Cannot discern number of fingers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left peripheral deficits:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Inferior and superior = 45˚ from transverse plane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal = 30˚ from sagittal plane</td>
<td></td>
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<tr>
<td>Temporal = 60˚ from sagittal plane</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Inappetence, tolerates only small intake</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Insomnia, racing thoughts, grief</td>
<td></td>
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</tbody>
</table>

Abbreviations: BMI, body mass index; BP, blood pressure; cm, centimeters; H, height; HR heart rate; kg, kilogram; LROM, left range of motion; O₂ sat, oxygen saturation in blood; OD, right eye; OS, left eye; ROM, range of motion; T, temperature; W, weight; WNL, within normal limits.
The niece, whose disease would be classified as moderate, described severe bilateral myalgia in the *recti femorus, vasti lateralis*, and *vasti medius*. She recounted pain of such severity that she was unable to walk her usual distances between villages to sell items for an income. Finally, the husband, who had severe disease, described articular pain severe in the morning and moderate during the day. Pain was localized to the cervical spine and symmetrically distributed among the glenohumeral joints; tibiofemoral joints; and the distal, medial, and proximal phalanges of the hands and feet. Sagittal cervical rotation was limited to <30 degrees in flexion and extension. The glenohumeral joints were limited at 90 degrees for vertical flexion and abduction. The tibiofemoral joints full flexion and extension but with pain. Pain of the phalanges of the hands and feet was described as moderate and relieved with motion.

**Figure 15.** Examination of Musculoskeletal Pain Site in an Ebola Virus Disease Survivor Identification of painful joints without evidence of local pathology.
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**Neuropsychiatric Sequelae**

The most severe neurologic symptom was reported by the niece (moderate disease), who reported persistent headaches that began after discharge from the ETU and continue more than two days each. The severity of the headaches left her bed-bound and unable to perform any activity of daily living,
Assessment of anxiety and depression in the mother and niece was limited to their willingness to discuss this type of symptom. The district health nurse characterized the mother as ‘acting depressed’ since her daughter died in October 2014. The husband described an inability to fall asleep and stay asleep. He said he has ‘racing thoughts’ and ‘feels grief’ over his wife’s death. He stated he was shunned by friends and community members upon his return from the ETU who refused to visit him or shake his hand. He said he felt ‘no reason to live’ upon arriving home and discovering the contents of his home had been burned.

**Ophthalmic Sequelae**

The mother, who had mild EVD, and the husband, who had more severe EVD, both reported ophthalmic sequelae. The mother described photophobia during peak daylight hours that require her to remain indoors. The husband described changes in visual acuity changes, pain, and hyper-lacrimation of the left eye during his ETU admission, which worsened after discharge. In the acute recovery phase, he had complete loss of vision and continuous hyperlacrimation. Subacutely, he regained vision but with a deficit of acuity that prevented him from performing community construction activities. In the physical examination, he had evidence of hyperlacrimation and demonstrated an inability to discern at a distance of 0.3 meters. In confrontational visual field testing, he demonstrated peripheral deficits of the left eye (Table 18, Appendix M).

**Functional Assessments**

The essential activities for daily living are listed in Table 19. Survivors were asked to associate a clinical symptom with their inability to perform their identified activities. Functional limitations among the three survivors were predominantly associated with arthralgias and myalgias. None of the survivors identified a psychiatric symptom as impeding their functional abilities, although the husband did report insomnia and nightmares about the loss of his wife and his time in the isolation unit.

Despite having mild disease, the mother demonstrated the most marked loss of function. Her premorbid function was rated at 100%, followed by 0% in the acute phase (\(t_1\)) and 10% in the subacute phase (\(t_2\)). She identified arthralgia as the reason for her loss of function, followed by fatigue and myalgia as secondary factors. She described an inability to hold farming tools due to pain and weakness of both hands. She was unable to make a fist and could not hold a knife or ladle to prepare food. On the days when her arthralgia was less severe, fatigue limited her functional ability.

The niece, who had moderate disease, had acute loss of function similar to the husband, 25% and 28%, respectively, but her subacute score was 53%, compared to 43% for the husband. She identified myalgia of the bilateral quadriceps muscles as the primary reason for functional decline, followed by fatigue and persist, debilitating headaches. In contrast, the husband attributed his function losses to an inability to see in his left eye, which improved from 0% in the acute recovery phase to 20% in the subacute recovery phase. Fatigue and weakness were prevented him from walking and farming, while arthralgias and loss of vision impeded his participation in community construction activities.
DISCUSSION

Following the 1995 outbreak in Kikwit, DRC, survivors reported arthralgias, myalgias, abdominal pain, hearing loss, extreme fatigue, and anorexia up to six months after the outbreak. At 21 months after the same outbreak, more than 60% of the survivors continued to experience symmetric

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Abbreviations: OS, left eye; $t$, time point.
polyarthropathies and myalgias. A more recent study conducted 22 years after the outbreak detected abnormal neurological exams, lower cognitive scores, and depression in survivors compared to their household controls. High viral titers cause sustained immune activation, with cell and tissue damage secondary to apoptosis and necrosis. These pathophysiologic processes have been postulated as the biologic bases of both the development and severity of PEVDS, but a causal mechanism or biomarker has yet to be identified.

Musculoskeletal Sequelae

Arthralgia and myalgia constitute the majority of reported complaints of pain and disability among survivors of the West Africa epidemic. Remarkably, the same symptom profile of arthralgias and large-muscle myalgias has been reported with consistence by survivors across all outbreaks of ebolaviruses. Arthralgia alone has been documented in up to 87% of survivors across three cohort studies. In nearly all cases, the patterns of arthralgia are symmetrical and involve multiple small and large joints. We observed the same pattern of symmetric pain distribution in the mother, whose 18 tender-point exam was positive at each of the 18 examined joints and, to a lesser extent, in the husband, who experienced severe cervical arthralgia and symmetrical arthralgia of the patellofemoral joints. Both had disabling pain that limited their cervical range of motion.

Two hypotheses have been proposed to explain the underlying pathophysiology of PEVDS. The first is that patients with high viral loads during the course of illness have persistence of RNA within immune privileged sites. This first hypothesis is contradicted by the recent finding that viral load on admission was not predictive of symptom frequency nor severity. A second hypothesis, which is more consistent with the distribution of symptoms both in the body and in those with less severe disease, is that EVD results in an overwhelming immune activation, including the destruction of cells and tissues that sustain damage from inflammatory cytokines. Other causalities have been proposed. In an animal study, double-stranded DNA, which is released from cells during destruction of tissues (e.g., rhabdomyolysis), and the presence of heat shock proteins in the synovium were associated with higher titers of autoantibodies. However, the underlying pathophysiology of musculoskeletal complaints in survivors remains unknown.

Neuropsychiatric Sequelae

Quiescent infection, recurrent neurologic disease, and post-EVD sequelae demonstrate an emerging range of neurovirulence and incomplete immune privilege of the CNS against EBOV. EBOV RNA has been detected in CSF and ocular fluid. Headaches; short-term memory impairment; tinnitus and hearing loss; altered sense of taste and smell; and confusion and hallucinations have been documented in survivors, with the most disabling symptoms noted in patients known to have had severe EVD.

Neurologists working with survivor cohorts have diagnosed localized deficits, such as homonymous hemianopia, tremors, gait abnormalities, muscle laxity, abnormal reflexes, and asthenias, which are
generally observed in post-stroke patients.\textsuperscript{30,31} Computed tomography imaging in Sierra Leone\textsuperscript{32} demonstrated inflammatory changes, including middle cerebral artery gliosis\textsuperscript{25}; meningoencephalitis\textsuperscript{26}; ischemic changes\textsuperscript{26,33}; atrophy of the cerebellar, parietal, and temporal lobes; and ventriculomegaly and hemorrhagic encephalitis.\textsuperscript{32} Each of these findings has the potential to be profoundly disabling. The broad range of neurosequelae cannot be attributed to one pathology. Viral persistence within the CNS, post-inflammatory nerve damage, and observational associations of altered consciousness, hemodynamic collapse, and cerebral hypoperfusion have been suggested.\textsuperscript{31}

\textit{Psychiatric Sequelae}

Chronic pain contributes to the frequency and severity of anxiety,\textsuperscript{34} a finding upheld by the World Mental Health survey, which found an association between the number of painful body sites and generalized anxiety disorder.\textsuperscript{35} In post-epidemic studies, computed tomography imaging detected evidence of atrophy in the cerebellar, parietal, and temporal lobes, all with the potential to be profoundly disabling and contributory to a depressive affect.\textsuperscript{32} Depression, anxiety, insomnia, grief, and fear are frequently reported by survivors who experience social isolation due to an inability to work and contribute to their communities.\textsuperscript{1,4,12,29,36,37} Finally, the history of the region cannot be overlooked. Superimposed on the traumas from decades of civil war, the prolonged and chaotic nature of the epidemic and loss of family and community members are certain to compound—and confound—post-EVD sequelae and recovery.

\textit{Ophthalmic Sequelae}

The complexity of diagnosing ophthalmic pathology lies in the requirements for visualization of the external and internal structures of the eye. Identification of lesions or structural abnormalities requires not only a clinical specialist, but advanced equipment and drugs. In African survivors, lesions of the retinal structures and along the axonal body of the optic nerve have been described,\textsuperscript{27,38} which are consistent with the development of conjunctivitis, uveitis, hyperlacrimation, and loss of visual acuity.\textsuperscript{5,22,39} In a Sierra Leonean cohort, 60\% of survivors reported ocular symptoms at 121 days after discharge,\textsuperscript{21,31} and nearly 20\% of survivors in West Africa experience vision problems.\textsuperscript{40} EBOV is neuroinvasive and causes widespread CNS inflammation. No conclusive evidence exists to determine the cause of ocular symptoms. Whether due to RNA persistence within the ocular chamber, or are the consequences of infection and inflammation, intermediate treatments and long-range therapies will be required to address ophthalmic sequelae associated with EBOV survivorship.

\textit{Limitations}

Assessment of physical symptoms is subject to bias on the part of the patient and the investigator. The interpretation of pain and disability involve personal perceptions of health and, therefore, are subject to inherent cultural biases. Our unique approach of residing in the village with the survivors was our attempt to overcome these biases by directly observing the context in which they experienced and preformed their activities of daily living.
Due to the emergency nature of the response, we were unable to perform clinical laboratory analyses to identify elevations in inflammatory biomarkers or cellular suggestive of immune-mediated disease processes. Additionally, we did not have access to the \( C_t \) values of the survivors from which to infer the association between viral load, EVD severity, and the development of symptoms. However, a recent finding from Wing, et al.\(^{17} \) found no association of viral load with development of PEVDS.

**The Research Agenda for Post-Ebola Virus Disease Syndrome**

*Longitudinal Cohort Studies*

The largest ever number of survivors from the West Africa epidemic was absolutely unanticipated at the outset of the epidemic, and the global cohort of survivorship will continue to expand as outbreaks of ebolavirus grow larger and more frequent. Our understanding of EVD is incomplete but our knowledge is evolving. Concerted efforts are underway in each of the West African countries to describe the syndrome, determine its causality, and identify treatment modalities. Areas of active investigation into the etiology(ies) of PEVDS include the role of global immune activation in cellular and tissue damage, the deposition of immune complexes in large and small joints, and the ability of EBOV to persist in immune-protected body sites, include the central nervous system. In Guinea, the French-led PostEboGui Study, a prospective, multidisciplinary cohort study, is investigating the long-term clinical, psychosocial, and viral outcomes of survivorship.\(^{13,29,41} \) Public Health England and the UK Ministry of Defence are operating survivor clinics throughout Sierra Leone, in addition to clinical trials to assess the safety and efficacy of favipiravir for the clearance of EBOV RNA from immune-privileged sites,\(^{42} \) and the use of convalescent plasma for treatment of PEVDS.\(^{43} \) The National Institute of Allergy and Infectious Diseases initiated the PREVAIL III trial in 2015 for longitudinal studies of survivors in Liberia,\(^{44} \) and an additional study, PREVAIL IV, is currently enrolling male survivors to assess the ability of experimental drug GS-5734 to eliminate EBOV RNA from semen.\(^{45} \) Understanding disease pathogenesis and identifying acute and chronic co-morbidities (e.g., malaria; history of stroke, diabetes, exposure to heavy metals) will identify endpoints in the clinical course of EVD for administration of therapies to mitigate disease severity and post-EVD sequelae.

**Therapeutics**

Treatment for the PEVDS is currently supportive and there is no definitive cure. Beyond recognizing the clinical features that are manifested as part of PEVDS, efforts must shift toward therapeutic measures that might be initiated during acute infection to minimize the incidence and severity symptoms among survivors. In addition, the scientific community must focus on effective therapeutic measures that may be useful in managing symptoms of PEVDS. Since the pathogenesis of the PEVDS is thought to be related to systemic injury resulting from the original infection, a sustained immune response, autoimmune-like inflammatory state, and viral persistence in specific anatomical locations (i.e., eye, brain, testes), treatment strategies directed at the inflammatory cascade and the immune system have been proposed as initial targets for new therapies.\(^{46} \) In 2015, the National Institute of Allergy and Infectious Diseases initiated the PREVAIL III trial\(^{47} \) to facilitate long-term follow-up of survivors and household contacts in Liberia. From this group, they are recruiting subjects for the PREVAIL IV trial, which will examine the ability of an experimental compound, GS-5734, to clear
persistent EBOV RNA from the semen in male survivors. In the immediate future, West African survivors will require an integrated system of care across the health services spectrum.

CONCLUSION

Post-Ebola virus disease syndrome is a constellation of multi-system, chronic debilitating symptoms experienced by survivors of infection with ebolaviruses, and is now reported in the majority of the 17,000 survivors of the West Africa epidemic. Research among EVD-infected patients and survivors reveals both acute and post-infectious complications, with no gains in defining the underlying pathogenesis. Our results provide person-level data on the experience of PEVDS, with an emphasis on the extent of disability associated with the symptoms. Together with other studies, these findings underscore the need for the international clinical and public health communities to continue investigations into the post-viral phase of infections with emerging tropical viruses. Through investment and development of health care and public health infrastructures for at-risk populations, we will expand our knowledge of EVD pathophysiology, identify therapeutic targets, and gain key insights for the continued development of vaccines.

REFERENCES


CHAPTER V
SUMMARY OF RESULTS AND CONTRIBUTION TO PUBLIC HEALTH PRACTICE

SUMMARY OF RESULTS

Chapter II. Multi-Pathogen Infections of Dengue Virus

Dengue presents as an AFI, typically characterized by fever, myalgia, and headache,1 and is indistinguishable from other tropical diseases, including leptospirosis, during the early-onset of symptoms. The difficulty, therefore, in diagnosing dengue has been the non-specific presentation of symptoms that mirrors other AFIs. We conducted a cross-sectional serosurvey of febrile patients presenting for treatment at two facilities in the Colombian State of Norte de Santander. Our objectives for this study were (1) to establish the first prevalence estimates of DENV MSIs and DENV–Leptospira co-infections in Norte de Santander, (2) identify hematologic indices associated with clinical severity, and (3) to construct a diagnostic algorithm to provide decisional support for clinicians in under-resourced settings.

Key Findings and Contribution to Public Health Practice

1) We estimated the prevalence of DENV MSI to be 14.6% and the prevalence of leptospirosis to be 1.4% in our hospital-based convenience sample. These are the first prevalence estimates for Norte de Santander, and our findings provide evidence of their presence in the pathogen landscape. However, our findings cannot estimate the burden of disease. Enhanced surveillance and research are required, yet laboratory capacity is the critical piece to addressing these issues.

2) We found no differences in clinical severity by SSI, MSI, or among patients co-infected with Leptospira. However, we found co-infections with multiple DENV serotypes, pathogenic Leptospira spp., and with HBV. An unexpected finding of a patient with dengue, leptospirosis, and HBV highlights the utility of case-study data to detect multi-pathogen infections, which contribute to alterations in the expected clinical presentation of disease. The impact of intra-host co-infection on clinical severity, and its potential to contribute to viral emergence, deserves further consideration. Larger studies, powered to detect differences by age-sex groups, are required to further explore the clinical impact of MSIs.

3) We were unable to construct a diagnostic algorithm to assist in clinical diagnostics in areas without laboratory capacity. Our findings underscore the challenge of dengue diagnosis: clinical presentation alone is insufficient. Increasing the availability of rapid diagnostics will be essential for initiating early treatment and defining the incidence of co-infections. Further, the study of coinfection must move beyond laboratory systems and examine the outcomes of disease among persons with complex immunologic profiles. The expanding application of molecular technologies for clinical diagnosis, including the use of next-generation sequencing, will detect and define the intra-host pathogen landscape. The clinical interpretation of the findings, and their translation into public health practice, will be required.
Chapter III. Familial Transmission of Zaire ebolavirus

We reconstructed and fully resolved the transmission events of EBOV transmission among geographically-dispersed family members in Liberia. The objectives were to (1) describe the how single-day exposures can potentiate an epidemic, and (2) elucidate the underlying ecological determinants (i.e., human behaviors and movement) that contribute to the dynamic nature of epidemic EBOV transmission.

Key Findings and Contribution to Public Health Practice

1) We defined key epidemiologic transmission parameters using individual exposure, transmission, and clinical data. We estimated the incubation period for multi- and single-day exposures to be 6.0 and 7.0 days, respectively. Our estimates are aligned with those calculated from a meta-analysis of available data up to the West Africa epidemic, which determined an incubation period of 6.22 days for all exposures. With the limitations inherent in small sample sizes, our estimates provide additional measures to contribute to the pool of existing resources from which transmission parameters can be estimated with increasing precision.

2) We identified how the inability to obtain an isolation bed for the source patient resulted in health-seeking behavior and long-range travel into and out of her community during her peak infectious phase of fatal EVD. Admission to a treatment center was a critical aspect of decelerating the incidence of EVD in West Africa. Between the months of July-November 2014, in regions where focused contact tracing was combined with patient isolation, the average time from detection to isolation decreased from 6.0 days to 1.5 days, the number of generations from a single source decreased from four to two, the median duration of localized outbreaks decreased from 53 to 25 days, survival increased from 13% to 50%. Our results provide the granular data to explain the individual-level and impact of these interventions, and support their use for future outbreaks of EVD.

3) We described how the cases, when they proceeded to the infectious phase of EVD, isolated themselves from large numbers of family members. They were provided food and water during their self-sequestration but did not generate additional secondary or tertiary infections. This was a critical intervention employed by the family and the community, as children were kept separate from sick parents. The care of a sick child is a resource-intensive endeavor, one in which many generations of family and community members become involved. It was a sick child that was the source of an outbreak in the DRC, and a sick child was the index case for what are probably in excess of 30,000 cases of EVD in West Africa. We argue that our results demonstrate the utility and effectiveness of home- and community-based isolation for future outbreaks, but it should be an emergency measure only during an interim waiting period for creation and expansion of isolation and treatment capacity.

4) Our unexpected finding of the probable contribution of sweat to epidemic transmission highlights an under-recognized mode of transmission. The assumption has been that EBOV transmission is
limited to blood and body fluids, without consideration of sweat. Previous reports have described a high concentration of virus in and around sweat glands. Previous reports have described a high concentration of virus in and around sweat glands. Together, this is an important finding with implications for driving ongoing and unrecognized virus transmission.

Chapter IV. Post-Ebola Virus Disease Syndrome

Post-Ebola virus disease syndrome is a constellation of multi-system, chronic debilitating symptoms experienced by survivors of infection with ebolaviruses, and is now reported in the majority of the 17,000 survivors of the West Africa epidemic. Incomplete recovery from tropical virus infection may contribute to additional transmission, poor case management, and functional decline amongst vulnerable populations lacking public health infrastructure and diagnostic methodologies. We conducted physical examinations and evaluations of functional status investigate clinical symptoms and chronicle the post-EVD disability among three survivors from the transmission chain described in Chapter III. Within the limitations of ongoing EBOV transmission, our aims were to characterize as fully as possible (1) the physical and psychiatric symptoms, (2) patterns of distribution by body system, and (3) impact on functional ability.

Key Findings and Contribution to Public Health Practice

1) We used repeated physical examinations to identify symptoms and provide highly-detailed descriptions of each affected body site. These data add to the accumulating evidence from ongoing investigations in West Africa and the US, and will contribute to systematic reviews and meta-analyses for future investigations of PEVDS among affected populations.

2) In concert with other studies, we, described a symmetrical distribution of pain consistent suggestive of an immune-mediated arthritis. These findings add epidemiologic support to the hypothesis that post-viral symptoms have a pathophysiologic bases in the immune-mediated aspect of EVD.

3) We used a vetted functional scale sensitive to cultural-specific measures of disability to assess functional decline between pre-EVD baseline status and 90- and 120-day intervals after discharge from ETU. Our results describe chronic sequelae and loss of function, emphasizing the need for clinical and public health engagement to elucidate the full spectrum of PEVDS. Understanding the patterns of disability among EVD survivors will our knowledge of EVD pathophysiology and identify targets for therapies and vaccines for use in future ebolavirus outbreaks.

FUTURE DIRECTIONS

Despite decades of research into dengue and the ebolaviruses, we lack the ability to fully explain, forecast, and prevent the emergence of these viruses and those to come. The risk of human infection from animals is persistent, wide-spread, and unpredictable. Until human exposure to infection can be more accurately predicted or prevented, the approach to managing future outbreaks will center on the
classic, response approach to control: early case detection and isolation; contact tracing and community engagement; environmental assessment and mitigation; and effective, equitable clinical care. Within its limitation and criticism of lacking scientific rigor, particularly with respect to generalizability, case study data are informative estimates from which clinical care is coordination, research plans developed, and policy decisions are made.

A vital next step is deliverable financial support for the development and sustainment of health services in the countries at risk for emerging zoonotic viruses. The Commission on a Global Health Risk Framework for the Future has underscored the importance of resilient national public health services as the first line of defense against future epidemics of EVD or other diseases. They recommended investment in essential health services with an emphasis on primary prevention and treatment, at an annual cost of $4.5 billion, to both strengthen the ability to recognize and respond to outbreaks, with also have sustained investment for the next generation of technologies for prediction, detection, and control. No country exists in isolation; borders are too porous, and travel too rapid. Containment and isolation are inadequate public health strategies. We do not lack the science to understand the consequences of maintaining weakened nation-states. In perhaps the greatest ongoing struggle, global health security remains an inexorable adjudgment of political will.

REFERENCES


APPENDIX A
PUBLISHED REPORTS OF DENGUE VIRUS MULTI-SEROTYPE INFECTIONS,
BY COUNTRY AND AUTHOR

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<td>Figueiredo, RM. (2011)</td>
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<td>DENV-3/4 (n=4)</td>
<td>NS</td>
</tr>
<tr>
<td>Marinho, PE. (2017)</td>
<td>PCR</td>
<td>DENV-2/3 (n=1)</td>
<td>No increased severity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-1/2/3 (n=1)</td>
<td></td>
</tr>
<tr>
<td>Rocco, IM. (1998)</td>
<td>Cell culture, ELISA, PCR</td>
<td>DENV-1/2 (n=1)</td>
<td>Mild disease</td>
</tr>
</tbody>
</table>

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ex., exported from; IFA, immunofluorescence assay; NS, not specified; PCR, polymerase chain reaction.
* References are listed by author last name at conclusion of table.
Table continued on page 94.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Diagnostic Methodology</th>
<th>Serotypes (Frequency)</th>
<th>Disease Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>China ex. Sri Lanka</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meiyu, F. (1997)</td>
<td>Cell culture PCR</td>
<td>DENV-2/4 ((n=8))</td>
<td>NS</td>
</tr>
<tr>
<td>Peng, W. (2005)</td>
<td>IFA PCR</td>
<td>DENV-2/3 ((n=1))</td>
<td>No increased severity</td>
</tr>
<tr>
<td><strong>India</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoop, M. (2010)</td>
<td>PCR</td>
<td>DENV-1/2 ((n=2)) DENV-2/3 ((n=18)) DENV-1/2/3 ((n=1))</td>
<td>NS</td>
</tr>
<tr>
<td>Bharaj, P. (2008)</td>
<td>PCR</td>
<td>DENV-1/3 ((n=4)) DENV-1/4 ((n=2)) DENV-2/3 ((n=1)) DENV-3/4 ((n=1))</td>
<td>DHF: 6/9 (67%)</td>
</tr>
<tr>
<td>Das, B. (2013)</td>
<td>PCR</td>
<td>DENV-2/3 ((n=14))</td>
<td>NS</td>
</tr>
<tr>
<td>Khan, SA. (2013)</td>
<td>ELISA PCR</td>
<td>DENV-1/3 ((n=11)) DENV-1/4 ((n=1)) DENV-2/3 ((n=1))</td>
<td>NS</td>
</tr>
<tr>
<td>Mehta, TK. (2018)</td>
<td>PCR</td>
<td>DENV-1/2 ((n=2)) DENV-2/3 ((n=10)) DENV-3/4 ((n=1))</td>
<td>Mild disease; lab values WNL</td>
</tr>
<tr>
<td>Rao, MRK. (2018)</td>
<td>PCR</td>
<td>DENV-2/3 ((n=74)) DENV-1/2/3 ((n=3))</td>
<td>DHF: 17/28 (60.7%), including 3/3 (100%) triple-serotype infections</td>
</tr>
</tbody>
</table>

Abbreviations: DHF, dengue hemorrhagic fever; ELISA, enzyme-linked immunosorbent assay; ex., exported from; IFA, immunofluorescence assay; NS, not specified; PCR, polymerase chain reaction; SD, severe disease; WNL, within normal limits.

* References are listed by author last name at conclusion of table.
Table continued on page 95.
<table>
<thead>
<tr>
<th>Author</th>
<th>Diagnostic Methodology</th>
<th>Serotypes (Frequency)</th>
<th>Disease Severity</th>
</tr>
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<tbody>
<tr>
<td><strong>India (continued)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reddy, MN.  (2017)</td>
<td>PCR</td>
<td>DENV-1/3 (n=9)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/3 (n=7)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>DENV-1/2/3 (n=8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-1/2/3/4 (n=2)</td>
<td></td>
</tr>
<tr>
<td>Savargaonkar, D.  (2018)</td>
<td>PCR</td>
<td>DENV-1/2 (NS)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/3 (NS)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>DENV-2/4 (NS)</td>
<td></td>
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<td>DENV-3/4 (NS)</td>
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<td></td>
<td>DENV-2/3/4 (NS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-1/2/3/4 (n=1)</td>
<td></td>
</tr>
<tr>
<td>Tazeen, A.  (2017)</td>
<td>PCR</td>
<td>DENV-1/2 (n=21)</td>
<td>SD: 3/21 (14.3%)</td>
</tr>
<tr>
<td>Vaddadi, K.  (2017)</td>
<td>ELISA  PCR</td>
<td>DENV-1/2 (n=7)</td>
<td>SD: 12/15 (80%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-1/3 (n=1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/4 (n=5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-3/4 (n=2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/4: 5/5 (100%)</td>
<td>classified DWWS</td>
</tr>
<tr>
<td>Vinodkumar, CS.  (2013)</td>
<td>PCR</td>
<td>DENV-1/2 (n=3)</td>
<td>DHF: 11/17 (64.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-1/3 (n=2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/3 (n=11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/4 (n=1)</td>
<td></td>
</tr>
<tr>
<td><strong>Indonesia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lardo, S.  (2016)</td>
<td>PCR</td>
<td>DENV-2/3 (n=1)</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Malaysia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dhanoa, A.  (2016)</td>
<td>PCR</td>
<td>DENV-1/2 (n=34)</td>
<td>SD more frequent among MSI patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-1/3 (n=5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DENV-2/3 (n=1)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DHF, dengue hemorrhagic fever; DWWS, dengue with warning signs; ELISA, enzyme-linked immunosorbent assay; ex., exported from; MSI, multi-serotype; NS, not specified; PCR, polymerase chain reaction; SD, severe disease.

* References are listed by author last name at conclusion of table.
Table continued on page 96.
<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Diagnostic Methodology</th>
<th>Serotypes (Frequency)</th>
<th>Disease Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexico</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Requena-Castro, R. (2017)</td>
<td>ELISA, PCR</td>
<td>DENV-1,2 ((n=9)) DENV-2/4 ((n=2)) DENV-1/2/3 ((n=1)) DENV-1/3/4 ((n=1)) DENV-1/2/3/4 ((n=1))</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Mexico ex. Indonesia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loroño-Piño, MA. (1999)</td>
<td>PCR</td>
<td>DENV-1/2 ((n=2)) DENV-1/3 ((n=4)) DENV-1/4 ((n=2)) DENV-2/3 ((n=2)) DENV-2/4 ((n=1)) DENV-3/4 ((n=1))</td>
<td>DHF: 3/12 (25%)</td>
</tr>
<tr>
<td><strong>Puerto Rico</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gubler, DJ. (1985)</td>
<td>CF, IFA, PRNT</td>
<td>DENV-1/4 ((n=1))</td>
<td>Mild disease</td>
</tr>
<tr>
<td><strong>New Caledonia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laille, M. (1991)</td>
<td>PCR</td>
<td>DENV-1/3 ((n=6))</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Peru</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mamani, E. (2010)</td>
<td>PCR</td>
<td>DENV-1/3 ((n=6))</td>
<td>DHF: 1/6 (16.7%)</td>
</tr>
<tr>
<td><strong>Sri Lanka</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissanayake, V. (2011)</td>
<td>PCR</td>
<td>DENV-1/3 ((n=9))</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: CF, complement fixation; DHF, dengue hemorrhagic fever; ELISA, enzyme-linked immunosorbent assay; ex., exported from; IFA, immunofluorescence assay; NS, not specified; PCR, polymerase chain reaction; PRNT, plaque reduction neutralization assay.

* References are listed by author last name at conclusion of table.
Table continued on page 97.
<table>
<thead>
<tr>
<th><strong>Author</strong> (Year)</th>
<th><strong>Diagnostic Methodology</strong></th>
<th><strong>Serotypes (Frequency)</strong></th>
<th><strong>Disease Severity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sriprapun, M. (2015)</td>
<td>PCR (urine)</td>
<td>DENV-2/4 ((n=1))</td>
<td>DHF</td>
</tr>
<tr>
<td>Maneekarn, N. (1993)</td>
<td>ELISA PCR</td>
<td>DENV-1/2 ((n=2))</td>
<td>NS</td>
</tr>
<tr>
<td>Chinnawirotpisan, P. (2008)</td>
<td>PCR</td>
<td>DENV-1/2 ((n=1)), DENV-2/4 ((n=1)), DENV-1/2/3 ((n=1))</td>
<td>DHF: DENV-2/4 only</td>
</tr>
<tr>
<td>Pongisiri, P. (2012)</td>
<td>PCR</td>
<td>DENV-3/4 ((n=1))</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Taiwan</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Wang, SF. (2016)</td>
<td>PCR</td>
<td>DENV-2/3 ((n=2))</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vietnam</strong></td>
<td></td>
<td></td>
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<tr>
<td>Binh, PT. (2009)</td>
<td>PCR</td>
<td>DENV-1/4 ((n=17)), DENV-2/3 ((n=1)), DENV-3/4 ((n=1))</td>
<td>No increased severity</td>
</tr>
</tbody>
</table>

Abbreviations: DHF, dengue hemorrhagic fever; ELISA, enzyme-linked immunosorbent assay; NS, not specified; PCR, polymerase chain reaction.

*References are listed by author last name at conclusion of table.*

**REFERENCES**


Rao MRK, Padhy RN, Das MK. Episodes of the epidemiological factors correlated with prevailing viral infections with dengue virus and molecular characterization of serotype-specific dengue virus circulation in eastern India. *Infect Genet Evol.* 2018;58:40-49.


APPENDIX C
PATIENT-SPECIFIC FUNCTIONAL SCALE

The Patient Specific Functional Scale

This useful questionnaire can be used to quantify activity limitation and measure functional outcome for patients with any orthopaedic condition.

Clinician to read and fill in below: Complete at the end of the history and prior to physical examination.

Initial Assessment:

I am going to ask you to identify up to three important activities that you are unable to do or are having difficulty with as a result of {your problem}. Today, are there any activities that you are unable to do or having difficulty with because of {your problem}? (Clinician: show scale to patient and have the patient rate each activity).

Follow-up Assessments:

When I assessed you on {state previous assessment date}, you told me that you had difficulty with (read all activities from list at a time). Today, do you still have difficulty with: (read and have patient score each item in the list)?

Patient-specific activity scoring scheme (Point to one number):

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to perform activity</td>
<td>Able to perform activity at the same level as before injury or problem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Date and Score)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
<tr>
<td>Additional</td>
<td></td>
</tr>
</tbody>
</table>

Additional

Additional

Total score = sum of the activity scores/number of activities
Minimum detectable change (90%CI) for average score = 2 points
Minimum detectable change (90%CI) for single activity score = 3 points


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APPENDIX D
LSU IRB APPROVAL—DETERMINANT FACTORS OF VIRAL DIVERSITY AND TRANSMISSION ECOLOGY OF DENGUE VIRUS IN COLOMBIA

ACTION ON PROTOCOL APPROVAL REQUEST

TO: Christopher Mores
Pathobiological Sciences

FROM: Robert C. Mathews
Chair, Institutional Review Board

DATE: September 17, 2013
RE: IRB# 3415

TITLE: Determinant Factors of Viral Diversity and Transmission Ecology of Dengue Virus in Colombia


Review type: Full ___ Expedited _X___ Review date: 9/18/2013

Risk Factor: Minimal ____ X ____ Uncertain _____ Greater Than Minimal_____

Approved ___ X ___ Disapproved__________

Approval Date: 9/18/2013 Approval Expiration Date: 9/17/2014

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 300

Protocol Matches Scope of Work in Grant proposal: (if applicable)

By: Robert C. Mathews, Chairman

PRINCIPAL INVESTIGATOR: PLEASE READ THE FOLLOWING – Continuing approval is CONDITIONAL on:

1. Adherence to the approved protocol, familiarity with, and adherence to the ethical standards of the Belmont Report, and LSU’s Assurance of Compliance with DHHS regulations for the protection of human subjects*
2. Prior approval of a change in protocol, including revision of the consent documents or an increase in the number of subjects over that approved.
3. Obtaining renewed approval (or submittal of a termination report), prior to the approval expiration date, upon request by the IRB office (irrespective of when the project actually begins); notification of project termination.
4. Retention of documentation of informed consent and study records for at least 3 years after the study ends.
5. Continuing attention to the physical and psychological well-being and informed consent of the individual participants, including notification of new information that might affect consent.
6. A prompt report to the IRB of any adverse event affecting a participant potentially arising from the study.
8. SPECIAL NOTE:

*All investigators and support staff have access to copies of the Belmont Report, LSU’s Assurance with DHHS, DHHS (45 CFR 46) and FDA regulations governing use of human subjects, and other relevant documents in print in this office or on our World Wide Web site at http://www.lsu.edu/irb

102
APPENDIX B
COLOMBIA SEROPREVALENCE STUDY ADULT CONSENT AND PEDIATRIC ASSENT FORMS, ENGLISH AND SPANISH

ADULT CONSENT FORM (ENGLISH)
Flesch–Kincaid Readability Scores: 6.0

Heterogeneity of Vector Exposure Alters Dengue Virus Transmission and Disease Risk Patterns

Description
You had been invited to participate in a study for an investigation about dengue virus and its vector. We are planning to study the virus serotypes and the antibodies against the virus that could be circulating in your blood.

Procedures
Your doctor has requested a diagnostic test to confirm an infection with dengue virus in your blood. We would like to request your permission to use the REMAINING serum in our research. Normally, this serum is discarded after the test is done. However, it will be of great use in our investigation and the results of this investigation will not change the treatment that your doctor has decided to give you. IF YOU ARE PREGNANT YOU WILL NOT BE INCLUDED IN THIS STUDY.

After completion of our main experiments, we would like to store part of your sample for future studies related to dengue. This sample will be frozen and stored with a CODE, your NAME WILL NOT BE INCLUDED on any material collected for this investigation. You can decide NOT to continue in this research at any moment and, all samples collected from you will be destroyed.

We would also like to have your permission to include the results of additional test (platelet count, red blood cell count) requested along your dengue confirmation test. We will not record any clinical procedure, treatment, past diseases or any identifiable information from your files. You may reject the access of your tests results at any time.

It is possible that in the future we can obtain new information, new products, or have any discoveries with potential commercial value. Any donor, in this case serum donor, will not be entitled to property rights on this information, product or discovery.

The results of this investigation will be obtained only for research purposes. You will not know the results of the test we will perform on your sample.

Risks and Benefits
We do not anticipate any risk or secondary effect for participating in this study. Since this study will not include any treatment, you will not receive any direct benefit for your participation. We cannot guarantee you will receive any benefit from this research.

Privacy
Your privacy will be protected to the extent legally possible. Information from this study may be published. We will not publish your name, address, or other information that would identify you personally or link your name with any of our results.
**Time**
Once the sample for the test requested by your doctor is taken, you will not be required to spend any extra time.

**Payment**
You will not receive any payment for participating in this study.

**Whom to call for questions:** You can call Carolina Cardenas at 317 300 4517 any time if I have any questions about study.

**Participant**
When I signed this form, it means that the study has been explained to me in the language that I understand. If I decide to take part of this study, I will receive a signed copy of this information and a written summary about the study. I will have the opportunity to ask questions about the study. My questions will have to be answer to my satisfaction before I signed this form. I can choose not to participate in the study, or I can quit the study at any time.

I know what will be done as part of the study. I also know the good and the bad (benefits and risks) that could happen if I take part in the study. I know that I can stop been part of the study and I will keep getting the usual medical attention.

I consent that my sample ____ or other tests results ____ will be used in this research.

I do NOT consent that my sample ____ or other test results ____ will be used in this research.

I consent that my sample will be stored for future investigations. _____

Printed Name of Participant __________________________  Date of Birth __________________________

Street Address _____________________________________  City __________________________

Printed Name of Person Conducting Consent Interview __________________________  Signature of Person Conducting Consent Interview __________________________

Printed Name of Witness __________________________  Witness Signature __________________________  Date __________________________
CONSENTIMIENTO INFORMADO PARA ADULTOS (SPANISH)

Descripción: Usted está invitado para participar en un estudio de investigación sobre el dengue y su vector. Nosotros planeamos estudiar los serotipos de dengue y los anticuerpos en contra del virus y de la saliva del vector encontradas en su sangre.

Procedimientos: A usted se le ha solicitado un examen de sangre para confirmar la infección por el virus del dengue. Nos gustaría que nos diera su permiso para estudiar el suero RESTANTE O QUE SOBRE de la realización de dicho examen. Ese suero sobrante normalmente es eliminado. Este estudio de investigación no cambiará su tratamiento médico. SI ESTAS EMBARASADA NO SERAS INCLUIDA EN ESTE ESTUDIO.

Después de completar nuestros exámenes iniciales, quisiéramos guardar el suero sobrante para hacer futuras investigaciones sobre el dengue. Ese suero sería congelado y almacenado con un número o CÓDIGO y NO SE INCLUIRA SU NOMBRE. En cualquier momento del estudio usted puede retirarse, y en ese caso su suero sería desechado.

Al mismo tiempo, quisiéramos su permiso para acceder a los resultados de otros exámenes que se pidieron junto con en examen de confirmación del dengue, con el UNICO propósito de obtener datos de hemograma (componentes de su sangre como plaquetas y glóbulos rojos). Es importante aclarar que no se tomará ninguna información personal como su nombre o dirección o cualquier otra información de enfermedades pasadas. Usted puede rechazar en cualquier momento la inclusión de estos exámenes.

Con el uso de su suero puede suceder que en el futuro se obtengan nuevos productos, exámenes o descubrimientos los cuales tienen potencial valor comercial. Los donantes de tejidos, en este caso el suero extraído de su sangre, no retienen ningún derecho de propiedad sobre esos productos. Por lo tanto, usted no recibirá ningún beneficio económico de esos productos, exámenes o descubrimientos. Los resultados del estudio realizado con su muestra serán usados únicamente con propósitos de investigación y usted no conocerá el resultado de dichas pruebas.

Riesgos y Beneficios
No se prevé riesgos asociados con este estudio. Como este no es un estudio sobre tratamiento, usted no recibirá ningún beneficio directo por su participación. Nosotros no podemos garantizarle que usted recibirá algún beneficio de este estudio.

Privacidad
Su privacidad será protegida al máximo posible. La información resultante de este estudio podría ser publicada. Nosotros no publicaremos su nombre, dirección o cualquier otra información que lo pueda ligar o identificar a usted con los resultados de esta investigación.

Gasto de Tiempo
Una vez se obtenga la muestra de sangre para realizar sus exámenes médicos, su participación en este estudio no requerirá de tiempo adicional para usted.

Pagos
Usted no recibirá pago alguno por participar en este estudio.
**A quien llamar por preguntas:** Usted puede llamar a Carolina Cárdenas al 317 3004517 a cualquier hora si tiene alguna pregunta sobre el estudio.

**Paciente**

Cuando yo firme este formato, significa que el estudio de investigación me ha sido explicado en un lenguaje que yo entiendo. Si yo decido participar, yo recibiré una copia de esta información y de un resumen del estudio. Yo tendrá la oportunidad de hacer preguntas sobre el estudio. Mis preguntas deberán ser respondidas a satisfacción antes que yo firme este formato. Yo puedo decidir no participar en el estudio o puedo renunciar a él en cualquier momento.

Yo sé que se evaluará en este estudio. Yo también se lo bueno y lo malo (riesgos y beneficios) que podría pasar si yo hago parte de este estudio. Yo sé que puedo dejar de ser parte de esta investigación sin dejar de recibir mi atención médica usual.

Marque los que considere pertinentes:

- Yo consiento en que mi muestra___o exámenes clínicos ___sean usados para esta investigación.
- Yo NO consiento en que mi muestra___o exámenes clínicos___sea usados para esta investigación.
- Yo consiento que mi muestra sea guardada para otras investigaciones: SI_____  NO ______

<table>
<thead>
<tr>
<th>Nombre del Paciente</th>
<th>Fecha de Nacimiento</th>
</tr>
</thead>
<tbody>
<tr>
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<table>
<thead>
<tr>
<th>Firma del Paciente</th>
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</thead>
<tbody>
<tr>
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</table>

<table>
<thead>
<tr>
<th>Barrio</th>
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</thead>
<tbody>
<tr>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nombre del Testigo</th>
<th>Firma del testigo</th>
<th>Fecha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Heterogeneity of Vector Exposure Alters Dengue Virus Transmission and Disease Risk Patterns

Description of the Study
Mosquitoes carry dengue virus. The purpose of this study is to look for virus and other molecules in the blood that are made when a person becomes sick. These molecules, called antibodies, are made by the body to protect against being sick.

Your child is being asked to participate in a study about dengue virus. Mosquitoes in your area carry this virus. When they bite people, this can cause a person to become sick with dengue. The data from this study will help to better control the mosquito population. It will also help us to understand how and when the body makes the molecules to protect against getting sick.

This study will test your child’s blood for virus to see if he or she has the virus. We will also see if he or she has the molecules to protect against getting sick. Study staff will take blood two times. The first time will be today, if you want your child to take part in the study. The second time will be in six months.

Possible Benefits
There is no benefit to you or your child for being in this study. The benefit of being in this study is the benefit of the community. It will also help guide mosquito control efforts and help people who get sick with dengue.

Possible Risks
The risk of side effects from drawing blood is small. Your child may have pain when the needle goes into the skin. Some people have a little bruising after the blood is taken.

Costs
There is no cost to be in this study.

Privacy
Your child’s privacy will be protected to the extent legally possible. Information from this study may be published. We will not publish your name or your child’s name, address, or other information that would identify you or your child or link you or your child to antibody results.

Contacting Study Personnel
You can call the study staff if you have questions about the study. You can also contact the study staff if you want your child to stop being part of the study. The contact information is:
(Insert site-specific investigator contact information.)

Consent
This study staff has been explained to me, and I freely consent to join this study. I understand the following:

- There are small risks from getting a blood draw.
- There are no direct benefits to me from being in this study.
- I may refuse to join or may drop out at any time.
<table>
<thead>
<tr>
<th>Printed Name of Participant</th>
<th>Date of Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature of Participant or Consenting Parent</td>
<td>Date/Time</td>
</tr>
<tr>
<td>Street Address</td>
<td>Barrio, City</td>
</tr>
<tr>
<td>Printed Name of Person Conducting Consent Interview</td>
<td>Signature of Person Conducting Consent Interview</td>
</tr>
<tr>
<td></td>
<td>Date</td>
</tr>
</tbody>
</table>
CONSENTIMIENTO INFORMADO PEDIATRICO (SPANISH)

Factores Determinantes de Diversidad Viral y Ecología de la Transmisión del Virus del Dengue en Colombia

Descripción
Usted está invitado para participar en un estudio de investigación sobre el dengue y su vector. Nosotros planeamos estudiar los serotipos de dengue y los anticuerpos en contra del virus y de la saliva del vector encontradas en su sangre.

Procedimientos
A usted se le ha solicitado un examen de sangre para confirmar la infección por el virus del dengue. Nos gustaría que nos diera su permiso para estudiar el suero RESTANTE O QUE SOBRE de la realización de dicho examen. Ese suero sobrante normalmente es eliminado. Este estudio de investigación no cambiará su tratamiento médico. SI ESTAS EMBARASADA NO SERAS INCLUIDA EN ESTE ESTUDIO.

Después de completar nuestros exámenes iniciales, quisiéramos guardar el suero sobrante para hacer futuras investigaciones sobre el dengue. Ese suero sería congelado y almacenado con un número o CÓDIGO y NO SE INCLUIRA SU NOMBRE. En cualquier momento del estudio usted puede retirarse, y en ese caso su suero sería desechado.

Al mismo tiempo, quisiéramos su permiso para acceder a los resultados de otros exámenes que se pidieron junto con en examen de confirmación del dengue, con el UNICO propósito de obtener datos de hemograma (componentes de su sangre como plaquetas y glóbulos rojos). Es importante aclarar que no se tomará ninguna información personal como su nombre o dirección o cualquier otra información de enfermedades pasadas. Usted puede rechazar en cualquier momento la inclusión de estos exámenes.

Con el uso de su suero puede suceder que en el futuro se obtengan nuevos productos, exámenes o descubrimientos los cuales tienen potencial valor comercial. Los donantes de tejidos, en este caso el suero extraído de su sangre, no retienen ningún derecho de propiedad sobre esos productos. Por lo tanto, usted no recibirá ningún beneficio económico de esos productos, exámenes o descubrimientos. Los resultados del estudio realizado con su muestra serán usados únicamente con propósitos de investigación y usted no conocerá el resultado de dichas pruebas.

Riesgos y Beneficios
No se prevén riesgos asociados con este estudio. Como este no es un estudio sobre tratamiento, usted no recibirá ningún beneficio directo por su participación. Nosotros no podemos garantizarle que usted recibirá algún beneficio de este estudio.

Privacidad
Su privacidad será protegida al máximo posible. La información resultante de este estudio podría ser publicada. Nosotros no publicaremos su nombre, dirección o cualquier otra información que lo pueda ligar o identificar a usted con los resultados de esta investigación.

Gasto de Tiempo
Una vez se obtenga la muestra de sangre para realizar sus exámenes médicos, su participación en este estudio no requerirá de tiempo adicional para usted.
**Pagos**
Usted no recibirá pago alguno por participar en este estudio.

**A quien llamar por preguntas:** Usted puede llamar a Carolina Cárdenas al 317 3004517 a cualquier hora si tiene alguna pregunta sobre el estudio.

**Paciente**
Cuando yo firme este formato, significa que el estudio de investigación me ha sido explicado en un lenguaje que yo entiendo. Si yo decido participar, yo recibiré una copia de esta información y de un resumen del estudio. Yo tendré la oportunidad de hacer preguntas sobre el estudio. Mis preguntas deberán ser respondidas a satisfacción antes que yo firme este formato. Yo puedo decidir no participar en el estudio o puedo renunciar a él en cualquier momento.

Yo sé que se evaluará en este estudio. Yo también se lo bueno y lo malo (riesgos y beneficios) que podría pasar si yo hago parte de este estudio. Yo sé que puedo dejar de ser parte de esta investigación sin dejar de recibir mi atención médica usual.

Marque los que considere pertinentes:

Yo consiento en que mi muestra _______ o exámenes clínicos _________ sean usados para esta investigación.

Yo NO consiento en que mi muestra ______ o exámenes clínicos_____ sea usados para esta investigación.

Yo consiento que mi muestra sea guardada para otras investigaciones: SI_____ No ______

<table>
<thead>
<tr>
<th>Nombre del Paciente</th>
<th>Fecha de Nacimiento</th>
</tr>
</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>Nombre del Acudiente</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Firma del Paciente o Acudiente</td>
<td>Fecha</td>
</tr>
<tr>
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</tr>
<tr>
<td>Barrio</td>
<td>Ciudad de Residencia</td>
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<tr>
<td>Nombre del Testigo</td>
<td>Firma del testigo</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Appendix C

## Centers for Disease Control and Prevention

### Dengue Case Investigation Report

- **CDC Dengue Branch and Puerto Rico Department of Health**
- **1324 Calle Canábean, San Juan, P.R. 00920-2860**
- **Tel.: (787) 706-2399, Fax: (787) 706-2496**

### Dengue Case Report Form

**FOR CDC DENGUE BRANCH USE ONLY**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Specimen #</th>
<th>Days post onset (DPO)</th>
<th>Type</th>
<th>Date Received</th>
<th>Specimen #</th>
<th>Days post onset (DPO)</th>
<th>Type</th>
<th>Date Received</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SAN ID</th>
<th>GCODE</th>
<th>S1</th>
<th></th>
<th>S2</th>
<th></th>
<th>S3</th>
<th></th>
<th>S4</th>
<th></th>
</tr>
</thead>
</table>

Please complete all sections

- **Hospitalized:** [ ] Yes [ ] No [ ] UNK
- **Name of Patient:** Last Name: ___________ First Name: ___________
- **If patient is a minor, name of father or primary caregiver:** Last Name: ___________ First Name: ___________
- **Mental Status Changes:** [ ] Yes [ ] No [ ] UNK

### Home Address

- **City, Town:** ___________
- **Urbanization or sector:** ___________
- **Street:** ___________ House / Apt. Number: ___________
- **Premise No.:** ___________ Box: ___________ P.O. Box: ___________
- **Road No.:** Km: ___________ Hm: ___________ Tel: ___________ Other Tel: ___________
- **Residence is close to:** Zip Code: ___________
- **Work address:**

### Physician who referred the case

- **Name of Healthcare Provider:** ___________
- **Phone number:** ___________
- **Email address:** ___________
- **Send laboratory results to:**

### Information about the person filling out this form

- **Name and title:** ___________
- **Name and address of employment:** ___________

### Patient’s Basic Information

- **Date of Birth:** ___________ Age: ___________ months Sex: [ ] M [ ] F

- **Day: ___________ Month: ___________ Year: ___________** OR **years: ___________**

### Must have the following information for sample processing

- **Date of first symptom:** ___________ ___________ ___________
- **Date specimen taken:**
  - **Serum:**  [ ] First sample [ ] Second sample [ ] Third sample
    - [ ]acute + 4-5 days of illness - check for virus [ ] acute + more than 5 days of illness - check for antibodies
  - **Tissue for fatal cases (specify):** ___________ ___________ ___________

### Additional Data

1. **How long have you lived in this city:** ___________
2. **Country of birth:** ___________
3. **Have you been diagnosed with dengue before:** [ ] Yes [ ] No [ ] UNK
4. **When diagnosed:** ___________ ___________ ___________
5. **During the 14 days before onset of illness, did you travel to other cities or countries:** [ ] Yes, another country [ ] Yes, another city [ ] No [ ] UNK
6. **WHERE did you TRAVEL?**

### Criteria for Dengue Hemorrhagic Fever (14) and shock (5)

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>UNK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fever (&gt;39°C)</td>
<td>Yes</td>
</tr>
<tr>
<td>2.</td>
<td>Platelets &lt;100,000/mm³</td>
<td>Yes</td>
</tr>
<tr>
<td>3.</td>
<td>Any hemorrhagic manifestation</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Petechiae</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Petechiae/Scchymosis</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Vomiting with blood</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Blood in stool</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Nasal bleeding</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Bleeding gums</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Blood in urine</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Vaginal bleeding</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Positive wrist (over 5% RBC/1µl or positive for blood)</td>
<td>Yes</td>
</tr>
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</table>

### Symptoms continued

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>UNK</th>
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<tbody>
<tr>
<td>4.</td>
<td>Evidence of capillary leak</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Pleural or abdominal effusion</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Palmar or palmar oozing</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Abdominal pain</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Burning sensation</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Cough</td>
<td>Yes</td>
</tr>
<tr>
<td>5.</td>
<td>Retinitis (red eyes)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Nasal congestion</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Swelling of the face</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Jowls</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Convulsion or coma</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Pregnancy</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Gull Yellow Fever Vaccine</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Year vaccinated</td>
<td>Yes</td>
</tr>
</tbody>
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## APPENDIX G
### ADULT AND PEDIATRIC CLINICAL LABORATORY REFERENCE RANGES

Adult American Board of Internal Medicine Clinical Laboratory Reference Values*  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Eryth) Erythrocytes</td>
<td>$4.2–5.9 \times 10^6/\mu L$</td>
</tr>
</tbody>
</table>
| (Hct) Hematocrit | female: 37%–47%  
male: 42%–50% |
| (Hb) Hemoglobin | female: 12–16 g/dL  
male: 14–18 g/dL |
| (Plt) Platelets | $150,000–450,000/\mu L$ |
| (Leu) Leukocytes | $4.0–11.0 \times 10^3/\mu L$ |
| (Neut) Neutrophils | 50%–70% |
| (Lymph) Lymphocytes | 30%–45% |
| (Mono) Monocytes | 0–6% |
| (Eos) Eosinophils | 0–3% |
| (Baso) Basophils | 0–1% |

Pediatric Clinical Laboratory Reference Values†  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
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</thead>
<tbody>
<tr>
<td>(Eryth) Erythrocytes</td>
<td>Age $\times 10^3/\mu L$</td>
</tr>
<tr>
<td>0–30 days</td>
<td>4.1–6.7</td>
</tr>
<tr>
<td>1 month</td>
<td>3.0–5.4</td>
</tr>
<tr>
<td>2–6 months</td>
<td>2.7–4.5</td>
</tr>
<tr>
<td>7 months–2 years</td>
<td>3.7–5.3</td>
</tr>
<tr>
<td>3–6 year</td>
<td>3.9–5.3</td>
</tr>
<tr>
<td>7–12 years</td>
<td>4.0–5.2</td>
</tr>
</tbody>
</table>
| 13–18 years | female 4.1–5.1  
male 4.5–5.3 |
| ≥19 years | female 4.2–5.4  
male 4.7–6.0 |

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Hct) Hematocrit</td>
<td>Age %</td>
</tr>
<tr>
<td>0–30 days</td>
<td>44–70</td>
</tr>
<tr>
<td>1 month</td>
<td>32–42</td>
</tr>
<tr>
<td>2–6 months</td>
<td>29–41</td>
</tr>
<tr>
<td>7 months–2 years</td>
<td>33–39</td>
</tr>
<tr>
<td>3–6 years</td>
<td>34–40</td>
</tr>
<tr>
<td>7–12 years</td>
<td>35–45</td>
</tr>
</tbody>
</table>
| 13–18 years | female 36–45  
male 37–49 |
| ≥19 years | female 36–46  
male 41–53 |

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Hb) Hemoglobin</td>
<td>Age g/dL</td>
</tr>
<tr>
<td>0–30 days</td>
<td>15.0–22.0</td>
</tr>
<tr>
<td>1 month</td>
<td>10.5–14.0</td>
</tr>
</tbody>
</table>
2–6 months 9.5–13.5
7 months–2 years 10.5–14.0
3–6 years 11.5–14.5
7–12 years 11.5–15.5
13–18 years female 12.0–16.0 male 13.0–16.0
≥19 years female 12.0–16.0 male 13.5–17.5

(Plt) Platelets Age, sex inclusive 150,000–450,000/μL.

(Leu) Leukocytes Age ×10⁹/μL
0–30 days 9.1–34.0
1 month 5.0–19.5
2–11 months 6.0–17.5
1–6 years 5.0–14.5
7–12 years 5.0–14.5
13–18 years 4.5–13.5
≥19 years 4.5–11.0

<table>
<thead>
<tr>
<th>Age</th>
<th>Neutrophils (Neut) (%)</th>
<th>Lymphocytes (Lymph) (%)</th>
<th>Monocytes (Mono) (%)</th>
<th>Eosinophils (Eos) (%)</th>
<th>Basophils (Baso) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–30 days</td>
<td>32–67</td>
<td>25–37</td>
<td>0–9</td>
<td>0–2</td>
<td>0–1</td>
</tr>
<tr>
<td>1 month</td>
<td>20–46</td>
<td>28–84</td>
<td>0–7</td>
<td>0–3</td>
<td>0–1</td>
</tr>
<tr>
<td>2–11 months</td>
<td>20–48</td>
<td>34–88</td>
<td>0–5</td>
<td>0–3</td>
<td>0–1</td>
</tr>
<tr>
<td>1–6 years</td>
<td>37–71</td>
<td>17–67</td>
<td>0–5</td>
<td>0–3</td>
<td>0–1</td>
</tr>
<tr>
<td>7–12 years</td>
<td>33–76</td>
<td>15–61</td>
<td>0–5</td>
<td>0–3</td>
<td>0–1</td>
</tr>
<tr>
<td>13–18 years</td>
<td>33–76</td>
<td>15–55</td>
<td>0–4</td>
<td>0–3</td>
<td>0–1</td>
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</tbody>
</table>

APPENDIX D
COLOMBIA SEROPREVALENCE SURVEY SAS CODE

******************************************
proc printto print=myprint;
proc printto log=mylog;
proc format;
value agecat
  1 = '<1'
  2 = '1-9'
  3 = '10-17'
  4 = '18-29'
  5 = '30-39'
  6 = '40-49'
  7 = '50-64'
  8 = '65+';
value agetwo
  0 = '<18'
  1 = '18+';
value serodenv
  0 = 'DENV neg'
  1 = 'DENV1 pos'
  2 = 'DENV2 pos'
  3 = 'DENV3 pos'
  4 = 'DENV4 pos'
  5 = 'DENV1,DENV2 pos'
  6 = 'DENV1,DENV3 pos'
  7 = 'DENV1,DENV4 pos'
  8 = 'DENV2,DENV3 pos'
  9 = 'DENV2,DENV4 pos'
  10 = 'DENV3,DENV4 pos'
  11 = 'DENV1,DENV2,DENV3 pos'
  12 = 'DENV1,DENV3,DENV4 pos'
  13 = 'DENV2,DENV3,DENV4 pos';
value combdenv
  0 = 'DENV neg'
  1 = 'DENV1 pos, only'
  2 = 'DENV2 pos, only'
  3 = 'DENV3 pos, only'
  4 = 'DENV4 pos, only'
  5 = 'combined DENV';
value pos
  0 = 'negative'
  1 = 'positive';
value multipos
  0 = 'mono inf'
  1 = 'multi inf';
value yesno
  0 = 'no'
  1 = 'yes';
value sex
  0 = 'female'
  1 = 'male';
value denvpos
  -1 = 'missing'
  0 = 'negative'
  1 = 'positive';
value cities
  1 = 'OCAÑA'
  2 = 'PATIOS'
  3 = 'OTHER';
value response
  1 = 'missing data'
  2 = 'data available';
value singcomb
  0 = 'denv neg'
  1 = 'denv single positive'
  2 = 'denv multi positive';
value sympdays
  0 = '<4 days ill'
  1 = '4+ days';
value serology
  0 = 'missing'
  1 = 'IgM pos'
  2 = 'IgG pos'
  3 = 'IgM & IgG pos';
data disser;
set new.new_jen_data;
if denvpos = . then delete;
*proc contents position data=disser;
*run;
*endsas;
************************************
* change character to numeric
* reset . to blank
************************************;
if DAYSSX = .' then DAYSSX = ' ';
DAYSSX_n = DAYSSX*1;
************************************
* agecat variable
************************************;
if age lt 1 then agecat = 1;
if age ge 1 and age lt 10 then agecat = 2;
if age ge 10 and age lt 18 then agecat = 3;
if age ge 18 and age lt 30 then agecat = 4;
if age ge 30 and age lt 40 then agecat = 5;
if age ge 40 and age lt 50 then agecat = 6;
if age ge 50 and age lt 65 then agecat = 7;
if age ge 65 then agecat=8;
************************************
* dichotomous age
************************************;
if age 18 then agetwo = 0;
if age ge 18 then agetwo = 1;
************************************
* fix error in denv2
************************************;
if denv2 = 2 then denv2 = 1;
************************************
* serotype DENV
************************************;
serodenv = 0;
if denvpos = . then serodenv = .;
if denv1 = 1 then serodenv=1;
if denv2 = 1 then serodenv=2;
if denv3 = 1 then serodenv=3;
if denv4 = 1 then serodenv=4;
if denv1 = 1 and (denv2 = 1 or denv2 = 2) then serodenv=5;
if denv1 = 1 and denv3 = 1 then
serodenv=6;
if denv1 = 1 and denv4 = 1 then
serodenv=7;
if denv2 = 1 and denv3 = 1 then
serodenv=8;
if denv2 = 1 and denv4 = 1 then
serodenv=9;
if denv3 = 1 and denv4 = 1 then
serodenv=10;
if denv1 = 1 and denv2 = 1 and denv3 = 1
then serodenv=11;
if denv1 = 1 and denv3 = 1 and denv4 = 1
then serodenv=12;
if denv2 = 1 and denv3 = 1 and denv4 = 1
then serodenv=12;
************************************
* combdenv
************************************;
combdenv = 0;
if denvpos = . then combdenv=.;
if denv1 = 1 then combdenvv=1;
if denv2 = 1 or denv2 = 2 then
combdenv=2;
if denv3 = 1 then combdenv=3;
if denv4 = 1 then combdenv=4;
if serodenv ge 5 then combdenv=5;
************************************
* neg, single pos, comb pos
************************************;
if combdenv = 0 then sing_comb = 0;
if combdenv ge 1 and combdenv le 4 then
sing_comb = 1;
if combdenv eq 5 then sing_comb = 2;
************************************
* sex variable
************************************;
if sex = 'F' then sex = 0;
if sex = 'M' then sex = 1;
************************************
* fix denvpos and denv
************************************;
if denvpos = . then denvpos = -1;
if denv1 = . then denv1 = -1;
if denv2 = . then denv2 = -1;
if denv3 = . then denv3 = -1;
if denv4 = . then denv4 = -1;

* cities variable

if (city = 'ABREGO' or city = 'BOGOTA' or city = 'CHICANOTA' or city = 'COCHIRA' or city = 'CONVENCION' or city = 'CUCUTA' or city = 'EL CARMEN' or city = 'GONZALES' or city = 'GUAMALITO' or city = 'HACARI' or city = 'LA ESPERANZA' or city = 'LA PLAYA' or city = 'RIO CESAR' or city = 'SAN PABLO' or city = 'TARRA' or city = 'TEORAMA') then cities = 3;
if city = 'OCAÑA' then cities = 1;
if city = 'PATIO'S' then cities = 2;

* response rate information

if denvpos = -1 then sero_response = 1;
if denvpos ge 0 then sero_response = 2;
if admit = . then clin_response = 1;
if admit ge 0 then clin_response = 2;
if rbc = . then blood_response = 1;
if rbc ge 0 then blood_response = 2;

* fix gingbleed

if gingbleed = -1 then gingbleed = .;

* serology

serology = 0;
if igm = 1 then serology = 1;
if igg = 1 then serology = 2;
if igm = 1 and igg = 1 then serology = 3;

* admit

if admit = 2 then admit = 1;

set disser;
if denvpos = -1 then delete;
data no_miss_denv;
if admit = . then delete;
run;
data single;
set disser;
if combdenv = 5 then delete;
data positive;
set disser;
if denvpos = 0 then delete;

* single or multiple pos

multi_pos = 0;
if serodenv ge 5 then multi_pos = 1;

* symptom days

symp_days = 0;
if dayssx ge 4 then symp_days=1;
data complete;
set positive;
if vomit = . then delete;
run;
*/
proc contents position data=disser;
*/
proc freq data=disser;
tables serodenv;
tables agecat;
tables sex;
tables lepto;
tables serodenv;
tables combdenv;
tables serology;
tables agecat*denvpos;
tables sex*denvpos;
tables city;
tables city*denvpos;
tables serology*denvpos;
tables city*serodenv;
tables lepto*denvpos;
tables serodenv*denvpos;
tables combdenv*denvpos;
format serodenv serodenv. agecat agecat.
denvpos denvpos. sex sex.
serology serology. combdenv
combdenv.;
run;
proc means data=disser;
  var age;
run;
proc sort data=disser;
  by denvpos;
run;
proc means data=disser;
  class denvpos;
  var age;
  format denvpos denvpos.;
run;
proc freq data=complete;
  tables multi_pos;
run;
proc sort data=complete;
  by sex;
run;
proc freq data=complete;
  tables multi_pos;
run;
proc sort data=complete;
  by vomit;
run;
proc freq data=complete;
  tables vomit*multi_pos/all;
  format multi_pos multipos. vomit yesno.;
run;
/*
proc logistic data=positive;
  model multi_pos = vomit;
  format multi_pos multipos. vomit yesno.;
run;
*/
proc sort data=complete;
  by symp_days;
run;
proc freq data=complete;
  tables symp_days*multi_pos/all;
  format symp_days sympdays. multi_pos multipos.;
run;
proc sort data=complete;
  by myalgia;
run;
proc freq data=complete;
  tables myalgia*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by retropain;
run;
proc freq data=complete;
  tables retropain*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by jntpain;
run;
proc freq data=complete;
  tables jntpain*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by rash;
run;
proc freq data=complete;
  tables rash*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by abdpain;
run;
proc freq data=complete;
  tables abdpain*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by ha;
run;
proc freq data=complete;
  tables ha*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by larvae;
run;
proc freq data=complete;
  tables larvae*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by diarrhea;
run;
proc freq data=complete;
  tables diarrhea*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by diarrhea;
run;
proc freq data=complete;
  tables diarrhea*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by fever;
run;
proc freq data=complete;
  tables fever*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by gingbleed;
run;
proc freq data=complete;
  tables gingbleed*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by agetwo;
run;
proc freq data=complete;
  tables agetwo*multi_pos/all;
  format multi_pos multipos. agetwo agetwo.;
run;
proc sort data=complete;
  by icterus;
run;
proc freq data=complete;
  tables icterus*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by hyperemia;
run;
proc freq data=complete;
  tables hyperemia*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by OLIGUR;
proc freq data=complete;
  tables oligur*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by petech;
proc freq data=complete;
  tables petech*multi_pos/all;
  format multi_pos multipos.;
run;
by nosebleed;
proc freq data=complete;
    tables nosebleed*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by hypotn;
proc freq data=complete;
    tables hypotn*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by tachy;
proc freq data=complete;
    tables tachy*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by bednet;
proc freq data=complete;
    tables bednet*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by admit;
proc freq data=complete;
    tables admit*multi_pos/all;
    format multi_pos multipos.;
run;
endssas;
*******************************************
*  response information
*******************************************;
proc freq data=disser;
    tables sero_response clin_response
    blood_response;
    format sero_response clin_response
    blood_response response.;
run;
******************************************************************************
*  frequencies with complete data
******************************************************************************;
******************************************************************************
**
*  all data including missing
******************************************************************************;
**;
proc means data=disser;
    var DENVPOS DENV1 DENV2
    DENV3 DENV4 IGM IGG LEPTO rbc
    PLT
    HB HCT WBC NEUT EOS LYMPH
    MONO BASO DAYSSX_n;
    title 'all data including missing';
proc means data=no_miss_all;
    var DENVPOS DENV1 DENV2
    DENV3 DENV4 IGM IGG LEPTO PLT
    HB HCT WBC NEUT EOS LYMPH
    MONO DAYSSX_n;
    title 'complete data';
proc means data=no_miss_denv;
    var rbc baso plt;
    title 'data for rbc, baso, plt - missing
denvpos taken out only';
proc freq data=no_miss_all;
    tables sex city cities dayssx_n ADMIT
    DENVCLASS FEVER MYALGIA
    GINGBLEED
    VOMIt Icterus RETROPAIN
    JNTPAIN HYPEREMIA RASH OLIGUR
    PETECH DIARRHEA NOSEBLEED
    ABDPAIN HA
    Hypotn Tachy Bednet
    Larvae LarvLoc DENVPOS DENV1
    DENV2 DENV3 DENV4 agecat;
    format agecat agecat. denvpos denv1 denv2
denv3 denv4 denvpos. cities cities.;
    title 'complete data';
run;
/****
proc means data=disser;
proc freq data=disser;
tables sex city dayssx_n ADMIT DENVCLASS FEVER MYALGIA GINGBLEED VOMIT ICTERUS RETROPAIN JNTPAIN HYPEREMIA RASH OLIGUR PETECH DIARRHEA NOSEBLEED ABDPAIN HA HYPOTN TACHY BEDNET LARVAE LARVLOC DENVPOS DENV1 DENV2 DENV3 DENV4 agecat cities;
format agecat agecat. denvpos denv1 denv2 denv3 denv4 denvpos. cities cities.;
run;
*/
***************************************
*  frequencies according to denvpos including combined denv  
***************************************;
proc freq data=no_miss_all;
tables (sex cities)*denvpos/chisq;
format denvpos pos. cities cities.;
title 'complete data';
run;
proc sort data=no_miss_all;
by sex;
run;
proc freq data=no_miss_all;
tables (cities FEVER MYALGIA GINGBLEED VOMIT ICTERUS RETROPAIN JNTPAIN HYPEREMIA RASH OLIGUR PETECH DIARRHEA NOSEBLEED ABDPAIN HA HYPOTN TACHY BEDNET LARVAE)*denvpos/chisq;
format denvpos pos. cities cities.;
title 'complete data';
run;
proc sort data=no_miss_all;
by vomit;
run;
proc freq data=no_miss_all;
tables vomit*denvpos/all;
format denvpos pos. vomit yesno.;
title 'complete data';
run;
proc logistic data=no_miss_all;
model denvpos = sex;
format denvpos pos. sex sex.;
run;
proc sort data=no_miss_all;
by vomit;
run;
proc logistic data=no_miss_all;
model denvpos = vomit;
format denvpos pos. vomit yesno.;
run;
******************************************************************************
single, multiple denv positive
proc sort data=no_miss_all;
  by sing_comb;
run;
proc freq data=no_miss_all;
  tables (sex cities)*sing_comb/chisq;
  format sing_comb singcomb. cities cities.;
  title 'complete data - single and multiple DENV pos';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model age=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean age';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model hb=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean hb';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model hct=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean hct';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model wbc=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean wbc';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model neut=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean neut';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model eos=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean eos';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model lymph=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean lymph';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model mono=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
  format sing_comb singcomb.;
  title 'Unadjusted ANOVA (sing_comb), mean mono';
run;
proc sort data=no_miss_denv;
  by sing_comb;
run;
proc anova data=no_miss_denv;
   class sing_comb;
model rbc=sing_comb;
   means sing_comb;
   means sing_comb/lsd;
format sing_comb singcomb.;
title 'Unadjusted ANOVA (sing_comb),
mean rbc';
run;
proc anova data=no_miss_denv;
   class sing_comb;
model plt=sing_comb;
   means sing_comb;
   means sing_comb/lsd;
format sing_comb singcomb.;
title 'Unadjusted ANOVA (sing_comb),
mean platelet';
run;
proc anova data=no_miss_denv;
   class sing_comb;
model baso=sing_comb;
   means sing_comb;
   means sing_comb/lsd;
format sing_comb singcomb.;
title 'Unadjusted ANOVA (sing_comb),
mean baso';
run;
endsas;
/*
---------------------------------------------------------------------
* frequencies according to denvpos only
---------------------------------------------------------------------;
proc sort data=single;
   by denvpos;
run;
proc freq data=single;
   tables (sex city hood)*denvpos/chisq;
   format denvpos pos.;
title 'frequencies according to singular denvpos';
run;
proc freq data=single;
   tables (FEVER MYALGIA GINGBLEED
   VOMIt ICTERUS RETROPAIN
   JNTPAIN HYPEREMIA RASH OLIGUR
   PETECH DIARRHEA NOSEBLEED
   ABDPAIN HA
   HYPOTN TACHY BEDNET
   LARVAE)*denvpos/chisq;
   format denvpos pos. FEVER MYALGIA
   GINGBLEED
   VOMIt ICTERUS RETROPAIN
   JNTPAIN HYPEREMIA RASH OLIGUR
   PETECH DIARRHEA NOSEBLEED
   ABDPAIN HA
   HYPOTN TACHY BEDNET
   LARVAE yesno.;
title 'frequencies according to singular denvpos';
run;
endsas;
/*
---------------------------------------------------------------------
* frequencies according to combdenv
---------------------------------------------------------------------;
proc sort data=single;
   by combdenv;
run;
proc freq data=single;
   tables (sex city hood)*combdenv/chisq;
   format combdenv combdenv.;
title 'frequencies according to singular
denvpos';
run;
proc anova data=single;
   class combdenv;
model age=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean age';
run;
proc anova data=single;
   class combdenv;
model rbc=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean rbc';
run;
proc anova data=single;
   class combdenv;
model plt=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean plt';
run;
proc anova data=single;
   class combdenv;
model hb=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean hb';
run;
proc anova data=single;
   class combdenv;
model hct=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean hct';
run;
proc anova data=single;
   class combdenv;
model wbc=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean wbc';
run;
proc anova data=single;
   class combdenv;
model neut=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean neut';
run;
proc anova data=single;
   class combdenv;
model eos=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean eos';
run;
proc anova data=single;
   class combdenv;
model lymph=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean lymph';
run;
data=single;
class combdenv;
model mono=combdenv;
  means combdenv;
  means combdenv/l=sd;
format combdenv combdenv.;
title 'Unadjusted ANOVA (combdenv),
mean mono';
run;
proc anova data=single;
class combdenv;
model baso=combdenv;
  means combdenv;
  means combdenv/l=sd;
format combdenv combdenv.;
title 'Unadjusted ANOVA (combdenv),
mean baso';
run;
proc freq data=single;
tables (FEVER MYALGIA GINGBLEED
  VOMIt ICTERUS RETROPAIN
  JNTPAIN HYPEREMIA RASH OLGUR
  PETECH DIARRHEA NOSEBLEED
  ABDPAIN HA
  HYPOTN TACHY BEDNET
  LARVAE)*combdenv/chisq;
format combdenv combdenv. FEVER
MYALGIA GINGBLEED
  VOMIt ICTERUS RETROPAIN
  JNTPAIN HYPEREMIA RASH OLGUR
  PETECH DIARRHEA NOSEBLEED
  ABDPAIN HA
  HYPOTN TACHY BEDNET
LARVAE yesno.;
title 'frequencies according to singular
denvpos';
run;
endsas;
*/
*******************************************************************************
* frequencies according to individual denv1-
4 among positives only
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean hb, positives only';
run;
proc anova data=positive;
   class combdenv;
   model hct=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean hct, positives only';
run;
proc anova data=positive;
   class combdenv;
   model wbc=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean wbc, positives only';
run;
proc anova data=positive;
   class combdenv;
   model neut=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean neut, positives only';
run;
proc anova data=positive;
   class combdenv;
   model eos=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean eos, positives only';
run;
proc anova data=positive;
   class combdenv;
   model lymph=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean lymph, positives only';
run;
proc anova data=positive;
   class combdenv;
   model mono=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean mono, positives only';
run;
proc anova data=positive;
   class combdenv;
   model baso=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
   mean baso, positives only';
run;
proc freq data=positive;
   tables (FEVER MYALGIA GINGBLEED
VOMIt ICTERUS RETROPAIN
JNTPAIN HYPEREMIA RASH OLI
PETECH DIARRHEA NOSEBLEED
ABDPAIN HA
HYPOTN TACHY BEDNET
LARVAE)*combdenv/chisq;
   format combdenv combdenv. FEVER
MYALGIA GINGBLEED
VOMIt ICTERUS RETROPAIN
JNTPAIN HYPEREMIA RASH OLI
PETECH DIARRHEA NOSEBLEED
ABDPAIN HA
HYPOTN TACHY BEDNET
LARVAE) yesno.;
title 'frequencies according to singular"
run;
endsas;
proc freq data=disser;
  tables agecat denvpos denv2 denv3
denv4 serodenv combdenv;
  format agecat agecat. serodenv serodenv.
  combdenv combdenv.;
run;
proc freq data=disser;
  tables city*denvpos;
  tables sex*denvpos;
run;
proc sort data=disser;
  by denvpos;
run;
proc means;
  class denvpos;
  var age rbc plt hb hct wbc neut eos lymph
  mono baso;
run;
/*
proc freq data=disser;
  tables denv2*(denv1 denv3 denv4
  serodenv);
  format serodenv serodenv. combdenv
  combdenv.;
run;
*/
proc sort data=disser;
  by combdenv;
run;
proc means data=disser;
  class combdenv;
  var age rbc plt hb hct wbc neut eos lymph
  mono baso;
format combdenv combdenv.;
run;
proc freq data=disser;
  tables combdenv*sex;
  format combdenv combdenv.;
/*
proc univariate freq data=disser;
  var age;
*/
run;
endsas;
**********************************************************************;
options ls=80 ps=60 nocenter;
libname new 'c:\jennifer\data';
options FORMCHAR="|----|+|---+=|/-\<>*";
filename myprint 'c:\jennifer\prog\jen6.lst';
filename mylog 'c:\jennifer\prog\jen6.log';
proc printto print=myprint;
proc printto log=mylog;
proc format;
  value agecat
    1 = '<1'
    2 = '1-9'
    3 = '10-17'
    4 = '18-29'
    5 = '30-39'
    6 = '40-49'
    7 = '50-64'
    8 = '65+';
value agetwo
  0 = '<18'
  1 = '18+';
value serodenv
  0 = 'DENV neg'
  1 = 'DENV1 pos'
  2 = 'DENV2 pos'
  3 = 'DENV3 pos'
  4 = 'DENV4 pos'
  5 = 'DENV1,DENV2 pos'
  6 = 'DENV1,DENV3 pos'
  7 = 'DENV1,DENV4 pos'
  8 = 'DENV2,DENV3 pos'
  9 = 'DENV2,DENV4 pos'
 10 = 'DENV3,DENV4 pos'
11 = 'DENV1,DENV2,DENV3 pos'
12 = 'DENV1,DENV3,DENV4 pos'
13 = 'DENV2,DENV3,DENV4 pos';
value combdenv
  0 = 'DENV neg'
  1 = 'DENV1 pos, only'
  2 = 'DENV2 pos, only'
  3 = 'DENV3 pos, only'
  4 = 'DENV4 pos, only'
  5 = 'combined DENV';
value pos
  0 = 'negative'
  1 = 'positive';
value multipos
  0 = 'mono inf'
  1 = 'multi inf';
value yesno
  0 = 'no'
  1 = 'yes';
value sex
  0 = 'female'
  1 = 'male';
value denvpos
  -1 = 'missing'
  0 = 'negative'
  1 = 'positive';
value cities
  1 = 'OCAÑA'
  2 = 'PATIOS'
  3 = 'OTHER';
value response
  1 = 'missing data'
  2 = 'data available';
value singcomb
  0 = 'denv neg'
  1 = 'denv single positive'
  2 = 'denv multi positive';
value sympdays
  1 = '<4 days ill'
  2 = '4+ days';
value altage
  1 = '<1 yr'
  2 = '1 yr'
  3 = '2-11 yrs'
  4 = '12-18 yrs'
  5 = '19-44 yrs'
  6 = '45+ yrs';
value serology
  0 = 'missing'
  1 = 'IgM pos'
  2 = 'IgG pos'
  3 = 'IgM & IgG pos';
value highlow
  0 = 'high'
  1 = 'low';
value lowhigh
  0 = 'low'
  1 = 'high';
value symptoms
  0 = 'no symptoms'
  1 = '<4 days ill'
  2 = '4+ days';
data disser;
  set new.new_jen_data;
  if denvpos = . then delete;
  ************************************
  * change character to numeric
  * reset . to blank
  ************************************;
  if DAYSSX = '.' then DAYSSX = ' ';
  DAYSSX_n = DAYSSX*1;
  *******************************
  * agecat variable
  *******************************;
  if age lt 1 then agecat = 1;
  if age ge 1 and age lt 10 then agecat = 2;
  if age ge 10 and age lt 18 then agecat = 3;
  if age ge 18 and age lt 30 then agecat = 4;
  if age ge 30 and age lt 40 then agecat = 5;
  if age ge 40 and age lt 50 then agecat = 6;
  if age ge 50 and age lt 65 then agecat = 7;
  if age ge 65 then agecat=8;
  ****************************************
  * dichotomous age
  ****************************************;
if age lt 18 then agetwo = 0;
if age ge 18 then agetwo = 1;

******************************************
* altage
******************************************;
if age lt 1 then altage = 1;
if age ge 1 and age lt 2 then altage = 2;
if age ge 2 and age lt 12 then altage = 3;
if age ge 12 and age lt 19 then altage = 4;
if age ge 19 and age lt 45 then altage = 5;
if age ge 45 then altage = 6;

***********************************
* fix error in denv2
***********************************;
if denv2 = 2 then denv2 = 1;

**********************************
* serotype DENV
**********************************;
serodenv = 0;
if denvpos = . then serodenv = .;
if denv1 = 1 then serodenv=1;
if denv2 = 1 then serodenv=2;
if denv3 = 1 then serodenv=3;
if denv4 = 1 then serodenv=4;
if denv1 = 1 and (denv2 = 1 or denv2 = 2)
th en serodenv=5;
if denv1 = 1 and denv3 = 1 then
serodenv=6;
if denv1 = 1 and denv4 = 1 then
serodenv=7;
if denv2 = 1 and denv3 = 1 then
serodenv=8;
if denv2 = 1 and denv4 = 1 then
serodenv=9;
if denv3 = 1 and denv4 = 1 then
serodenv=10;
if denv1 = 1 and denv2 = 1 and denv3 = 1
then serodenv=11;
if denv1 = 1 and denv3 = 1 and denv4 = 1
then serodenv=12;

********************************************
* combdenv
********************************************;
combdenv = 0;
if denvpos = . then combdenv=.;
if denv1 = 1 then combdenv=1;
if denv2 = 1 or denv2 = 2 then
combdenv=2;
if denv3 = 1 then combdenv=3;
if denv4 = 1 then combdenv=4;
if serodenv ge 5 then combdenv=5;

***************************************
* neg, single pos, comb pos
***************************************;
if combdenv = 0 then sing_comb = 0;
if combdenv ge 1 and combdenv le 4 then
sing_comb = 1;
if combdenv eq 5 then sing_comb = 2;

**************************************
* sex variable
**************************************;
if sex = 'F' then sex = 0;
if sex = 'M' then sex = 1;

*************************************
* fix denvpos and denv
*************************************;
if denvpos = . then denvpos = -1;
if denv1 = . then denv1 = -1;
if denv2 = . then denv2 = -1;
if denv3 = . then denv3 = -1;
if denv4 = . then denv4 = -1;

*************************************
* cities variable
*************************************;
if (city = 'ABREGO' or city = 'BOGOTA' or
city = 'CHICANOTA' or
city = 'COCHIRA' or city =
'CONVENCION' or city = 'CUCUTA' or
city = 'EL CARMEN' or city =
'GONZALES' or city = 'GUAMALITO' or
city = 'HACARI' or city = 'LA
ESPERANZA' or city = 'LA PLAYA' or
city = 'RIO CESAR' or city = 'SAN PABLO' or city = 'TARRA' or city = 'TEORAMA') then cities = 3;
if city = 'OCANÃ' then cities = 1;
if city = 'PATIOS' then cities = 2;

* response rate information
if denvpos = -1 then sero_response = 1;
if denvpos ge 0 then sero_response = 2;
if admit = . then clin_response = 1;
if admit ge 0 then clin_response = 2;
if rbc = . then blood_response = 1;
if rbc ge 0 then blood_response = 2;

* fix gingbleed
if gingbleed = -1 then gingbleed = .;
if gingbleed = 2 then gingbleed = 1;

* serology
serology = 0;
if igm = 1 then serology = 1;
if igg = 1 then serology = 2;
if igm = 1 and igg = 1 then serology = 3;

* indicator variables for denv
if denv1 = 1 then denv_one = 1;
denv_two = 0;
if denv2 = 1 then denv_two = 1;
denv_three = 0;
if denv3 = 1 then denv_three = 1;
denv_four = 0;
if denv4 = 1 then denv_four = 1;
denv_comb = 0;
if serodenv ge 5 then denv_comb = 1;
sing_denv = 0;
if serodenv ge 1 and serodenv lt 5 then sing_denv = 1;
multi_denv = 0;
if serodenv ge 5 then multi_denv = 1;

* indicator variable for cities
city1 = 0;
if cities = 1 then city1 = 1;
city2 = 0;
if cities = 2 then city2 = 1;

* symptom days
if dayssx_n eq . then symp_days = 0;
if dayssx_n ge 0 and dayssx_n lt 6 then symp_days=1;
if dayssx_n ge 6 then symp_days=2;

* symptom days cut at the median = 3 days
if dayssx_n ge 0 and dayssx_n lt 4 then sympdays2 = 1;
if dayssx_n ge 4 then sympdays2 = 2;

* account for missing
sympdays3 = sympdays2;
if dayssx_n = . then sympdays3 = 0;

* indicator variables
symptom1 = 0;
symptom2 = 0;
if sympdays3 = 1 then symptom1 = 1;
symptom2 = 0;
if sympdays3 = 2 then symptom2 = 1;

***;
* note: rbc missing 126
if rbc lt 5.0 then rbc2 = 1;
if rbc ge 5.0 then rbc2 = 0;
* plt cut at 100 (approx median) with 0 = plt gt 100

if plt ge 0 and plt lt 100 then plt2 = 1;
if plt ge 100 then plt2 = 0;

* HB cut at 13 (approx median) with 0 = 13+

if hb ge 0 and hb lt 13 then hb2 = 1;
if hb ge 13 then hb2 = 0;

* hct cut at 40 (median) with 0 = hct ge 40

if hct ge 0 and hct lt 40.0 then hct2 = 1;
if hct ge 40.0 then hct2 = 0;

* wbc cut at 4.0 (median) with 0 = wbc ge 4.0

if wbc ge 0 and wbc lt 4.0 then wbc2 = 1;
if wbc ge 4.0 then wbc2 = 0;
if wbc ge 0 and wbc le 5.0 then wbc3 = 1;
if wbc gt 5.0 then wbc3 = 0;

* alternate designation of case status for wbc2

if wbc2 = 1 then wbc_alt = 0;
if wbc2 = 0 then wbc_alt = 1;

* neut cut at 55 (median) with 0 = neut ge 55

if neut ge 0 and neut lt 55 then neut2 = 1;
if neut ge 55 then neut2 = 0;

* note: EOS missing 56

* lymph cut at 40.0 (median) with 0 = lymph ge 40.0

if lymph ge 0 and lymph lt 40.0 then lymph2 = 1;
if lymph ge 40.0 then lymph2 = 0;

* note: mono missing 115

* note: baso missing 126

* subset data to principal components data set

* continuous variables clinical plus age

data principal;
set disser;
keep dayssx_n plt hb hct wbc neut lymph age;
run;

* signs and symptoms for combdenv

tables myalgia gingbleed vomit icterus retropain jntpain hyperemia rash oligur petech diarrhea nosebleed abdpain ha hypotn tachy;
format myalgia gingbleed vomit icterus retropain jntpain hyperemia rash oligur petech diarrhea nosebleed abdpain ha hypotn tachy yesno.;
run;
proc freq data=disser;
tables (myalgia gingbleed vomit icterus retropain jntpain hyperemia rash oligur petech diarrhea nosebleed abdpain ha hypotn tachy);
format myalgia gingbleed vomit icterus retropain jntpain hyperemia rash oligur petech diarrhea nosebleed abdpain ha hypotn tachy yesno.;
run;
proc freq data=disser;
tables (myalgia gingbleed vomit icterus retropain jntpain hyperemia rash oligur petech diarrhea nosebleed abdpain ha hypotn tachy);
format myalgia gingbleed vomit icterus retropain jntpain hyperemia rash oligur petech diarrhea nosebleed abdpain ha hypotn tachy yesno.;
run;
tachy)*combdenv;
format combdenv combdenv. myalgia
gingbleed vomit icterus retropain jnptpain
hyperemia
    rash oligur petech diarrhea nosebleed
abdpain ha hypotn
tachy yesno.;
run;
endsas;
********************************************
*  means of clinical variables by denv pos or
neg
********************************************;
proc sort data=disser;
    by denvpos;
run;
proc means data=disser;
    var dayssx_n plt hb hct wbc neut lymph age
    rbc;
run;
proc ttest data=disser;
    class denvpos;
    var dayssx_n plt hb hct wbc neut lymph age
    rbc;
    format denvpos denvpos.;
run;
proc sort data=disser;
    by combdenv;
run;
proc anova data=disser;
    class combdenv;
    model hct = combdenv;
    means combdenv;
    means combdenv/lsd;
    format combdenv combdenv.;
run;
proc anova data=disser;
    class combdenv;
    model wbc = combdenv;
    means combdenv;
    means combdenv/lsd;
    format combdenv combdenv.;
run;
proc anova data=disser;
    class combdenv;
    model neut = combdenv;
    means combdenv;
    means combdenv/lsd;
    format combdenv combdenv.;
run;
proc anova data=disser;
    class combdenv;
    model lymph = combdenv;
    means combdenv;
    means combdenv/lsd;
    format combdenv combdenv.;
run;
proc anova data=disser;
    class combdenv;
    model wbc = combdenv;
    means combdenv;
    means combdenv/lsd;
    format combdenv combdenv.;
run;
proc freq data=disser;
    tables combdenv*wbc_alt;
    format combdenv combdenv. wbc_alt
    lowhigh.;
run;
proc freq data=disser;
    tables agetwo*wbc_alt;
    tables sex*wbc_alt;
    tables symp_days*wbc_alt;
    tables sympdays3*wbc_alt;
    tables cities*wbc_alt;
format wbc_alt lowhigh. agetwo agetwo. sex
sex. cities cities.
sympdays3 symptoms.;
proc sort data=disser;
by combdenv;
proc anova data=disser;
class combdenv;
model wbc = combdenv;
means combdenv;
means combdenv/lsd;
format combdenv combdenv.;
run;
proc sort data=disser;
by sympdays3;
run;
proc anova data=disser;
class sympdays3;
model wbc = sympdays3;
means sympdays3;
means sympdays3/lsd;
format sympdays3 symptoms.;
run;
proc logistic data=disser;
model wbc_alt(event='low') = denv_one
denv_two denv_three denv_four denv_comb;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. sympdays2 sympdays.
title 'crude odds ratio for sympdays covariate';
run;
proc logistic data=disser;
model wbc_alt(event='low') = agetwo;
format wbc_alt lowhigh. agetwo agetwo.
denv_two denv_three denv_four denv_comb
pos. agetwo agetwo.;
title 'crude odds ratio for age covariate';
run;
proc logistic data=disser;
model wbc_alt(event='low') = sex;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. sex sex.;
title 'crude odds ratio for sex covariate';
run;
proc logistic data=disser;
model wbc_alt(event='low') = symp_days;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. sympdays2 sympdays.
title 'crude odds ratio for sympdays covariate';
run;
proc logistic data=disser;
model wbc_alt(event='low') = symptom1
symptom2;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. symp_days sympdays.
title 'crude odds ratio for sympdays covariate';
run;
proc logistic data=disser;
model wbc_alt(event='low') = city1 city2;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos.;
title 'crude odds ratio for cities covariate';
run;
proc logistic data=disser;
model wbc_alt(event='low') = denv_one
denv_two denv_three denv_four denv_comb
age two;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. agetwo agetwo.;
title 'model of wbc and denv adjust for age';
run;
proc logistic data=disser;
model wbc_alt(event='low') = denv_one
denv_two denv_three denv_four denv_comb
sex;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. sex sex.;
title 'model of wbc and denv adjust for
sex';
run;
proc logistic data=disser;
model wbc_alt(event='low') = denv_one
denv_two denv_three denv_four denv_comb
symp_days;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos.;
title 'model of wbc and denv adjust for
symptom days';
run;
proc logistic data=disser;
model wbc_alt(event='low') = denv_one
denv_two denv_three denv_four denv_comb
symptom1 symptom2;
format wbc_alt lowhigh. denv_one
denv_two denv_three denv_four denv_comb
pos. symp2days sympdays.;
title 'model of wbc and denv adjust for
symptom days';
run;
******************************************************************************
*  principal components analysis on
continuous data
******************************************************************************;
proc corr data=principal;
title 'principal components analysis';
run;
proc princomp data=principal;
run;
proc factor data=principal simple corr;
run;
******************************************************************************
*  analysis of IgM, IGG, IgM and IgG by
symptomology
******************************************************************************;
*  check distribution of clinical variables
******************************************************************************;
/*
proc univariate freq data=disser;
var RBC PLT HB HCT WBC NEUT EOS
LYMPH MONO BASO;
run;
proc univariate freq data=disser;
var hb;
run;
*/
proc sort data=disser;
by denvpos;
proc means data=disser;
class denvpos;
var dayssx_n;
format denvpos denvpos.;
title 'analysis of IgM, IGG, IgM and IgG by
days symptom and denvpos';
run;
*/
/*
proc sort data=disser;
  by denvpos igm;
proc means data=disser;
  by igm;
  class denvpos;
  var dayssx_n;
  format denvpos denvpos. igm pos.;
run;
proc sort data=disser;
  by denvpos igg;
proc means data=disser;
  by igg;
  class denvpos;
  var dayssx_n;
  format denvpos denvpos. igg pos.;
run;
*/
/*
proc sort data=disser;
  by denvpos serology;
run;
proc freq data=disser;
  tables denvpos serology;
format denvpos denvpos. serology serology.;
proc freq data=disser;
  tables denvpos serology;
format denvpos denvpos. serology serology.;
proc means data=disser;
  by denvpos;
  class serology;
  var dayssx_n;
  format denvpos denvpos. serology serology.;
run;
proc anova data=disser;
  by denvpos;
  class serology;
  model dayssx_n = serology;
  means serology;
  means serology/lsd;
  format denvpos denvpos. serology serology.;
run;
*/

* tables plt2 hb2 hct2 wbc2 neut2 lymph2
proc freq data=disser;
  tables combdenv*plt2;
  tables combdenv*hb2;
  tables combdenv*hct2;
  tables combdenv*wbc2;
  tables sing_comb*wbc2;
  tables combdenv*wbc3;
  tables combdenv*wbc_alt;
  tables combdenv*neut2;
  tables combdenv*lymph2;
  format combdenv combdenv. sing_comb singcomb. plt2 hb2 hct2 wbc2 neut2 lymph2 highlow. wbc_alt highlow.;
run;

* logistic regression
proc logistic data=disser;
  model plt2(event='low') = denv_one
denv_two denv_three denv_four
denv_comb;
  format plt2 highlow. denv_one denv_two
denv_three denv_four denv_comb pos.;
  title 'model of plt and denv';
run;
proc logistic data=disser;
  model hb2(event='low') = denv_one
denv_two denv_three denv_four
denv_comb;
  format hb2 highlow. denv_one denv_two
denv_three denv_four denv_comb pos.;
  title 'model of hb and denv';
run;
proc logistic data=disser;
  model hct2(event='low') = denv_one
denv_two denv_three denv_four
denv_comb;
  format hct2 highlow. denv_one denv_two
denv_three denv_four denv_comb pos.;
  title 'model of hct and denv';
run;
format hct2 highlow. denv_one denv_two denv_three denv_four denv_comb pos.;
  title 'model of hct and denv';
run;
proc logistic data=disser;
  model neut2(event='low') = denv_one denv_two denv_three denv_four denv_comb;
  format neut2 highlow. denv_one denv_two denv_three denv_four denv_comb pos.;
  title 'model of neut and denv';
run;
proc logistic data=disser;
  model lymph2(event='low') = denv_one denv_two denv_three denv_four denv_comb;
  format lymph2 highlow. denv_one denv_two denv_three denv_four denv_comb pos.;
  title 'model of lymph and denv';
run;
endzas;
proc logistic data=disser;
  model wbc2(event='high') = denv_one denv_two denv_three denv_four denv_comb;
  format wbc2 highlow. denv_one denv_two denv_three denv_four denv_comb pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model wbc3 = denv_one denv_two denv_three denv_four denv_comb;
  format wbc3 highlow. denv_one denv_two denv_three denv_four denv_comb pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model wbc_alt(event='low') = denv_one denv_two denv_three denv_four denv_comb;
  format wbc_alt lowhigh. denv_one denv_two denv_three denv_four denv_comb pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model wbc2 = sing_denv multi_denv;
  format wbc2 highlow. denv_one denv_two denv_three denv_four denv_comb sing_denv multi_denv pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model plt2 = sing_denv multi_denv;
  format plt2 highlow. denv_one denv_two denv_three denv_four denv_comb sing_denv multi_denv pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model hb2 = sing_denv multi_denv;
  format hb2 highlow. denv_one denv_two denv_three denv_four denv_comb sing_denv multi_denv pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model hct2 = sing_denv multi_denv;
  format hct2 highlow. denv_one denv_two denv_three denv_four denv_comb sing_denv multi_denv pos.;
  title 'model of wbc and denv';
run;
proc logistic data=disser;
  model neut2 = sing_denv multi_denv;
  format neut2 highlow. denv_one denv_two denv_three denv_four denv_comb sing_denv multi_denv pos.;
  title 'model of wbc and denv';
run;
format wbc2 highlow. denv_one denv_two
denv_three denv_four denv_comb
    sing_denv multi_denv pos.;
title 'model of wbc and denv';
run;
proc logistic data=disser;
model neut2 = denv_one denv_two
denv_three denv_four denv_comb;
    format neut2 highlow. denv_one denv_two
denv_three denv_four denv_comb pos.;
title 'model of neut and denv';
run;
proc logistic data=disser;
model lymph2 = denv_one denv_two
denv_three denv_four denv_comb;
    format lymph2 highlow. denv_one
denv_two denv_three denv_four denv_comb
    pos.;
title 'model of lymph and denv';
run;
endsas;
*************************************************
*   construct logistic regression models
*************************************************;

data no_miss_denv;
    set disser;
    if denvpos = -1 then delete;

data no_miss_all;
    set no_miss_denv;
    if admit = . then delete;

data single;
    set disser;
    if combdenv = 5 then delete;

data positive;
    set disser;
    if denvpos = 0 then delete;

*************************************************
*   single or multiple pos
*************************************************;
multi_pos = 0;
    if serodenv ge 5 then multi_pos = 1;

***************************************************************************
*   symptom days
***************************************************************************;
    symp_days = 0;
    if dayssx ge 4 then symp_days=1;

data complete;
    set positive;
    if vomit = . then delete;
run;
proc univariate freq data=positive;
    var age;
    title 'for denv positive only';
run;
proc freq data=positive;
    tables agecat altage;
    format agecat agecat. altage altage.;
run;
proc means data=positive;
    var age;
run;
proc freq data=positive;
    tables multi_pos;
    format multi_pos multipos.;
run;
proc freq data=positive;
    tables multi_pos*serodenv;
    format multi_pos multipos. serodenv
    serodenv.;
run;
endsas;

proc contents position data=disser;
*/
proc freq data=disser;
    tables serodenv;
    tables agecat;
    tables sex;
    tables agecat*denvpos;
tables city;
tables city*denvpos;
tables city*serodenv;
  format serodenv serodenv. agecat agecat.
  denvpos denvpos. sex sex.;
run;
proc freq data=complete;
  tables multi_pos;
run;
proc sort data=complete;
  by sex;
run;
proc freq data=complete;
  tables sex*multi_pos/all fisher;
  format sex sex. multi_pos multipos.;
run;
/**
  proc logistic data=positive;
    model multi_pos = sex;
    format multi_pos multipos. sex sex.;
run;
  */
proc sort data=complete;
  by vomit;
run;
proc freq data=complete;
  tables vomit*multi_pos/all;
  format multi_pos multipos. vomit yesno.;
run;
/**
  proc logistic data=positive;
    model multi_pos = vomit;
    format multi_pos multipos. vomit yesno.;
run;
  */
proc sort data=complete;
  by symp_days;
run;
proc freq data=complete;
  tables symp_days*multi_pos/all;
  format symp_days sympdays. multi_pos multipos;
run;
proc sort data=complete;
  by myalgia;
run;
proc freq data=complete;
  tables myalgia*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by retropain;
run;
proc freq data=complete;
  tables retropain*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by jntpain;
run;
proc freq data=complete;
  tables jntpain*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by rash;
run;
proc freq data=complete;
  tables rash*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by abdpain;
run;
proc freq data=complete;
  tables abdpain*multi_pos/all;
  format multi_pos multipos.;
run;
proc sort data=complete;
  by ha;
run;
proc freq data=complete;
  tables ha*multi_pos/all;
  format multi_pos multipos.;
proc sort data=complete;
    by larvae;
run;
proc freq data=complete;
    tables larvae*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by diarrhea;
run;
proc freq data=complete;
    tables diarrhea*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by diarrhea;
run;
proc freq data=complete;
    tables diarrhea*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by diarrhea;
run;
proc freq data=complete;
    tables diarrhea*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by fever;
run;
proc freq data=complete;
    tables fever*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by fever;
run;
proc freq data=complete;
    tables fever*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by hypotn;
run;
proc freq data=complete;
    tables hypotn*multi_pos/all;
    format multi_pos multipos.;
run;
proc sort data=complete;
    by tachy;
run;
proc freq data=complete;
tables tachy*multi_pos/all;
format multi_pos multi_pos.;
run;
proc sort data=complete;
by bednet;
proc freq data=complete;
tables bednet*multi_pos/all;
format multi_pos multi_pos.;
run;
endsas;
 *******************************************
*  response information
 *******************************************;
proc freq data=disser;
tables sero_response clin_response blood_response;
tables sero_response*clin_response;
tables sero_response*blood_response;
tables blood_response*clin_response;
format sero_response clin_response blood_response response.;
run;
 ********************************************
*  frequencies with complete data
 ********************************************;
 ********************************************
*  all data including missing
 ********************************************;
proc means data=disser;
var age DENVPOS DENV1 DENV2 DENV3 DENV4 IGM IGG LEPTO rbc PLT HB HCT WBC NEUT EOS LYMPH MONO BASO DAYSSX_n;
title 'all data including missing';
proc means data=no_miss_all;
var age DENVPOS DENV1 DENV2 DENV3 DENV4 IGM IGG LEPTO PLT HB HCT WBC NEUT EOS LYMPH MONO BASO DAYSSX_n;
title 'all data including missing';
proc means data=disser;
var age DENVPOS DENV1 DENV2 DENV3 DENV4 IGM IGG LEPTO rbc PLT HB HCT WBC NEUT EOS LYMPH MONO BASO DAYSSX_n;
title 'all data including missing';
 proc means data=no_miss_all;
var age DENVPOS DENV1 DENV2 DENV3 DENV4 IGM IGG LEPTO PLT HB HCT WBC NEUT EOS LYMPH MONO BASO DAYSSX_n;
title 'all data including missing';
 proc means data=disser;
var age DENVPOS DENV1 DENV2 DENV3 DENV4 IGM IGG LEPTO rbc PLT HB HCT WBC NEUT EOS LYMPH MONO BASO DAYSSX_n;
title 'all data including missing';
 proc means data=no_miss_all;
 var rbc basal plt;
by denvpos;
run;
proc freq data=no_miss_all;
tables (sex cities)*denvpos/chisq;
format denvpos pos. cities cities.;
title 'complete data';
run;
proc ttest data=no_miss_all;
class denvpos;
var age HB HCT WBC NEUT EOS
LYMPH MONO;
format denvpos pos.;
title 'complete data';
run;
proc sort data=no_miss_denv;
by denvpos;
proc ttest data=no_miss_denv;
class denvpos;
var rbc plt baso;
format denvpos pos.;
title 'data for rbc, baso, and plt - missing
denvpos taken out';
run;
proc freq data=no_miss_all;
tables (cities FEVER MYALGIA
GINGBLEED VOMIt ICTERUS
RETROPAIN JNTPAIN HYPEREMIA
RASH OLIGUR PETECH DIARRHEA
NOSEBLEED ABDPAIN HA
HYPTN TACHY BEDNET
LARVAE)*denvpos/chisq;
format denvpos pos. cities cities.;
title 'complete data - single and multiple
DENV pos';
run;
proc sort data=no_miss_all;
by vomit;
proc freq data=no_miss_all;
tables vomit*denvpos/all;
format denvpos pos. vomit yesno.;
run;
proc logistic data=no_miss_all;
model denvpos = vomit;
format denvpos pos. vomit yesno.;
run;

******************************************************************************
* single, multiple denv positive
******************************************************************************;
proc sort data=no_miss_all;
by sing_comb;
proc freq data=no_miss_all;
tables (sex cities)*sing_comb/chisq;
format sing_comb singcomb. cities cities.;
title 'complete data - single and multiple
DENV pos';
run;
proc anova data=no_miss_all;
class sing_comb;
model age=sing_comb;
means sing_comb;
means sing_comb/lsd;
format sing_comb singcomb.;
title 'Unadjusted ANOVA (sing_comb),
mean age';
run;
proc anova data=no_miss_all;
class sing_comb;
model hb=sing_comb;
means sing_comb;
means sing_comb/lsd;
format sing_comb singcomb.;
title 'Unadjusted ANOVA (sing_comb),
mean hb';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model hct=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean hct';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model wbc=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean wbc';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model neut=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean neut';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model eos=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean eos';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model lymph=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean lymph';
run;
proc anova data=no_miss_all;
  class sing_comb;
  model mono=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean mono';
run;
proc sort data=no_miss_denv;
  by sing_comb;
run;
proc anova data=no_miss_denv;
  class sing_comb;
  model rbc=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean rbc';
run;
proc anova data=no_miss_denv;
  class sing_comb;
  model plt=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean platelet';
run;
proc anova data=no_miss_denv;
  class sing_comb;
  model baso=sing_comb;
    means sing_comb;
    means sing_comb/lsd;
format sing_comb singcomb ;
  title 'Unadjusted ANOVA (sing_comb),
  mean baso';
run;
endsas;
/*
******************************************************************************
* frequencies according to denvpos only
singular
******************************************************************************;
proc sort data=single;
   by denvpos;
run;
proc freq data=single;
   tables (sex city hood)*denvpos/chisq;
   format denvpos pos.;
   title 'frequencies according to singular
denvpos';
run;
proc ttest data=single;
   class denvpos;
   var age RBC PLT HB HCT WBC NEUT
   EOS LYMPH MONO MONO BASO;
   format denvpos pos.;
   title 'ttest by covariates according to
singular denvpos';
run;
proc freq data=single;
   tables (FEVER MYALGIA GINGBLEED
   VOMIt ICTERUS RETROPAIN
   JNTPAIN HYPEREMIA RASH OLGUR
   PETECH DIARRHEA NOSEBLEED
   ABDPAIN HA HYPOTN TACHY
   BEDNET LARVAE)*denvpos/chisq;
   format denvpos pos. FEVER MYALGIA
   GINGBLEED VOMIt ICTERUS
   RETROPAIN JNTPAIN HYPEREMIA
   RASH OLGUR PETECH DIARRHEA
   NOSEBLEED ABDPAIN HA
   HYPOTN TACHY BEDNET LARVAE
   yesno.;
   title 'frequencies according to singular
denvpos';
run;
endsas;
*/
/*
******************************************************************************
* frequencies according to combdenv
singular individual denv1-4
******************************************************************************;
proc sort data=single;
   by combdenv;
run;
proc freq data=single;
   tables (sex city hood)*combdenv/chisq;
   format combdenv combdenv.;
   title 'frequencies according to singular
denvpos';
run;
proc anova data=single;
   class combdenv;
   model age=combdenv;
   means combdenv;
   means combdenv/lsd;
   format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean age';
run;
proc anova data=single;
   class combdenv;
   model rbc=combdenv;
   means combdenv;
   means combdenv/lsd;
   format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean rbc';
run;
proc anova data=single;
   class combdenv;
   model plt=combdenv;
   means combdenv;
   means combdenv/lsd;
   format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean plt';
run;
proc anova data=single;
   class combdenv;
   model hb=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean hb';
run;
proc anova data=single;
   class combdenv;
   model hct=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean hct';
run;
proc anova data=single;
   class combdenv;
   model wbc=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean wbc';
run;
proc anova data=single;
   class combdenv;
   model neut=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean neut';
run;
proc anova data=single;
   class combdenv;
   model eos=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean eos';
run;
proc anova data=single;
   class combdenv;
   model lymph=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean lymph';
run;
proc anova data=single;
   class combdenv;
   model mono=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean mono';
run;
proc anova data=single;
   class combdenv;
   model baso=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
      mean baso';
run;
proc freq data=single;
   tables (FEVER MYALGIA GINGBLEED VOMiIt ICTERUS RETROPAIN JNTPAIN HYPEREMIA RASH OLIGUR PETECH DIARRHEA NOSEBLEED ABDPAIN HA HYPOTN TACHY BEDNET LARVAE)*combdenv/chisq;
   format combdenv combdenv. FEVER MYALGIA GINGBLEED VOMiIt ICTERUS RETROPAIN JNTPAIN HYPEREMIA RASH OLIGUR PETECH DIARRHEA NOSEBLEED ABDPAIN HA
HYPOTN TACHY BEDNET LARVAE
yesno.;
title 'frequencies according to singular
denypos';
run;
endsas;
*/
******************************************************************************
* frequencies according to individual denv1-4 among positives only
******************************************************************************;
proc sort data=positive;
   by combdenv;
run;
proc freq data=positive;
   tables (sex city hood)*combdenv/chisq;
   format combdenv combdenv.;
   title 'frequencies according to singular
denypos, positives only';
run;
proc anova data=positive;
   class combdenv;
   model age=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean age, positives only';
run;
proc anova data=positive;
   class combdenv;
   model rbc=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean rbc, positives only';
run;
proc anova data=positive;
   class combdenv;
   model plt=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean plt, positives only';
run;
proc anova data=positive;
   class combdenv;
   model hb=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean hb, positives only';
run;
proc anova data=positive;
   class combdenv;
   model hct=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean hct, positives only';
run;
proc anova data=positive;
   class combdenv;
   model wbc=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean wbc, positives only';
run;
proc anova data=positive;
   class combdenv;
   model neut=combdenv;
   means combdenv;
   means combdenv/lsd;
format combdenv combdenv.;
   title 'Unadjusted ANOVA (combdenv),
mean neut, positives only';
run;
proc anova data=positive;
class combdenv;
model eos=combdenv;
  means combdenv;
  means combdenv/lsd;
format combdenv combdenv.;
title 'Unadjusted ANOVA (combdenv), mean eos, positives only';
run;
proc anova data=positive;
  class combdenv;
  model lymph=combdenv;
  means combdenv;
  means combdenv/lsd;
format combdenv combdenv.;
title 'Unadjusted ANOVA (combdenv), mean lymph, positives only';
run;
proc anova data=positive;
  class combdenv;
  model mono=combdenv;
  means combdenv;
  means combdenv/lsd;
format combdenv combdenv.;
title 'Unadjusted ANOVA (combdenv), mean mono, positives only';
run;
proc anova data=positive;
  class combdenv;
  model baso=combdenv;
  means combdenv;
  means combdenv/lsd;
format combdenv combdenv.;
title 'Unadjusted ANOVA (combdenv), mean baso, positives only';
run;
proc freq data=positive;
  tables (FEVER MYALGIA GINGBLEED VOMIt ICTERUS RETROPAIN JNTPAIN HYPEREMIA RASH OLIGUR PETECH DIARRHEA NOSEBLEED ABDPAIN HA HYPOTN TACHY BEDNET LARVAE)
yesno.;
title 'frequencies according to singular denvpos, positives only';
run;
endsas;
proc freq data=disser;
  tables agecat denvpos denv1 denv2 denv3 denv4 serodenv combdenv;
  format agecat agecat. serodenv serodenv. combdenv combdenv.;
run;
proc freq data=disser;
  tables city*denvpos;
  tables sex*denvpos;
run;
proc sort data=disser;
  by denvpos;
run;
proc means;
  class denvpos;
  var age rbc plt hb hct wbc neut eos lymph mono baso;
run;
proc freq data=disser;
  tables denvpos*(serodenv combdenv);
  format serodenv serodenv. combdenv combdenv combdenv.;
run;
/*
proc freq data=disser;
  tables denv2*(denv1 denv3 denv4 serodenv);
  format serodenv serodenv.;
run;
*/
proc sort data=disser;
by combdenv;
run;
proc means data=disser;
   class combdenv;
   var age rbc plt hb hct wbc neut eos lymph mono baso;
format combdenv combdenv.;
run;
proc freq data=disser;
   tables combdenv*sex;
format combdenv combdenv.;
/*
proc univariate freq data=disser;
   var age;
*/
run;
endsas;
<p>| ID | Key | Gender | Age | Municipality | DENV | DENV1 | DENV2 | DENV3 | DENV4 | IGM | IGG | LEPTO | DATEDRAW | ERYTH | PLT | HB | HCT | LEU | NEUT | EOS | LYMPH | MONO | BASO | DPO | ADMIT | MYALGIA | GINGBLEED | VOMIT | ICTERUS | RETROPAIN | HYPEREMIA | RASH | OLIGUR | PETECH | DIARRHEA | NOSEBLEED | ABDPAIN | HA | HYPOTN | TACHY |
|----|-----|--------|-----|--------------|------|-------|-------|-------|-------|-----|-----|-------|-----------|-------|-----|----|-----|-----|-----|-----|------|------|-----|-----|-------|------------|--------|-------|--------|--------|------|--------|------|------|-------|--------|----------|--------|-----|-------|------|------|----------|--------|-----|-------|------|
| 1  | M  | 18    | OCAÑA | 0  | 0  | 0  | 0  | 0  | 0  | 11/06/13 | 4.2 | 137  | 12.5  | 37.9  | 8.8  | 91.9 | 1.1 | 4.3 | 1.8 | 0.9  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  | .  |</p>
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APPENDIX E
EBOLA VIRUS DISEASE STUDY IRB APPROVAL

ACTION ON PROTOCOL CONTINUATION REQUEST

TO: Christopher Mores
Pathobiological Sciences

FROM: Dennis Landin
Chair, Institutional Review Board

DATE: June 10, 2016

RE: IRB# 3610

TITLE: Operations and research infrastructure augmentation plan for increased support to stop the transmission of the Ebola virus in Port Loko and improve public health response to disease future threats

New Protocol/Modification/Continuation: Continuation

Review type: Full ___ Expedited X ___ Review date: 6/10/2016

Risk Factor: Minimal _____ X _____ Uncertain _________ Greater Than Minimal _________

Approved _____ X _____ Disapproved _________

Approval Date: 6/10/2016  Approval Expiration Date: 6/9/2017

Re-review frequency: (annual unless otherwise stated)

Number of subjects approved: 200

LSU Proposal Number (if applicable): 43128

Protocol Matches Scope of Work in Grant proposal: (if applicable) ___

By: Dennis Landin, Chairman

150
APPENDIX K
POST-EBOLA VIRUS DISEASE STUDY CONSENT FORM

POST-EBOLA SYNDROME: INVESTIGATING THE CLINICAL PROFILE OF PAIN AND DISABILITY AMONG SURVIVORS OF EBOLA VIRUS DISEASE IN BOMI COUNTY, LIBERIA

Principal Investigator: Christopher Mores
Co-investigator: Jennifer Giovanni

Description: You are a survivor of Ebola virus disease. Since you left the ETU, you have told us that you have pain, tiredness, and sadness. In February, I examined your body and asked you questions about your health. Now I ask your permission to do another physical exam and to ask you more questions about your health. The purpose of the physical exam and the questions is to learn where your body hurts, how your life has changed, and how nurses and doctors can learn about post-Ebola syndrome in people who survived Ebola.

Procedures: I will look at parts of your body, including your hands and feet, your neck, and your muscles. I will press on them to ask if they hurt. I will touch parts of your skin and ask you if you feel pain. I will ask you to move your arms and to walk. I will also take your blood pressure and ask you questions about how you feel. I will ask you if it is easy or hard to farm, to cook, and to walk in your village. I will ask how you got Ebola.

The examination will not go inside your body. I will not take your blood.

Risks and benefits: There is no risk to your body or health by being a part of this study. There is no benefit to you or your body by being part of this study. There is no treatment for Ebola or the pains people have when they survive Ebola. You will not get any treatment by being part of this study. The information I get from you will help nurses and doctors understand why survivors of Ebola have pain and tiredness. This will help us understand how to take care of people who survive Ebola in the future.

Privacy: You name will not be given to any person. The name of your village will not be given to any person. I know your name and village, and I will keep it private for just me. Information about how you got Ebola, your treatment, and the pain you have will be published. I will not give any information that will tell people who you are.

Time: This exam and the questions will take about 1 hour.

Payment: You will not get money for being in this study.

If you have questions: You can call me, Jennifer, on my Liberia mobile. The number is +231 88 618 7306. When I am in America, you can call me on my American mobile. The number in America is +001 _______ ________ ________

Participant: I am signing this form because the study was told to me in a language I understand. I know that I will be asked questions about my body and when I had Ebola. I do not have to be in this study. If I want to
be in this study, I will have a copy of this paper. I know there is no treatment for Ebola. I know my name and village name will not be given to anyone.

**Photos (“snaps”):** I may ask to take snaps of your exam. I will not take pictures of your face. If the snaps are used in the research, your name and face will not be in the snap.

By putting your name or mark below:

- It is okay for snaps of me to be taken and published. _________________________
- It is NOT okay for snaps of me to be taken and published. _________________________

______________________________________________________________________________
Your Name or Mark  Your Date of Birth or Age

______________________________________________________________________________
Date of Exam  Your Mobile Number

______________________________________________________________________________
Witness Printed Name or Mark

______________________________________________________________________________
Signature of Nurse Doing Your Exam  Liberian Nursing License Number
Cross-checking notes from survivor interviews with retained prescription medications from pharmacy in Monrovia.

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APPENDIX F
HISTORY AND PHYSICAL EXAMINATION, HUSBAND, LIBERIA, 2015

General appearance is that of a well-appearing, 40 year-old, muscular African male. Posture is erect; appears thin for height/weight. Demeanor, facial expressions appropriate to clinical setting. Maintains eye contact. Intellect congruent w/ educational level. A&O x 3. Immediate, recent, remote memory intact.

PMI: (1) 2009 admission for blood transfusion s/t malaria, (2) 2003 surgery for repair of LLL bisection of tibialis anterior during the civil war.

MEDICATIONS: None.

ALLERGIES: Cotrimoxazole; reports acute, intense dermal itching.

FAMILY HISTORY: Older brother with diabetes. Mother has chronic lower back pain. No h/o HTN or vascular disease.


HEAD AND NECK: General appearance is normal cephalic shape proportional to body. Hair closely shaven. Facial features symmetrical, appropriately placed. No pain reported upon palpation of skull. Lymph nodes, thyroid not palpated; no masses, swelling observed.

EYES: Eyelids are smooth, lashes appropriately placed. PERRLA. Conjunctivae pale w/o exudates; sclerae moist, white with intermittent brown coloration. Lacrimal ducts moist without tearing. Cornea smooth, transparent w/ symmetric light reflex. Six cardinal positions of gaze (CN III, IV, VI) intact w/ smooth movement. No nystagmus. Fundoscopic exam not performed.

Peripheral fields not equal. Right eye has normal confrontation, peripheral visual fields, and distal acuity. Left eye has decreased confrontation, marked distal acuity deficit with inability to discern the number of fingers presented. Left peripheral deficits: inferior and superior ≈ 45° from transverse plane, nasal ≈ 30° from sagittal plane, and temporal ≈ 60° from sagittal plane.

ENT

Ears: Pinnae symmetrical, appropriately placed on head. No pain upon palpation. Sensation equal bilaterally. Internal exam not performed.

Nose: No discharge, obstruction. Septum is midline. Nares symmetrical, clear, patent. Internal exam, smelling test for CN I not performed.

Mouth/pharynx: Lips dry, brown, symmetrical. Mucosa moist, pink, w/o lesions. Teeth present, clean; gums well-attached to dentitia. No pain with mastication. Swallow normal. Internal exam not performed.


154
CARDIOVASCULAR: RRR. No gallop, rub, or murmur.

ABDOMEN: Integument smooth, intact w/o lesions, color congruent w/ rest of body; normal hair distribution. Soft, flat, bowel sounds audible x 4 quadrants. Nontender to light, deep palpation. BS, liver not examined.

MUSCULOSKELETAL: Posture is erect and stable in sitting, standing, walking positions. Vertebral column straight w/o visible lordosis, kyposis. Head appears midline between shoulders. Muscle mass, tone equal bilaterally. Joints are defined w/o edema. Patient has full ROM of bilateral knees but demonstrates pain at extremes of flexion/extension. ROM of shoulders limited by pain at extension above shoulder level. Grip, UE strength strong, equal bilaterally; however, arthralgia of wrist, elbow, and shoulder joints made assessment difficult. Patient has extreme sensitivity to palpation over the lumbo-sacral area; retracts from slight pressure. From L3-L5, pain ascends the vertebral column to T7-T9. Patient indicates severe pain from C1-C7 with limited ROM of head in all four directions. Pain is concentrated over the splenius capitus and occipitalis muscles with a visible pain response upon palpation. For emphasis, the patient experiences relief from pain only when all the joints are in their most neutral positions without motion. Patient denies myalgias, although the muscle fibers in immediate proximity to the joints are mildly painful. In fact, it is the arthralgias that fatigue him, not muscular fatigue. All joints, including the fingers and toes, are painful, limiting the ability to perform DALYs, especially farming. When discussing his pain, the patient becomes visibly saddened.

NEUROLOGIC

Mental status: The patient is alert, attentive, and oriented. Speech is clear and fluent with good repetition, comprehension, and recall.

Motor: Patient moves all extremities. Coordination, accuracy of movement equal bilaterally, upper, lower. Gait even w/ alternating arm movement. No loss of balance. No tremor, abnormal or extraneous movements. Gait is steady with normal step, base, arm swing, and turning. Heel and toe walking are normal.

Reflexes: Not examined.

Sensory: Grossly normal to light touch, pressure. Senses are intact in fingers and toes. No neuropathy or paraesthesia.

Cranial Nerves

CN II: Visual fields are full to confrontation.

CN III, IV, VI: At primary gaze, there is no eye deviation. When the patient is looking to the left, the right eye does not adduct. When the patient is looking up, the right eye does not move up as well as the left. She develops horizontal diplopia in all directions of gaze especially when looking to the left. Convergence is impaired.

CN V: Corneal responses intact bilaterally. Facial sensations intact on right side. Tactile sensation diminished over the three branches.

CN VII: Face is symmetric with normal eye closure and smile.

CN VII: Hearing is normal with to whispering. Answers questions appropriately.

CN IX, X: Not examined.

CN XI: Head turning intact.

CN XII: Not examined.

GENITOURINARY: Not examined. Patient states he occasionally awakens with an erection of height and firmness equal to pre-EVD status. Sexual intercourse not attempted discharge from ETU. Rectal/prostate exam deferred.
VITA

Jennifer Giovanni is an infectious disease epidemiologist whose career has incorporated bench research, clinical practice, public health policy, and global health field work. She has worked globally across academic, federal, military, and non-governmental milieus. She holds a Master of Public Health in infectious disease epidemiology from Emory University and a Master of Science in critical care and public health nursing from the University of California, Los Angeles. She served as a US Peace Corps volunteer and a commissioned officer with the US Public Health Service and the US Air Force.