EMERGING INFECTIOUS DISEASES WITH LIMITED TREATMENT OPTIONS: THE CASE OF EBOLA HEMORRHAGIC FEVER IN UGANDA AND SHIGA TOXIN

PRODUCING ESCHERICHIA COLI IN THE UNITED STATES

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Title

Emerging Infectious Diseases With Limited Treatment Options: The Case Of Ebola

Hemorrhagic Fever In Uganda And Shiga Toxin-producing Escherichia Coli in the United States

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ABSTRACT

Emerging infectious diseases are diseases that newly emerge in a population or change the frequency or spatial distribution of their occurrence. Ebola Hemorrhagic Fever (EHF) and Shiga Toxin-producing *Escherichia coli* (STEC) infections are among diseases that emerged in the 1970s. The two diseases have limited treatment options with no vaccines. This paper is based on two case studies. The first case study utilized data from the 2007/2008 EHF outbreak in Uganda and investigated the epidemiological and clinical aspects of the outbreak. The second case study was based on a study done on STEC isolates collected from beef cattle at the North Dakota State University Research Extension Center in Dickinson. The study investigated the prevalence of the common pathogenic STEC serotypes. The driving factors for the emergence of EHF and STEC, their prevention and control strategies and their challenges were discussed based on the case studies.

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ABSTRACTii
ACKNOWLEDGEMENTS i
LIST OF TABLES
LIST OF FIGURES
LIST OF ABBREVIATIONSit
1. INTRODUCTION
2. OBJECTIVES
3. LITERATURE REVIEW
3.1. Ebola Hemorrhagic Fever (EHF)
3.2. Shiga Toxin-producing <i>Escherichia coli</i> (STEC)
4. METHODOLOGY 12
4.1. Methodology for objective 1 1.
4.2. Materials and methods for objective 2 1.
4.3. Materials and methods for objective 3 1:
4.4. Methodology for objective 41
5. RESULT AND DISCUSSION
5.1. The driving factors for the emergence of infectious diseases
5.2. Epidemiological and clinical aspects of the 2007-2008 EHF outbreak in Budibugyo, western Uganda
5.3. Prevalence of STEC O45, O145, O157, O111, O103, O113, O121 and O26 serotypes in beef cattle at NDSU research extension center, Dickinson

TABLE OF CONTENTS

5.4.	Prevention and control of emerging infectious diseases	
6. CC	ONCLUSIONS	44
6.1.	Conclusion for objective 1	44
6.2.	Conclusion for objective 2	44
6.3.	Conclusion for objective 3	45
6.4.	Conclusion for objective 4	
7. SU	JMMARY	
8. RE	EFERENCES	49
APPEN HEMO	NDIX. CASE DEFINITIONS USED DURING THE 2007/2008 EBOLA DRRHAGIC FEVER OUTBREAK IN BUNDIBUGYO, UGANDA	69

LIST OF TABLES

<u>Tabl</u>	le	Page
1.	Socio-demographic characteristics of EHF cases in Bundibugyo, Uganda, August 2007-January 2008	25
2.	Clinical presentations of EHF cases in Bundibugyo, Uganda, August 2007- January 2008	26
3.	Result of bivariate analysis of the risk factors associated with acquisition of EHF in Bundibugyo, Uganda August 2007– January 2008	27
4.	Results of final logistic regression model for the associations between patient survival, clinical presentations and age groups in Bundibugyo, Uganda August 2007 January 2008	7 – 27

LIST OF FIGURES

Fig	ure	Page
1.	Epidemic curve for the EHF outbreak in Bundibugyo, Uganda, August 2007- January 2008	23
2.	The spatial distribution of EHF outbreak by sub-county in Bundibugyo, Uganda, August 2007-January 2008	24

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
BSE	Bovine Spongiform Encephalopathy
BSL	Biosafety Level
CDC	Centers for Disease Control and Prevention
CFR	Case Fatality Rate
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of the Congo
EHF	Ebola Hemorrhagic Fever
FAO	Food and Agricultural Organization
FATUS	Foreign Agricultural Trade of the United States
GP	Glycoprotein
НС	Hemorrhagic Colitis
HUS	Hemolytic Uremic Syndrome
IgG	Immunoglobulin G
IHR	International Health Regulation
IDSR	Integrated Disease Surveillance and Response

NDSU	North Dakota State University
NIAID	National Institute of Allergy and Infectious Diseases.
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
SARS	Severe Acute Respiratory Syndrome
STEC	Shiga Toxin-Producing Escherichia coli
USDA	United States Department of Agriculture
US GAO	United States Government Accountability Office
VTEC	Verocytotoxin-producing Escherichia coli
WHO	World Health Organization

1. INTRODUCTION

Emerging infectious diseases are diseases that newly emerge in a population or change the frequency or the spatial distribution of their occurrence (Woolhouse, 2002). Emerging infectious diseases include diseases caused by viruses, bacteria, parasites and fungi (Guerrant and Blackwood., 1999). From the 1407 pathogen species that affect humans, 13% were classified as emerging and re-emerging pathogens (Woolhouse and Gowtage-Sequeria., 2005). Among the emerging and re-emerging pathogen species, 37% are RNA viruses. Most emerging and reemerging pathogens have multiple host species (Woolhouse and Gowtage-Sequeria., 2005), and 75% of them are zoonoses (Taylor et al., 2001).

The occurrence of emerging infectious diseases is not random; rather, there are different factors that contribute to the emergence of infectious diseases including ecological factors, change in human behavior, international travel, change in food production system, globalization of trade, microbial evolution and the interface between animals and humans (Morse, 1995; Wobeser, 2002). Acquired Immune Deficiency Syndrome (AIDS), Ebola Hemorrhagic Fever (EHF), Rift Valley Fever, *E. coli* O157:H7 and Bovine Spongiform Encephalopathy (BSE) are examples of emerging infectious diseases (Morse, 1995).

EHF is a highly contagious and fatal zoonotic disease emerged in 1976 (WHO, 1978b). According to the National Institute of Allergy and Infectious Diseases (NIAID), Ebola virus is a category A priority pathogen that has high death rate, causes social anxiety and can be easily transmitted (NIAID, 2012). Often, the virus is introduced to human population when people contact infected primates and duikers (Leroy et al., 2004). Despite the studies that have been conducted, currently, there is no approved treatment or vaccine for EHF (CDC, 2010).

Verotoxin-producing *E. coli* were discovered in 1977 (Konowalchuk et al., 1977). They were named Shiga Toxin-Producing *E. coli* (STEC) by O'Brein et al. (1982). STEC are category B pathogens that can be spread slightly and cause low death rate (NIAID, 2012). The reservoirs of the bacteria are ruminants (Oporto et al., 2008). STEC is usually transmitted through contaminated food and water (Tauxe, 1997). Like EHF, STEC has limited treatment options. There is evidence to suggest that, using antibiotics to treat children with STEC infection increases the risk of developing Hemolytic Uremic Syndrome (HUS) (Wong et al., 2000), a syndrome characterized by hemolytic anemia, low platelet count and renal injury (CDC, 2012a). In addition, *E. coli* O157 has shown antibiotic resistance (Schoeder et al., 2002). Therefore, prevention is the most important public health intervention for both EHF and STEC.

The purpose of this paper was to discuss the driving factors associated with emergence of infectious diseases, the prevention and control strategies as well as the challenges in managing emerging infectious diseases using the two case studies conducted on EHF in Uganda and STEC in the United States (US). The former study was done to understand the epidemiological and clinical aspects of the 2007/2008 EHF outbreak in Bundibugyo, Uganda while the study on STEC used multiplex Polymerase Chain Reaction (PCR) to determine the prevalence of the common pathogenic STEC in *E. coli* samples collected from cattle in North Dakota, US.

2. OBJECTIVES

- 1. To discuss the driving factors of emerging infectious diseases.
- To investigate the epidemiological and clinical aspects of the 2007/2008 EHF outbreak in Uganda.
- To investigate the prevalence of the common pathogenic STEC serotypes in beef cattle at North Dakota State University Research Extension Center.
- 4. To discuss the general approaches to prevention and control of EHF and STEC and their challenges.

3. LITERATURE REVIEW

3.1. Ebola Hemorrhagic Fever (EHF)

3.1.1. Overview of EHF

EHF is an emerging zoonosis that was discovered in 1976 in the Democratic Republic of Congo (DRC) around the river "Ebola" after which the disease is named (Leroy et al., 2004; WHO, 1978b). The disease is caused by a single stranded RNA virus in the family *Filoviridae* (Klenk and Feldman, 2004). As summarized by the U.S. Centers for Disease Control and Prevention (CDC, 2010), Ebola virus has five sub-types; Ebola Zaire (DRC), Ebola Sudan (Sudan), Ebola Ivory Coast (Ivory Coast), Ebola Bundibugyo (Uganda), and Ebola Reston (Reston), all named after places where they were first documented. The first four sub-types cause hemorrhagic fever in humans while the last subtype causes disease in nonhuman primates (CDC, 2010). The natural reservoir of EHF is not known with certainty but it is suspected to be some species of fruit bats (Leroy et al., 2005).

EHF is one of the most deadly hemorrhagic fevers with case fatality rate (CFR) ranging from 34% to 88% (Wamala et al., 2010; WHO, 1978b). EHF is transmitted from person to person through contact with blood, tissue, body fluids, and secretions of infected persons. It is also transmitted through contaminated needles and fomites. Direct contact with bats, infected primates and duikers is thought to be the common way of transmission for index cases of outbreaks (Francesconi et al., 2003). The incubation period of the disease varies from 2 to 21 days. Clinical manifestations include sudden onset of fever, weakness, headache, vomiting, diarrhea, internal and external bleeding. Currently, there is no treatment or vaccine for EHF (CDC, 2010).

3.1.2. Natural history of EHF

Ebola virus is in the family *Filoviridae* that includes Ebola and Marburg viruses. The virus has single stranded negative sense RNA genome. The replication of the virus is thought to be similar to other negative sense RNA viruses but it is not fully understood (Klenk and Feldman, 2004). The virus causes outbreaks irregularly, which affects humans, non-human primates and duikers. However, until today the way the virus resides in the environment between the outbreaks is unknown. Some studies implied that some species of fruit bats are the reservoir but it was not possible to isolate the virus from the bats (Leroy et al., 2005). The way by which the virus transmits from the reservoir to humans or to primates is also unstated. Nevertheless, it was observed that contact with infected primates transmits the virus to humans and an outbreak in primates is usually followed by an outbreak in humans (Pourrut et al., 2005; Nkoghe et al., 2005). The two major ways of transmission of EHF from person to person are through direct contact with sick or dead persons or the body fluids and through iatrogenic transmission. Air borne transmission has not been documented (Baron et al., 1983).

The virus infects cells of the endothelium, kidney, liver, spleen and lymph nodes. In cell culture, the virus was found to infect every cell except lymphocytes. After infecting the cells, the virus replicates at a high rate (Klenk and Feldman, 2004; Sullivian et al., 2003). As summarized by Sullivan and colleagues, when Ebola virus infects monocytes and macrophages, it makes them release proinflammatory cytokines like TNF α (Tumor Necrosis Factor alpha) which increases the inflammatory response and results in increased permeability of endothelial cells. In addition, the virus infects the endothelial cells and causes cell death. The combination of the two factors leads to bleeding, decrease in blood volume and shock. The inflammatory response causes fever which is the cardinal sign of EHF (Sullivian et al., 2003). After the pathological

sequel, the outcome of the infection depends on the immune response, the level of monocytes and macrophages damage and the level of circulating antibody (Baize et al., 2002). Once survived during the first two weeks period, the patient recovers slowly. It was shown that patients who recovered from EHF had detectable level of antibodies for few years (Ksiazek et al., 1999). After recovery, patients suffer from long-term health problems such as weight loss, weakness and psychological problems (Wendo, 2001).

3.1.3. Epidemiology of EHF in humans

It has been about 36 years since EHF was first recognized in the DRC in 1976 (WHO, 1978b). According to the CDC report, there was no reported outbreak of EHF in Africa from 1979 until 1994, when it reemerged in Gabon. Since 1994, EHF outbreaks were reported more frequently encompassing new geographical areas and new subtypes of the virus (CDC, 2010). EHF outbreaks have some patterns of occurrence in terms of weather conditions, with most outbreaks occurring during the rainy and short dry season (Monath, 1999) and lasting for months (Lamunu et al., 2004).

According to CDC, outbreaks of EHF have occurred about 19 times in Eastern, Central and Western Africa. Democratic Republic of Congo has had five outbreaks of EHF. Gabon and Uganda have had four outbreaks each and Sudan and Republic of Congo have had three outbreaks each ranging from a single case to hundreds of cases. A single case of EHF was reported in Ivory Coast and imported cases were reported in other countries (CDC, 2010).

In Uganda, the first EHF outbreak occurred in 2000 with 425 cases and 224 deaths (CFR 53%) in districts of Mbarara, Gulu and Masindi. The outbreak was caused by Ebola Sudan. It lasted for about 18 weeks and the index case was unknown (Lamunu et al., 2004). The second outbreak occurred in Bundibugyo district with 192 suspected cases and 39 deaths (CFR 34%). A

new subtype of Ebola virus (Ebola Bundibugyo virus) caused the outbreak (Wamala et al., 2010). The third outbreak that involved a single case occurred in May 2011 in Luwero district, Central Uganda. A 12 year old girl presented with acute febrile illness and hemorrhagic manifestations. She was diagnosed with Ebola hemorrhagic fever of Sudan sub-type (WHO, 2011). The fourth outbreak started in July 2012 in Kibale district and 16 deaths were reported (WHO, 2012)

Based on data from previous outbreaks, ecological and geographical distribution of Filoviruses was mapped. It was found that EHF occurs along humid tropical rain forests in Central, Western and Eastern Africa (Peterson et al., 2004, Monath, 1999). The common subtype in outbreaks was Ebola Zaire followed by Ebola Sudan, with Bundibugyo and Ivory Coast Ebola viruses reported only once. Cases of Ebola Reston have been detected in Philippines, U.S. and Italy (Acheson, 2007). As summarized by Pourrut and his colleagues, the distribution of Ebola virus subtypes have some geographical patterns in that Ebola Sudan commonly occurs in East Africa while Ebola Zaire occurs in Central Africa (Pourrut et al., 2005).

The 1976 EHF outbreak affected every age groups from infants to elderly people with higher number of cases in young adults between 15 and 45 years of age (WHO, 1976b). Similarly, in the 1995 outbreak in the DRC, the mean age of the patients was 37 years with age range of 2 months to 71 years. Therefore, EHF affects every age group. Regarding the mortality, Khan and colleagues observed a significant difference in the mean age of survivors and the dead, with dead patients being older (Khan et al., 1999). However, the disease is also very fatal in children under five years of age (Mupere et al., 2001).

Even though, Ebola affects both genders, women aged 15-29 were the most affected group in the 1976 EHF outbreak in DRC, which was associated with the injections they received

at a hospital (WHO, 1978b). Generally, women were more affected than men in most of the outbreaks (WHO, 1978b; Okware et al., 2002; Khan et al., 1999).

3.1.4. Occurrence of EHF in animals

Most EHF outbreaks in humans were associated with death of primates and duikers. During EHF outbreaks in humans and great apes in Gabon and the Republic of Congo, small vertebrates and birds were collected in effort to identify the Ebola virus reservoir. Only fruit bats were found to have asymptomatic infection but isolation of the virus from the bats was not possible (Leroy et al., 2005). Allela and others investigated dogs living around villages where humans EHF outbreaks occurred; dogs were tested for Ebola antibodies and 31.8% of the dogs were found to be positive implying that dogs can be infected with Ebola virus (Allela et al., 2005). Therefore, Ebola virus possibly has multiple hosts.

3.1.5. Virulence of Ebola viruses

Subtypes of Ebola virus have different levels of virulence. Ebola Zaire subtype is associated with higher death rate (88%) compared to Ebola Sudan which had a death rate of 53% in 1976 (WHO, 1978a; WHO, 1978b). McCormick and his colleagues compared the cytopathic effect, infectious dose and extent of pathologic lesions of Ebola Zaire with Ebola Sudan and showed that Ebola Zaire was more virulent than Ebola Sudan (McCormick et al., 1983). In another study, Ebola Zaire was found to be more virulent than Ebola Bundibugyo in terms of viral replication and tissue damage (Gupta et al., 2010). Therefore, Ebola Zaire is the most virulent subtype of Ebola virus.

The initial development of vaccines for EHF is underway with more expected efforts (Phoolcharoena et al., 2001; Geisbert et al., 2002). In addition, an antiviral drug Cyanovirin N is being investigated and has been found to decrease the progress of the disease in mice by binding

to an envelope glycoprotein (GP) which the Ebola virus uses for viral entry in to the host cell (Barrientos et al., 2003). However, there is no approved treatment or vaccine for EHF at this time (CDC, 2010).

3.2. Shiga Toxin-producing Escherichia coli (STEC)

3.2.1. Overview of STEC

Escherichia coli are gram-negative bacteria in the family *Enterobacteriaceae*. They are part of the normal flora of the large intestine in humans and cattle. They are harmless bacteria unless they get some genetic materials that make them become pathogenic and virulent (Baron, 1996). Verotoxin producing *E. coli* were discovered in 1977 (Konowalchuk et al., 1977). In 1982, O'Brien et al. published a paper that established how some strains of *E. coli* produced shiga-like toxins or toxins similar to the one produced by *Shigella dysenteriae* type 1. Shiga Toxin-producing *E. coli* (STEC) or Verocytotoxin-producing *E. coli* (VTEC) are *E. coli* strains that produce shiga-like toxins (O'Brien et al., 1982). STEC produce two immunologically different toxins: shiga toxin 1 and shiga toxin 2 which are important for their pathogenesis. The shiga toxins and the genes that encode for the shiga toxins help in laboratory diagnosis of STEC (Acheson and Keusch, 1996).

The reservoirs of STEC are ruminants (Oporto et al., 2008). The most common vehicle for STEC is contaminated food and water. Therefore, STEC infections have been categorized as emerging food borne zoonoses (Tauxe, 1997). The pathogen can also be transmitted through contact with infected animals, feces of infected persons or contaminated environment (Brooks et al., 2005). The pathogen has a low infectious dose of less than 100 cells and can survive the acidic environment in the stomach (Tilden, 1996; Paton and Paton, 1998).

As reviewed by Paton and Paton (1998), there is a huge variation in the mechanism by which STEC attaches to the intestinal wall and in the acid tolerance of the bacteria. Moreover, there is variation in the amount of shiga toxin they produce (Marques et al., 1986). Those factors affect the pathogenicity and virulence of the bacteria. Therefore, the diseases caused by STEC range from mild diarrhea to severe disease such as HUS (Johnson et al., 2006). Shiga toxins are often associated with HUS and Hemorrhagic Colitis (HC) (Acheson and Keusch, 1996).

STEC infections have limited treatment options; treating STEC infections with some groups of antibiotics activates the genes that encode for shiga toxins thereby increasing toxin production and the risk of developing HUS (Kimmitt et al., 2000; Johnson et al., 2006). Wong et al., (2000) also found a strong association between use of antibiotics and HUS. Therefore, use of antibiotics is not recommended in the treatment of STEC infections.

3.2.2. Epidemiology of STEC infections in humans

In terms of seasonality, STEC commonly occurs in summer (Besser et al., 1999). The seasonality of the disease agrees with the pattern of shedding of the bacteria; STEC are shed more commonly in hot months than cold months (Chapman et al, 1997). Since cattle are the reservoir for STEC, studies have found an association between cattle population and the incidence of STEC infections in humans. The incidence of STEC infection in humans was higher in areas with high cattle population and in areas where manure was used for agricultural practices (Frank et al., 2008). Similarly, other studies showed that the incidence is higher in rural areas where people have frequent contact with cattle (Michel et al., 1999).

Mead and his colleagues estimated that about 110,220 cases of STEC infections occur each year in the US (Mead et al., 1999). A study done in Nebraska also showed that 1.2% of stool samples collected from patients with gastroenteritis were positive for STEC (Fey et al. 2000). Moreover, STEC are causing frequent food borne outbreaks. STEC infections are also the leading cause of HUS in the U.S. (Neill et al, 1987). According to CDC foodborne outbreak online database, there were four confirmed STEC outbreaks in North Dakota from 1998 to 2009. Three of the outbreaks were caused by *E. coli* O157 while one was caused by *E. coli* O111 (CDC, 2012e). Therefore, STEC infections are significant public health problem in the U.S.

3.2.3. Epidemiology of STEC in cattle

The prevalence of STEC in feedlot cattle had been reported to be as high as 92% (Renter et al., 2004). The prevalence depends on factors such as housing, cattle density, feed of the cattle and the geographical regions (Synge et al., 2003; Paton and Paton, 1998). A study done to examine the prevalence of STEC in seven domestic animals observed that sheep, goats and cattle were the common domestic animal reservoirs of STEC (Beutin et al, 1993). Among the animals, the prevalence in sheep was the highest, followed by goats and cattle. The other domestic animals such as dogs, pigs and cats showed low prevalence of STEC. Chickens were negative for STEC. The study also indicated that 60% of the isolated bacteria were human pathogens (Beutin et al, 1993). In addition, a study has found that deer was a reservoir of STEC (Keene et al., 1997). Therefore, STEC infects multiple hosts.

Due to the high prevalence of STEC in food animals, STEC is a common food contaminant in foods of animal origin. Samadpour et al. (1994) tested meat, poultry and seafood samples for shiga-like toxin genes and reported that 17% of the food samples were positive for shiga toxins. Due to the occurrence of STEC in meat and its impact on public health and food safety, USDA Inspection Service (FSIS) tests ground beef for *E. coli* O26, O45, O103, O111, O121, O157 and O145 (USDA, 2012b).

3.2.4. Virulence factors and serotypes of STEC

STEC differ from other groups of *E. coli* by the genes they possess. Those genes are stx1 and stx2 genes which encode for the toxins that are important in their pathogenesis. *E. coli* groups that have those genes produce toxins that result in severe clinical diseases such as HUS and HC (DebRoy and Maddox, 2001; Andrade et al., 2012). In addition, there are other virulence factors involved in the pathogenesis of STEC; one of which is the protein intimin which is responsible for the intimate attachment of the bacteria to intestinal mucosa. Intimin causes effacing lesions in the intestinal mucosa and is encoded by a gene called *aeaA* (Blanco et al., 2003). Nonpathogenic *E. coli* do not have those genes that enable pathogenic *E. coli* to cause disease.

There are about 200 STEC serotypes grouped as *E. coli* O157:H7 and non-O157 *E. coli*. Non-O157 *E. coli* include a variety of serotypes mainly O26, O45, O103, O111, O121, and O145 (Blanco et al., 2003). In addition to *E. coli* O157, *E. coli* O26, O45, O103, O111, O121, and O145 are commonly isolated from patients with STEC infections (CDC, 2012b; Brooks et al., 2005).

4. METHODOLOGY

4.1. Methodology for objective 1

For the first objective, information was obtained through Google scholar and PubMed search engine using the key phrases emerging infectious diseases, driving factors for emerging infectious diseases, factors contributing to emergence of infectious diseases, emergence of EHF and emergence of STEC. Articles published by different peer-reviewed journals, governmental websites and publications of health service organizations published between 1980 and 2012 were used as sources of information.

4.2. Materials and methods for objective 2

4.2.1. Data source

Epidemiological data collected by the Ugandan Ministry of Health (MOH) during the 2007- 2008 EHF outbreak were reviewed. A team of health workers who were providing service to EHF patients collected the data from November 29, 2007 to February 20, 2008 following the case definition set by the epidemic response team (Appendix 1). Overall, 192 individuals were identified as suspected cases of EHF. Later, 42 (22%) were classified as confirmed cases (laboratory test positive), 74 (38.5%) were probable cases (no laboratory test done) and 76 (39.5%) were non- cases (laboratory test negative). Detailed information on data collection and laboratory investigations used was published earlier (Wamala et al., 2010).

4.2.2. Assumptions made on the data

The case categories assigned by the outbreak investigation team (Wamala et al., 2010) were used for this study. Confirmed cases (positive laboratory test result) and probable cases (those with typical clinical presentations but no laboratory test was done) were considered as cases of EHF. The descriptive part of the results section of the study was based on probable and confirmed cases.

For the analysis of the risk factors, individuals who tested negative for EHF were categorized as non-cases and were used as the control groups. Confirmed and probable cases were categorized as cases of EHF, due to the similarity in their case fatality rates and clinical presentations. The analysis on the determinants of patient prognosis was completed by comparing the clinical presentation and age of the cases that survived to those that succumbed among the confirmed and probable case categories.

4.2.3. Data analysis

4.2.3.1. Descriptive epidemiology

An epidemic curve was generated using Epi info software version 3.5.3 to show the distribution of the cases over time. The recorded date of onset of fever was displayed on the X-axis and the number of cases on the Y-axis. The epidemic curve was plotted for probable and confirmed cases of EHF. Spatial mapping for distribution of cases by sub-county was done using Geographic Information System (GIS) mapping software GCS WGS. Age, gender, occupation, clinical presentations and outcome of the illness were among the variables used to describe cases by person using Epi info software version 3.5.3.

4.2.3.2. Chi square analysis

For the analysis of the risk factors for acquiring EHF, the category of the cases was used as dependent variable. Probable and confirmed cases were compared against the non-case (control group). Independent variables included attending funerals, having contact with suspected cases, visiting hospitals within the past three weeks and history of travel. For the analysis of the associations between clinical presentations, age of cases and prognosis of the patients; outcome of the disease (survival or death) was used as dependent variable and the clinical features and age were used as independent variables. Cases with missing values on the clinical signs were excluded from the analysis. The chi square analysis was done using SAS 9.2.

4.2.3.3. Logistic regression

In addition to Chi Square analysis, logistic regression was applied to determine the risk factors associated with acquiring the disease and the significant factors associated with prognosis of the cases. Forward selection procedure as described by Hosmer and Lemeshow (2000) was used for the analysis. The data were analyzed using SAS 9.2 system with a P value of < 0.05 as benchmark for significance.

4.2.4. Limitation of the case study

The limitations of this study might be from the data used. Some information in the data were incomplete and the quality of the data might be poor as a secondary data. In addition, information for some of the patients who died before the confirmation of the outbreak was obtained from relatives and family members, which could cause information bias.

4.3. Materials and methods for objective 3

4.3.1. Samples

The STEC samples used in this study were isolated from an earlier study that examined fecal samples and rectal swabs collected from feedlot cattle at North Dakota State University (NDSU) research extension center at Dickinson. Although these cultures have at least one of the shiga toxin genes, their serotype was unknown. Those STEC had been frozen and stored at (-80^oC) in the department of Veterinary and microbiological Sciences at NDSU. These samples were inoculated on Tryptic Soy Agar (TSA) media and incubated at 37^oC for 24 hours.

4.3.2. DNA extraction and template preparation from *E. coli* isolates

Single Cell Lysing Buffer (SCLB) protocol was used to extract DNA for the PCR, as done by Marmur (1961). The SCLB master mix consisted of 10 μ l of 20 mg/ml proteinase K (Amresco) and 990 μ l of 10 mM Tris HCL and 1mM EDTA (TE) buffer solution. A single isolated colony was suspended in 40 μ l of SCLB buffer in a 0.2 microcentrifuge tube. The lysis procedure consisted of lysing the cells at 80°C for 10 minutes and then cooling the cells to 55°C for 10 minutes in a thermo cycler. Following this, 80 μ l of sterile double distilled water was added to the suspension and centrifuged for 30 seconds at 4500 x g. The samples were stored at -20°C until further analysis could be completed.

4.3.3. Polymerase Chain Reaction (PCR) Assay

Multiplex PCR was used to serotype the isolates using *wzx* (O antigen flippase) genes as described by DebRoy et al. (2011). The primers used for each bacterium were taken from DebRoy et al., (2011). Primers for O26 F, caatgggcggaaattttaga, R ataattttctctgccgtcgc; for O45 F tgcagtaacctgcacgggcg, R agcaggcacaacagccactact; for O103 F, ttggagcgttaactggacct, R gctcccgagcacgtataaag; for O111 F, tgtttcttcgatgttgcgag, R gcaagggacataagaagcca; for O113 F tgccataattcagagggtgac, R aacaaagctaattgtggccg; for O121 F tccaacaattggtcgtgaaa, R agaaagtgtgaaatgcccgt; for 145 F ttcattgtttgctgctg, R ggcaagctttggaaatgaaa; and for O157 F tcgaggtacctgaatctttccttctgt, R accagtcttggtgctgtctgaca were used. An equal volume of each primer (100mM) was mixed and 1 μ L of the composite primer was used with the QIAGEN Multiplex kit as described by the manufacturer (QIAGEN, Valencia and CA) to give a volume of 23 μ L. The positive controls were prepared from all serogroups while the negative control was prepared from water. A volume of 2 μ L of the DNA templates from the samples, positive control and water were mixed with the master mix separately. Initial denaturation was done at 95°C for

15 minutes. Subsequent denaturation was done at 94°C for 30 seconds, annealing at 57°C for 1.5 minutes followed by extension at 72°C for 1.5 minutes and the final extension at 72°C for 10 minutes. The amplified PCR product was stained with a DNA loading dye (EZ-Vision One, Amresco, Solon, OH) and electophoresed in agarose gel (1.5%) for 45 minutes at 175 volt as described in DebRoy et al. (2011). Finally, the gel was observed under Ultra Violet light.

4.3.4. Limitation of the methodology

Preferential amplification could be one of the limitations of the Multiplex PCR assay used for this study. In addition, the multiplex PCR assay detects only eight serotypes therefore, it was not possible to serotype the other isolates.

4.4. Methodology for objective 4

For the fourth objective, information was obtained through Google Scholar and PubMed search engine using the key phrases prevention and control of emerging infectious diseases, emerging infectious disease surveillance, infection prevention, prevention of EHF and STEC and the challenges of emerging infectious disease control. Articles published by different peer reviewed journals, governmental websites and publications of health service organizations between 1970 and 2012 were used as sources of information.

5. RESULT AND DISCUSSION

5.1. The driving factors for the emergence of infectious diseases

5.1.1. Discussion

5.1.1.1. Pathogen factors

There are different genetic factors that contribute to the emergence and re-emergence of infectious diseases. Often, RNA viruses cause emergence and re-emergence of infectious diseases (Woolhouse and Gowtage-Sequeria, 2005). For instance, RNA viruses, such as influenza virus, are reported to a have higher mutation rate, which is the reason for their high evolution rate (Parvin et al., 1986). The emergence of influenza virus is associated with mutation, which occurs spontaneously and reassortment, which involves two strains of the virus from two different hosts. Because of the two genetic processes, a virulent strain emerges (Morse and Schluederberg, 1990). Therefore, mutation, reassortment and the ability of the virus to infect multiple hosts contribute to the emergence of a new influenza virus (Cleaveland et al., 2001). Similarly, the emergence of STEC is thought to be due to acquisition of virulence genes from phage and plasmids. Plasmids are responsible for virulence factors such as hemolysin while phages are responsible for the shiga toxins. The combination of the virulence factors acquired through those genetic processes result in a new bacteria with high virulence (Reid et al., 2000).

5.1.1.2. Host factors

Globally, there is an increasing trend in the number of immunocompromised people due to factors such as population aging, Human Immunodeficiency Virus (HIV) infection, chronic diseases, malnutrition and medical treatments such as chemotherapy and organ transplantation (Morris and Potter, 1997). Immunosuppression is another factor that contributes to emergence of infectious diseases. The immunosuppression in HIV patients is a driving factor for the emergence of diseases such as Kaposi's Sarcoma (CDC, 1982) and oral candidiasis caused by *Candida dubliniensis* (Coleman et al., 1999). *C. dubliniensis* is a recently described opportunistic pathogen that is closely related to *C. albicans* but differs from it with respect to its epidemiology, certain virulence characteristics, and the ability to develop fluconazole resistance *in vitro*. *C. dubliniensis* has been linked to oral candidiasis in AIDS patients, although it has recently been associated with invasive disease (Gutiérrez et al., 2002).

Decreased prevalence of Enteropathogenic *Escherichia coli* (EPEC) infections in the US is thought to be associated with increased incidence of STEC (Beutin, 2006). Because a study has shown that, the immunity against EPEC infection can prevent against STEC infection (Palmeira et al., 2005). Therefore, lack of immunity to EPEC might contribute to the increased susceptibility to STEC infections in the U.S.

5.1.1.3. The interface between wildlife, livestock and humans

Studies have shown that the interaction between wildlife and humans increases the risk of transmission of infectious diseases from the wild animals to humans or vice versa (Wobeser, 2002). For instance, the occurrence of an outbreak of *Mycobacterium tuberculosis* in mongooses in Botswana was an example of how human pathogens could be transmitted to wildlife (Alexander et al., 2002). Alternatively, the interface between humans and wildlife could be a source of infectious disease to humans, especially in tropical rain forests where the interface has increased due to intrusion on forests and ecotourism (Wolfe et al., 1998). Outbreaks of EHF in humans were reported to be preceded by outbreak of EHF in duikers and primates because of the practices of handling infected animals (Leroy et al., 2004). Once the virus is introduced to the human population through contact with wildlife, it spreads by person-to-person transmission (Leroy et al., 2004).

The use of indigenous animals as food source has contributed to the emergence of infectious diseases. The emergence of EHF has been associated with the consumption of primates as food source (Rizkalla, 2007) and the emergence of Severe Acute Respiratory Syndrome (SARS) was linked to consumption of civet cats and ferret badgers (Weinhold, 2004). Similarly, humans are affected by pathogens that normally live in livestock. The reservoirs for STEC are domestic ruminants (Oporto et al., 2008). People get the disease when they are exposed through contact or food. The fact that food animals are the reservoirs for those pathogens is a big problem to food safety (Tauxe, 1997).

5.1.1.4. Globalization of trade and transport

The movement of animals and people is one of the most important factors in the spread of infectious diseases. For instance, the movement of *E. coli* O157 shedding cattle has contributed to the spread of these bacteria (Lui et al., 2007). The introduction of Ebola Reston virus to the U.S. through imported monkeys from Philippines and the introduction of Bovine Spongiform Encephalopathy (BSE) case to the U.S. (Pearson et al., 1996, Animal and Plant Inspection Service et al., 2004) are some of the examples of how movement of animals could contribute to the spread infectious diseases. There is a huge international live animal trade worldwide. According to the data from Foreign Agricultural Trade of the U.S. (FATUS), US imported 1,450,996,963 live calves and cattle in 2011(USDA, 2011); this kind of live animal trade can increase the risk of disease spread.

Similarly, the movement of people contributes to the spread of disease. Imported case of EHF in South Africa (Arthur, 2002) and imported cases of *E. coli* O104 in the US (Alexander et al., 2012) are examples of the effect of movement of people in the emergence of infectious diseases in new territories.

5.1.1.5. Agricultural practices

The use of animal tissue in animal feed has contributed to the emergence of BSE, a neurological disease that affects cattle. The disease spreads through feed contaminated with animal tissues and can affect people (Orriss, 1997). Furthermore, mass production of food animals increases the risk of spread of infectious diseases. Diseases spread more easily in the large scale animal production systems than the traditional animal production system. The waste of animals produced by mass production systems is also another source for infectious agents, which exposes farmers, their workers and the environment to pathogens and contributes to the spread and emergence of infectious diseases (Graham et al., 2008).

The use of some groups of antibiotics, such as quinolones in animals, is believed to increase the spread of shiga toxin genes thereby increasing the prevalence of STEC in cattle (Kimmitt et al., 2000). Moreover, the use of antibiotics in agriculture for growth promotion or treatment is believed to increase antibiotic resistance in human pathogens. For instance, the emergence of antibiotic resistant *E. coli* and *Salmonella* is thought to be due to the use of antibiotics in agriculture (Van den Bogaard and Stobberingh, 1999). Therefore, current agricultural production methods contribute to emergence of infectious diseases.

5.1.1.6. Food production systems

Mass production and distribution of food is a challenge to food safety. Contamination of a food product can cause outbreaks affecting large geographical areas and large number of people. Recent STEC outbreaks that occurred in the U.S. involved most of the states and large number of people (Sanders, 1999, Tauxe, 1997). In addition, the current food production system increases the number of food products used as vehicles for food borne pathogens. For instance, with cattle as the reservoirs of *E. coli* O157, the pathogen is expected in animal food products

like beef. However, the food vehicle for the pathogen has broadened to include sprouts, apple cider and other foods (Tauxe, 1997).

5.2. Epidemiological and clinical aspects of the 2007-2008 EHF outbreak in Budibugyo, western Uganda

5.2.1. Results

5.2.1.1. Case distribution

Among the 192 EHF suspected cases, 42 (21.9%) were positive for laboratory test and were classified as confirmed cases; no test was done for 74 (38.5%) individuals and they were categorized as probable cases while 76 (39.6%) had negative test results and were grouped as non-case.

5.2.1.2. Temporal distribution of the outbreak

The onset of disease for the first case was identified retrospectively as August 1, 2007 (Figure 1). The number of cases peaked in the nineteenth week of the outbreak and then decreased until the end of the outbreak in February 2008. The last cases of the outbreak were reported on January 2, 2008 (Figure 1).

5.2.1.3. Spatial distribution of the outbreak

All EHF cases were from the Bundibugyo district in Western Uganda. Confirmed and probable cases were reported in seven sub-counties with high percentage of cases in Kasitu 62 cases (54.4%), Bundibugyo town council 24 cases (21%) and Bubukwanga 17 cases (15%) (Figure 2). The three sub-counties are geographically adjacent to each other (Figure 2). Among the sub-counties, the highest death rate was observed in Kasitu sub-county 17 deaths (45% of all deaths); this was the county where the index case of the outbreak had been living. The other sub-

counties with relatively higher death rates were Bundibugyo town council 8 deaths (21%) and Bubukwanga 7 deaths (18.4%).



Figure 1. Epidemic curve for the EHF outbreak in Bundibugyo, Uganda, August 2007-January 2008 (N=113). Source of data: Ugandan Ministry of Health

5.2.1.4. Clinical presentations

Fever, headache and abdominal pain were the most common signs and symptoms experienced by the patients. More than 97% of cases had fever as shown in Table 2. Information was collected on the history of bleeding of the patients and 72 (62.1%) had at least one form of bleeding while 44 (37.9%) of patients had no bleeding. Of the 108 cases of EHF for whom the data was available, 59(54.6%) experienced difficulty of breathing.



Figure 2. The spatial distribution of EHF outbreak by sub-county in Bundibugyo, Uganda, August 2007-January 2008, (N=114). Source of data: Ugandan Ministry of Health

Socio-demographic	No of cases (%)	No of fatal Cases (%)
Variables		
Age group		
0-10	6(5.2)	4(10.3)
11-20	20(17.2)	4(10.3)
21-30	29(25)	6(15.4)
31-40	22(19)	5(12.8)
41-50	23(19.8)	9(23)
51-60	11(9.5)	8(20.5)
61-70	5(4.3)	3(7.7)
Gender		
Female	51(44)	16(41)
Male	65(56)	23(59)
Occupation		
Children	9(7.8)	5(12.8)
Farmer	40(34.5)	13(33.3)
Health care staff	16(13.8)	6(15.4)
House wife	15(12.9)	7(18)
Students	14(12.1)	2(5.1)
Others	22(19)	6(15.4)

Table 1. Socio-demographic characteristics of EHF cases in Bundibugyo, Uganda, August 2007-January 2008 (N=116). Source of data: Ugandan Ministry of Health

5.2.1.5. Risk factors for acquiring EHF

In bivariate analysis, the risk factors that had significant association with acquisition EHF were attending funerals, having physical contact with suspected cases and visiting hospital prior to illness (Table 3). However, in a final logistic regression model developed for the risk factors associated with acquiring EHF; Only attending funerals was significantly (OR 2.88(1.4- 5.9), P<0.0037) associated with acquiring EHF.

Clinical presentations	No of cases with clinical signs and symptoms (%)	No of cases for which information is available
Fever	111(97.4%)	114
Headache	98(89.1%)	110
Abdominal pain	98(88.3%)	111
Vomiting	96(84.2%)	111
Diarrhea	94(83.2%)	113
Loss of appetite	88(80.7%)	109
Difficulty of swallowing	61(56.5%)	108
Difficulty of breathing	59(54.6%)	108
Skin rash	57(50.4%)	113
Bleeding eyes	52(47.3%)	110
Black stool	42(37.8%)	111
Hiccups	37(33.3%)	111
Vomiting of blood	24(21.6%)	111
Bleeding nose	23(20.9%)	110
Bleeding gums	18(16.4%)	110
Bleeding from vagina	9(10.3%)	87
(non-menstrual)		
Bleeding of injection site	10(9.6)	104

Table 2. Clinical presentations of EHF cases in Bundibugyo, Uganda, August 2007-January 2008 (Source of data: Ugandan Ministry of Health)
Variable	Category	OR (95% CI)	p-value
Attended funerals	Yes vs. No	2.97(1.51-5.87)	.0008*
Had contact with suspected cases	Yes vs. No	3.51(1.73-7.09)	.0002*
Hospital visit prior to illness	Yes vs. No	2.13(1.09-4.13)	.0132*

Table 3. Result of bivariate analysis of the risk factors associated with acquisition of EHF in Bundibugyo, Uganda August 2007 – January 2008 (N=192). Source of data: Ugandan Ministry of Health

5.2.1.6. Determinants of patient prognosis

The age group of cases was categorized into two groups, as shown in Table 4. Children under the age of 10 years were excluded from the two age groups on the assumption that they did not have equal exposure to the virus compared to older age groups. Children under the age of 10 are less likely to get involved in funeral activities, which is one of the significant risk factors for acquiring the disease. Both age group of cases and difficulty of breathing were significantly associated with the prognosis of cases in logistic regression model (Table 4). The odds of having difficulty of breathing was three times higher among patients who died compared to patients who did not die. Both age group and difficulty of breathing were significantly associated with prognosis of patients

Table 4. Results of final logistic regression model for the associations between patient survival, clinical presentations and age groups in Bundibugyo, Uganda August 2007- January 2008 (N=99). Source of data: Ugandan Ministry of Health

Variables	Category	OR (95% CI)	P-value
Age group	11-40 vs. 40-70	2.70 (1.05-6.97)	.0393*
Difficulty of breathing	Yes vs. No	3.08 (1.13-8.35)	.027*

5.2.2. Discussion

Most EHF outbreaks started during the short dry season and rainy season, but not during the long dry season (Monath, 1999). In the 2007/2008 EHF outbreak in Bundibugyo, the index case was detected in August which is part of the short dry season and lasted for months by maintaining its transmission. The Bundibugyo outbreak, lasted about 6 months. The previous outbreak that occurred in Uganda started in October 2000, a rainy season lasted for about 13 weeks but it affected 425 individuals and caused 224 deaths (Lamunu et al., 2004).

The outbreak occurred in Bundibugyo district, which is located in western Uganda at the border with Democratic Republic of Congo where previous outbreaks of EHF occurred (WHO, 1978b, Wamala et al., 2010). EHF outbreaks occur in humid tropical rain forests of Africa (Peterson et al., 2004). The Semuliki National Park, which is part of the tropical rainforests is located in the Bundibugyo district. The district shares ecological and geographical similarity with places where previous outbreaks occurred (Wamala et al., 2010, Peterson et al., 2004).

A new subtype of Ebola virus caused the outbreak (Towner et al., 2004). The CFR for this outbreak was relatively low compared to other outbreaks caused by Ebola Zaire and Ebola Sudan subtypes which had CFR of about 88% and 53%, respectively (WHO, 1978b, WHO, 1978a). In experimental settings, Ebola Bundibugyo was found to be less pathogenic compared to Ebola Zaire (Gupta et al., 2010); that may be one of the reasons for the lower CFR observed. In addition, community based case detection, the establishment of isolation wards and burial teams might have helped prevent further transmission and death (Wamala et al., 2010). In the 2001-2002 outbreak of EHF in Uganda, those measures helped a lot in the containment of that outbreak (Lamunu et al., 2004).

28

Concerning the death rates among the case categories, both confirmed and probable cases experienced similar CFR. However, six of the laboratory negative patients (not cases) also died. This could be explained by the weak immune response of the cases. Baize and colleagues found that the immune response in fatal cases of EHF is so weak that Ebola virus specific IgG and IgM antibodies are not detected (Baize et al., 1999). Therefore, the fatal cases might have been EHF cases that tested negative due to limited immune response. This observation highlights the limitation of the case definition. Pittalis and colleagues tested the sensitivity of the clinical case definition set by the World Health Organization (WHO) and found that only 58% of EHF cases fulfilled the clinical case definition. They pointed out that the use of the case definition in case detection may result in false negative cases. Therefore, a more comprehensive and sensitive case definition may be needed for future outbreak investigation (Pitallis et al., 2009). Similarly, laboratory tests based on IgG may not be reliable in detecting new cases because a study has shown that the IgG can be detected for years in convalescent patients (Ksiazek et al., 1999).

The EHF outbreak affected every age group. Even though, the number of cases was few in the age groups 0-10 and 51 and above, the death rates were higher in those two age groups. As reported in previous outbreaks (Scott et al., 1999), there were higher numbers of cases in the age group 11-50 years; however, this group experienced lower CFR compared to the elderly and young children. In one study, it was reported that being above the age of 18 years was one of the risk factors for EHF intrafamilial transmission (Scott et al., 1999). The higher number of cases in the age group 11-50 might be due to the exposure of those groups to the risk factors of EHF like attending funerals, caring for patients and travelling (Francesconi et al., 2003). In this outbreak, only a few children acquired EHF which is in agreement with previous studies (Mupere et al., 2001, Scott et al., 1999)]. The death rates varied among the age groups, with relatively higher

CFR among the elderly (>51 years) possibly due to lower innate and humoral immunity level and/or presence of other chronic diseases related to old age (Plowden et al., 2004, McGlauchlen and Voge, 2003). In agreement with previous outbreaks (Mupere et al., 2001) higher death rate was observed among children under the age of 5 years compared to older age groups.

Unlike other outbreaks where women were affected more than men, (WHO, 1978a, Okware et al., 2002) few women acquired and died of EHF in the Bundibugyo outbreak. The control measures applied as part of the outbreak response might have shielded women and decreased their risk of exposure to EHF. Usually, women experience higher infection rate and CFR than men possibly due to their cultural role in caring for the sick and preparing bodies for funerals (Hewlett and Amola, 2003). Inadition, health workers are at higher risk of acquiring EHF. This is because EHF usually manifests itself as a common acute febrile illness in the early stages. Therefore, health workers who care for those patients without barrier nursing and proper protective equipment will acquire the disease. The 1976 outbreaks of EHF in Zaire and Sudan were amplified by iatrogenic and hospital based transmission that killed health workers and patients (WHO, 1978b; Mupere et al., 2001).

In the 2007/2008 EHF outbreak in Bundibugyo, attending funerals was one of the significant risk factors for acquisition of EHF. Funerals in some parts of Uganda have cultural norms; women wash the dead body to prepare it for burial. People who attend the burial ceremony are given a chance to touch the dead body for the last time; a practice referred to as the "love touch" (Hewlett and Amola, 2003). This norm makes funeral ceremonies a significant risk factor for the acquisition EHF because succumbed patients have higher viral loads compared to survived patients (Sanchez et al., 2004). Other forms of contact with cases of EHF can transmit the disease but funerals are the most important one. Similar to other studies, having contact with

patients was a significant risk factor for transmission of the disease in this outbreak. Any contact with EHF patient such as shaking hands, caring for the patient or any other physical contact can transmit the disease. However, contact during the early stage of the disease is reported to have lower risk of infection as compared to the late stage of the disease (Francesconi et al., 2003, Baron et al., 1983).

By excluding children under 10, due to their protection from risk factors such as funerals, age group of patients was significantly associated with outcome of EHF infection with older patients exhibiting higher CFR. Other studies have reported age to be significantly associated with survival of patients. The mean age of fatal cases was significantly higher than the age of the survivors (Khan et al., 1999; Sadek et al., 1999).

5.3. Prevalence of STEC O45, O145, O157, O111, O103, O113, O121 and O26 serotypes in beef cattle at NDSU research extension center, Dickinson

5.3.1. Results

Primers for *E. coli* O45, O145, O157, O111, O103, O113, O121, 026 were used for the multiplex PCR. Of the 198 STEC isolates tested, 184 (93%) were negative to the multiplex PCR assay; that is, they were STEC as each had at least one shiga toxin gene but were not among the eight serotypes that commonly cause disease in human (O45, O145, O157, O111, O103, O113, O121, 026. Only 14 (7%) tested positive for the multiplex assay. Among the positive isolates six were identified as having the *wzx* gene for O121, four had the *wzx* gene for O103, two had the *wzx* gene for O45 and two had the *wzx* gene for O111 and O145 each.

5.3.2. Discussion

In this study, only 7% of the *E. coli* isolates tested were positive for at least one of the eight STEC serotypes included in the multiplex PCR assay. The low prevalence of the eight

STEC serotypes reported in this study was not unexpected because STEC serotypes are numerous. Studies have shown that there are numerous STEC serotypes with 174 O antigens and 53 H antigens (Scheutz et al., 2004). Similarly, Blanco et al., (2003), reported about 200 serotypes of STEC. Therefore, the low prevalence might be due to the variation of the serotypes. Ninety three percent of the STEC isolates were in different serotypes other than the 8 STEC serotypes we tested for. Therefore, it is not surprising to have 198 STEC isolates of which only 7% tested positive for the eight STEC serotypes that are commonly isolated from human. The prevalence of the STEC we tested for also differs depending on different factors. Different prevalence rates were reported for both *E coli* O157 and non O157 *E. coli*, depending on the type of animal tested, the season and the animal production system (Hussien, 2007). Lack of a study with similar methodology made comparisons difficult.

The variability of the pathogens may pose a big challenge in the control and prevention of the disease. The variability decreases the chance of detecting all serotypes of the bacteria when they cause disease in human because the pathogenic *E. coli* do not have phenotypes in common that would enable us to detect them at once (Magni, 2008). The variability challenge the immune system; studies have shown that people living in areas where there is large population of cattle have some antibodies to some STEC serotypes (Michel et al., 1999). Moreover, the variation makes it difficult to produce effective vaccines against the different serotypes.

The amount of shiga toxins produced by different serotypes affects the pathogenicity of STEC. A study has shown that pathogenic STEC, such as *E. coli* O157, produce high levels of shiga toxin (Marques et al., 1986). Those STEC that are commonly isolated from the cattle and are not part of the common pathogenic *E. coli* serotypes in human may not cause disease if they

produce low levels of shiga toxins. Also, it is possible that those STEC are nonpathogenic because some studies have shown that the presence of shiga toxin alone may not cause disease; rather other virulence factors which are used for attachment may be needed (Levine et al, 1987). In agreement with Levine et al. hypothesis, another study (Samadpour et al., 2002) hypothesized that the high prevalence of STEC in food samples but the absence of human cases related to the STEC may be due to the fact that some STEC are nonpathogenic. In addition, the acid resistance of the bacteria may play a role in its pathogenicity. Paton and Paton (1998) summarized that there is a huge difference in the acid tolerance of STEC, which influence their pathogenicity.

The 7% prevalence of the serotypes that are commonly isolated from patients has a public health impact. Because, food is produced in large volume and introduction of a small quantity of pathogenic bacteria can lead to contamination of a large volume of food. For bacteria like *E. coli* O 157 where the infectious dose is small, even small quantities of the pathogen can cause food contamination and disease (FDA, 2012).

Targeting *wzx* gene, one of the genes that encodes for O antigen of STEC for serotyping some STEC using PCR, has shown good specificity and sensitivity (Fratamico et al., 2005). It also can be used to easily serotype STEC in foods and human fecal samples. Multiplex PCR is an assay that can detect serotypes of *E. coli* which commonly cause disease in human. The assay has good sensitivity and specificity (Debroy et al., 2011). The challenges in detecting and testing non-O157 STEC can be alleviated by the development of reliable tests that enable us to detect different serotypes of the bacteria in one reaction. This type of test would make the detection and confirmation of outbreaks easier and quicker.

33

5.4. Prevention and control of emerging infectious diseases

5.4.1. Discussion

The methods used to control and prevent infectious diseases depend heavily on the characteristic of the disease. Haydon et al. (2002) suggested three approaches to infectious disease prevention and control that aimed at the reservoir of the disease, the mode of transmission of the disease and the susceptible host. The reservoir of an infectious disease can be wild animal, domestic animal or human. The reservoirs of some emerging infectious diseases, like EHF and Rift valley fever are unknown (Leroy et al., 2005, Oelofsen and Van der Ryst, 1999). For the purpose of this paper, the term reservoir refers to any source of infectious diseases diseases including non-reservoir sources.

5.4.1.1. Prevention and control of EHF

Pike and his colleagues (Pike et al., 2010) developed a model that depicts how animal pathogens that initially infect only animals move through five stages to become exclusively human pathogens. The pathogens start to infect humans causing primary infections. As people are exposed to them, they progress through the steps to become an exclusively human pathogen (Pike et al., 2010). In the earlier stage of the diseases where animals have a role in transmitting and maintaining infection, the control and prevention of the disease should focus on the animal that serves as source of infection or reservoir.

In emerging infectious disease with reservoir of wild animal like EHF, prevention methods should focus on the interface between human and wild animals which occurs as a result of agricultural practice, wild animal petting, ecotourism, live animal trade, bush meat trade and consumption (Leroy et al., 2004, Chomel et al., 2007). Avoiding contact with wild animals and their products decreases the risk of disease transmission; therefore, health education is crucial to provide information on the risk of EHF while contacting the reservoirs (Pike et al., 2010). Because of its importance in the economy and food security of some communities, hunting is one of the challenging areas in addressing wildlife-human interface. Hence, it requires multi-faceted actions including regulatory actions that ban hunting and promote wildlife conservation (Milner-Gulland et al., 2003). Intensive multi-sectoral actions help prevent human-wildlife interface and the introduction of viruses of wildlife origin to human population.

However, when action at the human-wildlife interface fails and the virus is introduced to the human population, prevention should focus on containment of the disease (Lamunu et al., 2004). International travel is frequently mentioned as an important factor in the spread of infectious diseases (Morse, 1995); there is no boundary for infectious diseases. Therefore, infectious diseases should be detected and contained as early as possible before they spread around the world. Surveillance is one of the most important tools to enable health officials detect and control infectious disease outbreaks timely.

Surveillance is an organized collection, analysis and distribution of data utilized for public health actions (WHO, 2008a). Intersectoral coordination between public health, animal health and wildlife conservation personnel in surveillance and response is the best approach to address emerging zoonotic diseases (Woolhouse, 2002). In case of EHF, an outbreak in human is usually preceded by an outbreak in primates (Leroy et al., 2005). Therefore, early detection of EHF outbreak in primates is important for prevention of outbreaks in human population. Public health services can utilize the information to take public health actions and to alert the public about the risks associated with infected primates.

Integrated disease surveillance and response (IDSR) is another part of the strategy aimed at strengthening surveillance systems at a national level. The integration of the surveillance activities results in efficient use of resources. Moreover, it enables countries to meet disease surveillance needs at international level (WHO, 2010). The collaborative effort at international level helps in early detection and control of emerging infectious disease. The WHO global disease surveillance, alert and response strategy was developed to build a strong surveillance program at the global level. One of its objectives is to prevent and control diseases that can cross boundaries. EHF, Rift valley fever and avian influenza are some of the diseases included in the global alert and response strategy. The control of the 2000/2001 EHF outbreak in Uganda was possible due to the collaborative effort of different organizations including the WHO global alert and response (Lamunu et al., 2004). In addition, under the International Health Regulation (IHR), WHO member countries are required to strengthen their surveillance system, report reportable conditions and diseases of global concern that occur inside or outside their territories within 24 hours. In addition, WHO has the responsibility to help countries strengthen their surveillance, diagnostic and other technical capacities (WHO, 2008a; WHO, 2006).

Both active and passive disease surveillance systems need to be utilized for surveillance of emerging infectious diseases (US GAO, 1999). Both systems need sensitive and specific case definitions. EHF and STEC have case definitions (PAHO, 2003; CDC, 2012a). However, the case definition set for EHF, has shown poor sensitivity and specificity (Pittalis et al., 2009). In the 2007/2008 EHF outbreak in Uganda, six individuals categorized as non-cases died, which underscores the limitation of the case definition. Therefore, case definitions with good sensitivity and specificity are needed for effective disease surveillance.

In addition, for disease surveillance to be effective there needs to be a well-developed diagnostic capacity. In the Bundibugyo EHF outbreak, cases of febrile illness with diarrhea (which were EHF cases) were detected through disease surveillance in the early stage of the

outbreak (Wamala et al., 2010). However, the lack of diagnostic capacity delayed the confirmation of the cases for months. If there were good diagnostic capacity, it would have been possible to contain the outbreak earlier.

After confirmation of an outbreak, prevention and control of infectious disease should focus on the transmission of the disease. The mode of transmission of the disease and the risk factors should be targeted in the disease prevention and control plan. For highly contagious disease like EHF, strict infection prevention protocol including isolation of cases, quarantine of contacts, establishment of community triage and burial services are required (Nkoghe et al., 2004; Lamunu et al., 2004). Hospitals had been reported as hot spots for dissemination of EHF. Health workers have lost their lives while caring for sick patients (WHO, 1978b). Therefore, training health workers on infection prevention and stockpiling of infection prevention kits will help in early containment of the disease. Personal protective equipment is important to prevent health service providers from infection (WHO, 2008b). The above-mentioned methods hinder the transmission of the pathogen to a susceptible host. However, prevention of transmission may not be always feasible. Thus, it is important to target the susceptible host in prevention.

For fatal diseases with no treatment such as EHF, the development of vaccine may help protect susceptible individuals from the disease (Geisbert et al., 2002). The increase in the frequency of occurrence of EHF in Africa (Lamunu et al., 2004; Wamala et al., 2010; WHO, 2011; WHO, 2012) and bioterrorism underpin the importance of vaccines. Vaccination is one of the cost effective strategies that led to the eradication of small pox (Henderson, 1999). Measles, pertussis, diphtheria and other vaccine preventable diseases are well prevented and controlled due to the development of effective vaccines (WHO, 2007). Similarly, there were ongoing efforts

37

to develop vaccines for EHF (Phoolcharoena et al., 2011; Geisbert et al., 2002). The development of the vaccine could help protect high-risk groups.

For disease prevention and control strategy to be cost effective and feasible, there is a need to identify high-risk groups. High-risk groups are a segment of the population that has more exposure to a disease than the general population (Gordon, 1983). Due to the ecological and geographical distribution of EHF, people who live in the tropical rainforest of Central and Eastern Africa and hunters in the same region are at higher risk of acquiring the diseases (Peterson et al., 2004; Rizkalla et al., 2007). Similarly, health workers are high-risk groups for EHF due to occupational exposure (Hewlett and Hewlett, 2005). Therefore, public health officials should label high-risk groups and incorporate them into their disease prevention and control plan. If all the stated prevention measures fail and the individual contracts EHF, supportive care such as fluid replacement, palliative care and treatment of immediate complications improve the patient's prognosis and comfort (CDC, 2010).

5.4.1.2. Prevention and control of STEC infections

EHF and STEC share some similarities. Both of them are emerging infectious diseases, zoonotic diseases and have limited treatment options. Regardless of their similarities, the prevention and control strategies are slightly different. The reservoirs of STEC are domestic ruminants that are used as food sources (Oporto et al., 2008). In this case, it is difficult to prevent the contact between people and those animals. Therefore, the prevention and control of STEC should start on the animals at the farm. The United States Government Accountability Office (US GAO) (2012) has recently identified some interventions that can decrease the prevalence of STEC in cattle before slaughter. Although most of them are still under investigation, bacteriophages, probiotics, sodium chlorate, prebiotics, vaccines, natural product extracts and

colicins are among the pre-slaughter interventions identified. Thus, FDA licensed only the bacteriophages and vaccines (US GAO, 2012). In an experimental study, vaccination of cattle in a feedlot decreased the shedding of *E. coli* O157 (Potter et al., 2004). Therefore, vaccination of cattle can help decrease the incidence of STEC in human by controlling the bacteria at the source. In addition, zoonotic diseases that are mostly associated with food animal need biosecurity and biocontainment measures at farms to prevent the transmission of disease to human and animal herds, and to prevent interspecies transmission of diseases (Graham et al., 2008).

At processing units such as slaughterhouses, Hazard Analysis Critical Control Points (HACCP) can be implemented to prevent contamination of food with STEC. In case of food contamination at the processing unit, microbiological testing can be applied to the food that is contaminated with STEC so that appropriate actions are taken (USDA, 1996). In addition to *E. coli* O157:H7, *E. coli* O26, O45, O103, O111, O121 and O145 were included in the zero tolerance policy of the US Department of Agriculture Food Safety and Inspection Service (USDA FSIS) in June 2012. Therefore, those bacteria are currently routinely tested in raw beef trim and contaminated samples are recalled (USDA, 2012a). The other important stage is food preparation at home or food services. The cooking internal temperature set for ground beef (160⁰F) by USDA FSIS to be used by food service establishments and consumers is another effort that can help reduce the incidence of the disease. In addition to the cooking temperature, consumer education addresses other ways of preventing food contamination (USDA, 2012a).

Other than the food vehicles, STEC can be transmitted from person to person (Belongia et al., 1993) implying the importance of personal hygiene to prevent the risk of fecal contamination. Individuals with STEC infection need supportive therapy such as administration

of intravenous fluids to decrease the risk of complications (Thorpe, 2004). To decrease susceptibility, future vaccinations may help protect high-risk groups. There is ongoing research to develop vaccine against shiga toxins to prevent the occurrence of severe and fatal complications that result due to shiga toxins (Uchida, 2003).

Countries set a list of notifiable diseases based on their public health priority. STEC is a notifiable disease in the US; health facilities and laboratories are expected to report cases of STEC to CDC, which generates a national database on the incidence and serotypes of the bacteria. Also, CDC monitors outbreaks of STEC (CDC, 2012a). Moreover, the establishment of FoodNet as a national foodborne disease surveillance network helped in monitoring the occurrence of foodborne pathogens (Scallan, 2007).

In addition to prevention and control methods discussed, STEC and EHF need more attention in terms of biosecurity due to their potential use in bioterrorism. CDC listed some of the causative agents of emerging infectious diseases such as Ebola virus, *E. coli* O157, Marburg Virus and Nipah virus as potential bioterrorism agents (CDC, 2012d). Like other health interventions, bioterrorism preparedness and response needs good surveillance, development of effective diagnostic tools, vaccines and drugs (McDade, 1999).

The existing knowledge for some emerging infectious diseases is limited. We have limited knowledge on the reservoir of Ebola virus (Leroy et al., 2005) and the pathogenesis of STEC infections (Paton and Paton, 1998). Therefore, extensive research is needed to make evidence-based public health interventions and policies. Detailed knowledge should be generated to enable better understanding of the pathogens, the natural history of the diseases, their pathogenesis and epidemiology. The generation of that basic knowledge will help in developing vaccines, diagnostic tools and guidelines for management and control of those diseases (WHO, 2005). Scientific research on zoonotic pathogens may help increase the knowledge of new pathogens and help predict the occurrence of infectious diseases in the future. The detection of antibodies against simian foamy virus in Cameroonian hunters showed how the viruses crossed species barriers (Wolfe et al., 2004). Moreover, it predicts the future risks associated with the viruses. As reviewed by Wolfe et al. (2005), knowledge of the factors that contribute to the emergence of disease, risk analysis for infectious disease transmission and development of microbiological techniques helps in predicting occurrence of future infectious diseases.

Zoonotic diseases such as Rabies, EHF, STEC and West Nile virus need the collaboration between public health and animal health departments. Communication between the two departments can help detect, control and monitor zoonotic diseases. The importance of the collaboration between expertise in animal health and human health is one of the lessons learned from the first West Nile virus outbreak in New York City (US GAO, 2000). In case of zoonotic diseases, laboratories in the field of public health and animal health should work together by sharing expertise and technologies (US GAO, 2000).

Challenges in management of emerging infectious diseases

Lack of diagnostic facilities delays the prevention and control of infectious diseases (Lamballerie and Colson, 2006). Moreover, the diagnosis and research of some of the emerging viruses like Ebola virus, Hendra virus, Nipah virus and Rift valley virus are difficult because they need Bio Safety Level 4 (BSL4) facilities that are found in limited numbers worldwide (CDC, 2012c). In the 2007, EHF outbreak, the first suspected case was reported on August 1, but there was no diagnosis made for the case; that would have helped in early control of the outbreak. Instead, the outbreak was confirmed on November 29, 2007 after the disease had spread (Wamala et al., 2010). Besides, diseases spread readily in areas with poor socioeconomic situation. One of the main factors that contributed to the spread of EHF was the poor infrastructure in the health facilities. Lack of personal protective equipment had exposed health workers to the deadly virus and had caused many deaths (WHO, 1978b).

The other factor that makes the control of infectious diseases difficult is the presence of multiple hosts for a pathogen. About half of the emerging and reemerging diseases affect more than three hosts (Woolhouse and Gowtage-Sequeria, 2005). For instance, EHF can infect duikers, dogs, bats, primates and humans (Allela et al., 2005, Leroy et al., 2005, Leroy et al., 2004). Similarly, STEC can infect goat, sheep, cattle and pigs, which increases the chance of survival and the interspecies dissemination of the bacteria (Beutin et al., 1993).

The other host factor that poses a challenge in the prevention and control of emerging infectious disease is the lack of previous exposure to a pathogen. Lack of previous exposure to a pathogen at population level can increase the susceptibility of a population to a disease. In the 1918 influenza pandemic large numbers of young people under the age of 30 were affected due to lack of previous exposure to the virus while elderly individuals were less affected due to previous exposure to the virus, and the presence of some level of immunity in their body. Therefore, lack of immunity to a pathogen is another challenge in control of infectious disease especially when it is a new pathogen (Ahmed et al., 2007).

Currently, both EHF and STEC do not have vaccines (CDC, 2010). Developing vaccines for emerging infectious diseases has many challenges. Lack of knowledge of the pathogen, lack of suitable animal model, inability to develop a vaccine that stimulates both humoral and cell mediated immunity and the side effects of the vaccines underdevelopment are some of the challenges in vaccine development for emerging diseases. Moreover, the number of species of the organism is another factor that challenges the development of vaccines. In diseases like EHF where the disease is caused by different Subtypes, it is difficult to develop a vaccine that can protect against all the subtypes of the virus (Barrett and Stanberry, 2009).

Emerging infectious diseases such as EHF and STEC have chronic complications. Patients of EHF in Uganda outbreak in 2000 experienced chronic complication like blindness, deafness, general weakness and many other psychological problems (Wendo, 2001). Therefore, the disease has a long-term effect on affected individuals. Similarly, HUS is one of the sequels of STEC infection which commonly occurs in children (Brooks et al., 2005). HUS is self-limiting however; sometimes it can cause chronic complications such as chronic renal failure, end stage renal failure and other neurological complications (Razzaq, 2006).

6. CONCLUSIONS

6.1. Conclusion for objective 1

The driving factors for emergence of infectious diseases are multifactorial. Factors associated with the pathogen, host factors, interface between animals and human, globalization of trade and transportation, agricultural and food production systems are among the factors that contribute to the emergence and reemergence of infectious diseases. Interface between wild animals and people, the ability Ebola virus to infect multiple hosts, the use of primates as food sources, trade and transportation contributed to the emergence of EHF. Similarly, the emergence of STEC infections had many contributing factors, which include the change in the genotype of *Escherichia coli*, agricultural practices, food production system and the lack of immunity against EPEC.

6.2. Conclusion for objective 2

An outbreak of EHF occurred in Bundibugyo, a district that shares ecological and geographical similarity with places where previous outbreaks occurred. The outbreak occurred between August 1, 2007 and January 2, 2008, during a short dry season followed by a rainy season when EHF outbreaks typically occur. Health workers were at higher risk of acquiring EHF possibly due to lack of proper protective attire. In addition, attending funerals was one of the significant risk factors for acquisition of EHF possibly due to some of the cultural norms practiced at funerals which place people, especially women, at higher risk of acquiring the infection. Among the clinical presentations, difficulty of breathing was the best predictor of patient prognosis. Age group of patients was significantly associated with the outcome of EHF infection with older patients exhibiting higher CFR. The Bundibugyo EHF virus exhibited a lower CFR than the previous EHF subtypes. Nevertheless, the outbreak affected 192 individuals

of which 116 were categorized as confirmed or probable cases with 39 deaths (CFR of 34%). Also, it took the Ministry of Health three months to respond to the outbreak underscoring the need for developing local capacity for rapid diagnosis and response to such public health emergencies.

From the perspective of infectious disease management, public health actions taken to halt the outbreak, including active case search in the community helped in preventing the transmission of EHF within a family or in the community pinpointing the importance of active surveillance during outbreaks. Furthermore as a contagious disease that is easily transmitted, infection prevention actions such as establishing funeral teams, providing personal protective equipment for caregivers and isolation of cases helped contain the outbreak and are the important lessons taken from this case study. However, delayed response to the outbreak, delay in case confirmation, lack of diagnostic capacity, delayed personal protective equipment supply and death of health workers due to exposure are areas that need to be improved and taken into consideration in the management of EHF.

6.3. Conclusion for objective 3

The prevalence of the eight STEC serotypes tested (O26, O45, O111, O103, O145, O121, O113 and O157) in beef cattle at North Dakota State University research extension center was 7%. Only a few STEC isolates were among the serotypes, which usually cause disease in human. The majority of the STEC isolates belonged to other serotypes which were not tested for, an indication of the numerousness in the serotypes. Those serotypes may or may not be pathogenic depending on the amount of shiga toxin they produce, the presence of other virulence factors and the ability to resist stomach acid. However, the 7% prevalence of the common pathogenic STEC

could have a significant public health impact exacerbated by the current agricultural and food production systems.

6.4. Conclusion for objective 4

The prevention and control of EHF and STEC can be implemented at three different areas: the reservoir host, the mode of transmission and the susceptible host. Preventing wildlifehuman interface could help prevent the introduction of the Ebola virus to human population. However, if the virus has already been introduced to human population, the intervention should focus on preventing the spread of the virus using infection prevention protocols. Early detection and control activities need a well-developed surveillance and diagnostic capacity. For people who are at risk of getting EHF such as health workers, a future vaccine could help protect them from the disease. For individuals who are already infected with the virus, supportive care could help increase the comfort and survival of the patient.

The prevention and control strategy of STEC focuses on the food animals that serve as the reservoir of the bacteria. Use of bacteriophages and vaccines are among the FDA-approved interventions that decrease the prevalence of STEC in cattle. HACCP is one of the interventions that are applied at the slaughtering facility to decrease risk of contamination of food products with STEC. Moreover, routine microbiological tests for *E. coli* O26, O45, O103, O111, O121, O157 and O145 on beef trim helps to detect contamination and take immediate action.

National and international disease surveillance and response are one of the major approaches that need emphasis in the prevention and control of those infectious diseases. Moreover, infection control, biosecurity measures and health education are important areas of emphasis. Furthermore, those new pathogens need extensive research in order to have detailed knowledge of how to prevent and control them. Development of vaccines can be targeted as a cost effective way of preventing and controlling of those diseases. However, there are inherent challenges in the prevention and control of those diseases. Limited knowledge of the new pathogens has limited the development of treatment and vaccines. The fact that the pathogen affects multiple hosts also challenges the prevention and control due to the interspecies spread of the diseases. In addition, the susceptibility of the human population to those diseases has increased due to the newness of the pathogens. Finally, the long-term complications of the diseases especially, STEC infections and EHF have a huge public health impact.

7. SUMMARY

Diseases emerge due to multiple factors. For EHF, human-wildlife interface is the major driving factor while for STEC the evolution of the bacteria through acquisition of genetic materials is the major driving factor for the emergence of the disease.

The infectivity and virulence of Ebola virus makes it a terrifying emerging infectious disease. The disease sporadically appears in human population and needs intensive control and prevention strategies to prevent the spread of the disease. The virus leaves human population by killing most of the human hosts. Only few with strong immune system can survive since the disease does not have treatment and vaccine. Avoiding contact with wild animals is the most efficient way to prevent the disease.

STEC is a relatively mild disease in terms of infectivity and virulence as compared to EHF. The risk of being infected with STEC is high in human population because the bacteria live in food animals and commonly contaminate our foods. The variability of the bacteria in terms of phenotype, virulence and serotype makes the prevention and control of the disease difficult. Lack of effective treatment and vaccine exposes infected individuals to severe complications. Therefore, the disease needs intensive preventive actions with special focus on food safety.

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APPENDIX. CASE DEFINITIONS USED DURING THE 2007/2008 EBOLA HEMORRHAGIC FEVER OUTBREAK IN BUNDIBUGYO, UGANDA

Suspected cases were individuals who had sudden onset of fever and at least four of the following symptoms in a resident or visitor to the affected sub-counties in Bundibugyo district: vomiting, diarrhea, abdominal pain, conjunctivitis, skin rash, unexplained bleeding from any body part, muscle pain, intense fatigue, difficulty of swallowing, difficulty of breathing, hiccups, or headache since August 1, 2007, OR sudden onset of fever in any person who had contact with a person with suspected, probable, or confirmed EHF, OR sudden death in a person in the community without any other explanation.

Probable cases were individuals who were suspected of EHF in any person (dead or alive) with at least three of the following symptoms; vomiting, diarrhea, or unexplained bleeding from any site, conjunctivitis, or skin rash; AND with an epidemiologic link to a person with probable or confirmed EHF, OR either no specimen collected for laboratory testing or a negative laboratory result in a specimen collected 0–3 days after onset of symptoms in a person with suspected EHF.

Confirmed cases were those who had laboratory confirmation of infection by isolation of virus from any body fluid or tissue, OR detection of viral antigen in any body fluid or tissue by antigen-detection ELISA, reverse transcription– PCR, or immunohistochemistry, OR demonstration of serum Ebola virus–specific IgG antibodies by ELISA, with or without IgM, in any person with suspected or probable EHF.