

Three ebola outbreaks in Uganda 2000-2011

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To my wife Daisy and children: Paul, Christopher, Gloria Brenda, Solome, Sammy and daughters Manelli, Sasha, Eva and William for their prayers and encouragement.

Contributors

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The Ministry Health

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Table of contents

Contributors	4
Table of contents	5
TABLES.....	7
Abbreviations	9
Abstract.....	10
Acknowledgements	12
Original papers.....	13
1.0 INTRODUCTION.....	15
1.1 Ebola and Marburg haemorrhagic fevers: historical perspective.....	15
1.1.1 Ebola haemorrhagic fever	15
1.1.2 Marburg haemorrhagic fever	16
1.2 Ebola virus	19
1.2.1 Reservoir	21
1.2.2 Susceptibility and transmission	23
1.3 Pathogenesis.....	24
1.4 Prevention against infection.....	24
1.4.1 Protecting the individual and the environment	24
1.4.2 Capacity for laboratory diagnosis.....	25
1.5. Uganda	27
1.5.1 National health care system.....	27
2.0 AIM AND STUDY OBJECTIVES.....	30
2.1 Aim	30
2.2 Rationale of the study	30
3. STUDY SUBJECTS AND METHODS.....	31
3.1 Study setting.....	31
3.2. Study sites and populations.....	31
3.3 Methods.....	35
3.3.1 Linkages between the papers.....	35
3.3.3 Methodology and tools for data collection.....	38
3.3.4 Methodology for data management and analysis	43
3.3.5 Planning and implementing the national response.....	44
3.3.6 Ethical clearance	51
4.0 SUMMARY OF RESULTS.....	52
4.1 Description of outbreak No 1: the Sudan ebolavirus, Gulu district, 2000	52
4.1.1 Outbreaks detection and verification: How and where did it all begin?	52
4.1.2 Epidemiological description of the outbreak.....	53
4.2 Outbreak No 2: The Bundibugyo ebolavirus outbreak, Bundibugyo district, Uganda, 2007.....	60
4.2.1 Description of the epidemic	60
4.2.2 Risk factors investigation and analysis (Paper III).....	64
4.2.3 Case fatality rate estimation	66
4.3 Outbreak No.3: Outbreak of Sudan Ebolavirus, Luwero district, Uganda, 2011	69
4.3.1 Outbreak Description and containment.....	69

4.3.2 Investigation of contacts and bats	69
4.4 Laboratory results	70
4.5. Delays in case detection and the community mobilisation	72
5.0 DISCUSSION	74
5.1. Challenges in early detection and isolation.....	74
5.2 Seasonality and zoonotic connection	76
5.3. Susceptibility and risk factors	76
5.4. Differences in severity and fatality	77
5.5. Limitations on data collection.....	78
5.6. Challenges in the application of tools for data collection.....	78
5.7 Ethics and contentious issues	80
6.0 LESSONS LEARNT AND CONCLUSION	81
REFERENCES.....	83
Appendix 1: Case investigation form.....	88
Appendix 2: Contacts recording sheet.....	93
Appendix 3: Contact tracing form (follow up)	94
Appendix 4: Algorithm for mobile team	95
Appendix 5: Surveillance flow chart	96
Appendix 6: Educational Facts Sheet	97
Appendix 7: Procedures for testing Ebola at the UVRI laboratory	97
Appendix 8: Algorithm for discharge.....	101
Appendix 9: Estimated cost of a district work plan against Ebola	102
Appendix 10: Extract from report on wildlife investigation in Mpigi near Luwero district, 2007	103
Appendix 11: Acknowledgement and list of partners at national and national level	104

TABLES

Table 1. Known outbreaks of ebola and marburg by location and year, 1967-2012.....	17
Table 2. The known genera and species of the filoviridae ^a	19
Table 3 Laboratory tests for filoviruses.....	26
Table 4: comparison of health indicators between uganda and norway, 2011	27
Table 5: organisation of public health care delivery, uganda.....	27
Table 6: POPULATION IN THE EBOLA AFFECTED districts, 2000-2011, UGANDA*	32
Table 7: SUMMARY OF THE OBJECTIVES, DESIGN, METHODOLOGY, DATA COLLECTION AND ANALYSIS	37
TABLE 8: ADAPTED CASE DEFINITION FOR EBOLA HAEMORRHAGIC FEVER ADAPTED UGANDA, 2007.....	39
Table 9: SUMMARY OF CASES AND DEATHS BY DISTRICT, UGANDA, 2000	53
TABLE 10: CUMULATIVE EBOLA CASES BY MOST AFFECTED PARISH, GULU DISTRICT, UGANDA, 2000	55
TABLE 11: ATTACK RATES/10,000 POPULATION AND RELATIVE RISK BY COUNTY, GULU DISTRICT, UGANDA, 2000	56
TABLE 12: DISTRIBUTION OF EBOLA CASESBY AGE GROUP, GULU OUTBREAK, UGANDA, 2000.....	57
TABLE 13: RELATIVE RISK OF DYING ACCORDING TO CLINICAL CONDITION ON ADMISSION,; RESULTS OF A UNIVARIATE ANALYSIS, GULU DISTRICT, UGANDA, 2000	58
Table 14: DISTRIBUTION OF CASES BY SUBCOUNTY,BUNDIBUGYO DISTRICT, UGANDA, 2007	62
Table 15: BIVARIATE ANALYSIS OF RISK FACTORS FOR EBOLA, BUNDIBUGYO, UGANDA, 2007.....	65
TABLE 16: ANALYSIS OF THE DERMOGRAPHIC AND THE CLINICAL CHARACTERISTICS OF THE LABORATORY CONFIRMED CASES BY SURVIVAL OR DEATH OUTCOME, BUNDIBUGYO DISTRICT, UGANDA, 2007	68
TABLE 17: LABORATORY RESULTS OF SUSPECTED EBOLA CASES, BUNDIBUGYO, UGANDA, 2007 ...	71
TABLE 18: PROPORTION OF SCREENED CASES REVALIDATED BY SUPERVISORS, GULU DISTRICT, UGANDA, 2000.....	71
Table 19: LEVEL AND TIMELINES OF DELAYS HINDERING DETECTION AND ACTION, UGANDA, 2000-2011 .	72
Table 20: ATTACK RATES AND CASE FATALITY IN GULU AND BUNDIBUGYO DISTRICTS	77

FIGURES

Figure 1 reported outbreaks of marburg and ebola in africa, 1967-2011	18
Figure 2 structure of the filovirus (a,b,c,dand e) and sghematic representationof a filovirus (d).....	20
Figure 3 the presumed life cycle of the ebola virus*	22
Figure 4 immunological responses to ebola virus infection.....	26

Figure 5 map of study sites by district, UGANDA.....	31
Figure 6 map of gulu district, UGANDA.....	32
Figure 7 map of bundibugyo district, UGANDA.....	33
Figure 8 rwenzori mountain slopes, difficult terrain, bundibugyo, uganda	33
Figure 9 people in bundibugyo town,at narrow vLLEYSOF RWENZORI MOUNTAINS, BUNDIBUGYO,UGANDA.....	34
Figure 10 BUNDIBUGYO HOSPITAL, UGANDA.....	34
Figure 11 MAP OF LUWERO DISTRICT, UGANDA	35
Figure 12 CONCEPTUAL FRAMEWORK OF THE STUDY.....	36
Figure 13 TIMELINES FOR DATA COLLECTION 2000-2011.....	38
Figure 14 SPECIMEN COLLECTION FROM DEAD MONKEY BY MOH/UWA, SEMULIKI GAME RESERVE,BUNDIBUGYO, UGANDA, 2008.....	43
Figure 15 ISOLATION UNIT, KIKYO HEALTH CENTRE, bUNDIBUGYO, UGANDA, 2007	45
Figure 16 NATIONAL TASK FORCE (L TO R) DR SAM OKWARE, HON MINISTER OF HEALTH, STEPHEN MALLINGA, BUNDIBUGYO, UGANDA, 2007	46
Figure 17 HEALTH CARE WORKER AND ATTENDANT DISINFECTING PATIENTS CLOTHES, BUNDIBUGYO, 2007.....	47
Figure 18 PROCEDURES AND TOOLS FOR BURIALS-DISINFECTION, TRANSPORTATION AND BURIAL, BUNDIBUGYO, 2007.....	48
Figure 19 NTF PLANNING MEETING (LEFT); COMMUNITY WORKERS FROM UGANDA RED CROSS, (RIGHT), 2007.....	49
Figure 20 SURVEILLANCE FLOW CHART FOR EBOLA	50
Figure 21 MEDIA SUPPORT AND COVERAGE, UGANDA.....	51
Figure 22 DAILY TREND NEW EBOLA CASES, GULU DISTRICT, 2000 ⁴	54
Figure 23 ATTACK RATES BY SEX AND AGE, GULU DISTRICT, UGANDA, 2000.....	57
Figure 24 DISTRIBUTION OF SYMPTOMS ON ADMISSION, GULU, UGANDA, 2000	58
Figure 25 CASE FATALITY FROM EBOLA BY WEEK, GULU,2000	60
Figure 26 EPIDEMIOLOGICAL CURVE OF NEW EBOLA CASES, BUNDIBUGYO, UGANDA, 2007	61
Figure 27 DISTRIBUTION OF CASES BY SYMPTOMS, BUNDIBUGYO, 2007	63
Figure 28 LABORATORY CONFIRMED CASES BY CRETARIA, BUNDIBUGYO, UGANDA, 2007.....	66
Figure 29 TIMELINES FOR THE EBOLA OUTBREAK, LUWERO, uGANDA, 2011	70
Figure 30 DEAD MONKEY IN FOREST IN MPIGI DISTRICT, UGANDA, 2008.....	72
Figure 31 PROBABLE CAUSES OF DELAY IN DETECTION AND ACTION, UGANDA	75

Abbreviations

ACF	Action Contre la Faim (French: Action Against Hunger)
AHSPR	Annual Health Sector Performance Report
AR	Attack Rate
BEBOV	<i>Bundibugyo ebolavirus</i>
BSL	Biosafety Security Level
C	Degrees Celsius
CDC	Centre for Disease Control and Prevention
CFR	Case Fatality Rate
CIEBOV	<i>Cote d'Ivoire ebolavirus</i>
CIH	Centre for International Health, Bergen, Norway
CTF	County Task Force
DRC	Democratic Republic of Congo
DTF	District Task Force
EHF	Ebola Haemorrhagic fever
ELISA	Enzyme linked immune-sorbent assay
EU	European Union
IDP	Internally Displaced Persons
Ig	Immunoglobulin
IMSC	Inter Ministerial Steering Committee
KEMRI	Kenya Medical Research Institute
LC1	Local Council 1 at Village level
LC2	Local Council 2 at Parish level
LC3	Local Council 3 at Sub-county level
LC4	Local Council 4 at County level
LC5	Local Council 5 at District level
LRA	Lord's Resistance Army
MOH	Ministry of Health
MSF	Médecins Sans Frontiers
NGO	Non-Governmental Organization
NTF	National Task Force
NUFU	Norwegian Programme for Development Research and Education
NUSAF	Northern Uganda Social Action Fund
PPE	Personal Protective Equipment
PTF	Parish Task Force
REBOV	<i>Reston ebolavirus</i>
RNP	Ribonucleoprotein
RT PCR	Reverse Transcriptase Polymerase Chain Reaction
SEBOV	<i>Sudan ebolavirus</i>
SITREP	Situation report for Ebola
SMS	Short Messages conveyed via mobile phones
STF	Sub-county Task Force
UNCST	Uganda National Council of Science and Technology
URC	Uganda Red Cross
USA	United States of America
USD	United States Dollars
UVRI	Uganda Virus Research Institute
UWA	Uganda Wildlife Authority
VTF	Village Task Force
WHO	World Health Organization
ZEBOV	<i>Zaire ebolavirus</i>

Abstract

Three separate outbreaks of Ebola associated with high fatality occurred in Uganda between 2000 and 2011. A country wide national response contained each epidemic with various degrees of success. The experiences challenges and successes are described in Gulu, Bundibugyo and Luwero outbreaks.

Objectives

The study linked the following objectives: to describe the three Ebola outbreaks and the national response in Uganda from 2000-2011; to establish the risk factors associated with the Bundibugyo ebolavirus outbreak, 2007; to estimate the case fatality rate related to the Bundibugyo ebolavirus outbreak, 2007.

Methods

A descriptive design study documented the three different outbreaks. The occurrence and epidemiological characteristics of each epidemic were defined and described based on the adapted case definition for Ebola haemorrhagic fever. Data and information was systematically collected from cases and contacts routinely using questionnaires covering personal, demographic and social parameters. Active case search, isolation, as well as community mobilisation for public education including the media were the major strategies used. Risk factors were studied using a case control design (Paper III) which compared cases and non-cases in a sub set of presumed cases identified at community level. A quantitative study of a subset of cases with only laboratory confirmed acute phase blood samples estimated the corresponding case fatality rate (Paper IV).

Results

Two large outbreaks of the Sudan ebolavirus and the Bundibugyo ebolavirus occurred in Uganda in 2000 (425 cases) and 2007 (116 cases) respectively, followed in 2011 by a single case outbreak in Luwero. Clinical characteristics were similar in all the three outbreaks: acute onset of high grade fever, severe headache and chest pain, abdominal pain, associated with some bleeding tendencies. Clustering of cases was common. The case definition helped in screening suspected cases but the major weakness of this approach was that it had a low specificity, and less than 50% of suspected cases identified by the community were confirmed as true Ebola patients. In Gulu, attack rates were higher among women than men (RR=1.6; 95% C.I. = 1.3; 1.9). Children between 5-14 years had the lowest attack rate. The risk increased with age and was highest at 60-64 years age group (RR =16.4; 95% CI = 9.4; 28.8). Case fatality was highest in the SEBOV subtype (53%; 95% CI = 47.8-57.5) and lowest in the BEBOV (33.6%; 95% CI 25.0-42.2; $p=0.005$). There was two fold increase in mortality (RR=1.8, p value <0.001) when bleeding manifestations occurred in patients with the Ebola Sudan subtype. Ebola is a highly fatal nosocomial infection. Delayed detection often resulted in spread of infection in health care settings. Some 31 health care workers in Gulu and another 14 in Bundibugyo were infected during the outbreaks. Direct contact with a known case (OR 7.4, 95% CI 2.9-19.3) was probably the major mode of spread as demonstrated in Bundibugyo. Sex differences were not associated with significant risk

factors (OR 1.3, 95% CI 0.7-2.5) and age (OR 1.3, 95% CI 0.7-2.7) unlike in the Gulu observation. Seasonality was observed in the three outbreaks- erupting between May and December, which coincides with the rainy and fruit season. Evidence of asymptomatic past infection was demonstrated among individuals with Ebola positive IgG in the districts of Gulu, Luwero and Mpigi. The known primary cases that started the outbreaks came from rural remote areas. A zoonotic connection was apparent but unclear although one monkey specimen was found positive for Ebola IgG. The study demonstrated weaknesses in infection control as isolation procedures were apparently less effective. Despite instituting isolation procedures, 64% of the 31 health care workers in Gulu were infected after the isolation units were established, thus showing gaps in procedures for infection control.1). Prompt detection and communication was demonstrated to be effective in containing the Luwero outbreak which resulted in the best outcomes. Strengthening laboratory capacity and surveillance, therefore, at national level and enhancing collaborative networks at regional and international levels is crucial for effective timely diagnosis and management. Ad hoc incentives improved staff commitment, demonstrating that better remuneration of health care workers may contribute to better performance. Involving local communities and the media in outbreak control activities supported community based surveillance and timely identification of cases, in areas without health care workers. Ethical principles were breached and waiver of informed consent was considered a practical option under these life threatening circumstances, demonstrating further the difficulties of doing research under emergency situations.

Conclusion

The experiences and challenges from the three Ebola outbreaks in Uganda have been described. Attempts were also made to establish the risk factors and severity. Prompt detection and communication yielded the best ideal outcome and timely containment. Not all was one hundred percentage perfect, but the Ministry of Health working alongside its partners and the community contained the outbreaks against the constraints of the low resource settings, sometime with delays but once promptly and effectively done

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Original papers

The thesis is based on the following papers:

Paper I

Okware SI, Omaswa FG, Zaramba S, Opio A, Lutwama JJ, Kamugisha J, Rwaguma EB, Kaggwa P, Lamunu M. An outbreak of Ebola in Uganda. *Trop Med Int Health* 2002; **7**:1068-75.

Paper II

Lamunu M, Lutwama JJ, Kamugisha J, Opio A, Namboozee J, Ndayimirije N, Okware S. Containing a haemorrhagic fever epidemic: the Ebola experience in Uganda (October 2000-January 2001). *Int J Infect Dis* 2004; **8**:27-37.

Paper III

Wamala JF, Lukwago L, Malimbo M, Nguku P, Yoti Z, Musenero M, Amone J, Mbabazi W, Nanyunja M, Zaramba S, Opio A, Lutwama JJ, Talisuna AO, Okware SI. Ebola hemorrhagic fever associated with novel virus strain, Uganda, 2007-2008. *Emerg Infect Dis* 2010; **16**:1087-92.

Paper IV

MacNeil A, Farnon EC, Wamala J, Okware S, Cannon DL, Reed Z, Towner JS, Tappero JW, Lutwama J, Downing R, Nichol ST, Ksiazek TG, Rollin PE. Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. *Emerg Infect Dis* 2010; **16**:1969-72.

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1.0 INTRODUCTION

Uganda has experienced three separate outbreaks in 2000, 2007 and 2011. The first outbreak erupted suddenly, taking us completely unawares. Around September 2000, an outbreak of Ebola erupted in the Gulu district in northern Uganda. During this time, I was at the Ministry of Health coordinating and managing epidemics and disasters at the national level. I was in charge of the national task force that coordinated the control of this new emerging highly fatal disease, which had already killed several nurses in Gulu regional hospital. The Gulu epidemic was the largest such single outbreak ever recorded. Gulu district was then a war torn area as fighting continued between rebel insurgents and government troops. The epidemic caused panic in the country and the region. Health care workers were scared. They threatened to abandon their normal duties in hospitals and other health facilities. The communities were frightened and shocked. Many were desperate and at a loss as to what should be done. Seven years later in 2007, the second Ebola outbreak occurred in Bundibugyo district in western Uganda. This outbreak was similar but it took long to detect because it was a different sub type species of Ebola.. In 2011 a third outbreak occurred in Luwero district, Uganda, after an interval of ten years. This epidemic was quickly controlled within days leaving a just a single fatality.

I was a member of the Ebola national task force which coordinated control efforts during the years the epidemics occurred. Several members of my team died and I am so lucky to have survived to tell the story. There were good lessons and also bad ones. There were some good experiences such as when we contained the outbreak in Luwero within days. Ideally this scenario is what should happen, should an outbreak occur. We also had bad experiences in Gulu and Bundibugyo districts, when we lost many health care workers including the hospital directors. For this we need knowledge about the disease and about how to manage similar future outbreaks. We also need to be prepared and take into account lessons from previous experiences. It is the sharing of these rare experiences and challenges that prompted me to study and document them for posterity. This is why I decided to write this thesis.

1.1 Ebola and Marburg haemorrhagic fevers: historical perspective

1.1.1 Ebola haemorrhagic fever

Ebola is a new emerging threat of public health in Africa. The high fatality and the continuous high risk faced by health care workers make it an important nosocomial infection. Ebola Haemorrhagic Fever (EHF) commonly known as Ebola is an acute infectious febrile illness that is associated with bleeding manifestations and very high fatality. It has no known treatment. In 1976, the first recognised outbreak of Ebola occurred near a river called Ebola, a tributary of the River Congo in the then Zaire, now the Democratic Republic of Congo. The causative agent was identified and found to be similar to the Marburg virus (Buchmeier et al. 1983; Cox et al. 1983).. Another severe haemorrhagic outbreak was reported that year in Yambuku, Zaire (Heymann et al. 1980). This outbreak was largely the result of the reuse of contaminated needles. A similar outbreak erupted in Nzara, in neighbouring Southern Sudan (WHO 1978; WHO 2004). It was speculated that six cotton factory workers and their relatives could have been the first

victims (WHO 1978). No new cases were reported until 1994, when a mild illness was reported from an ethnologist who had performed a post-mortem on a dead chimpanzee in Tai Forest in Cote d'Ivoire (Le Guenno et al. 1995; Formenty et al. 1999). In 1995, a large community outbreak reemerged in Kikwit, DR Congo (Dowell et al. 1999; Khan et al. 1999). The transmission intensified following a major surgical operation on an infected laboratory worker (Dowell et al. 1999). Early in 1994, 3 more outbreaks were reported in the north eastern provinces of Gabon (Georges et al. 1999; Khan et al. 1999). The epidemic escalated and crossed the border into the Republic of the Congo, where it caused a large outbreak in 2002 which spread to several areas including Mbomo (Georges et al. 1999). In 2004, Southern Sudan was affected again by another minor outbreak (WHO 2004). In 2007 and 2008, a large outbreak was reported in Luebo, DR Congo (Leroy et al. 2009). On 30th August 2007, 103 persons were reported with Ebola in the village of Kampugwu in Eastern DR Congo. The outbreak started from a funeral of two local chiefs. The outbreak was caused by the Bundibugyo Ebola virus and confirmed in November of the same year (WHO, 2008). In 2011 a single case of Ebola occurred in Luwero district in Uganda. The highly fatal Sudan and Zaire Ebola viruses caused most of these outbreaks. Haemorrhagic fever outbreaks from 12 countries reported in Africa by 2010, eight were due to Marburg while 18 were caused by Ebola. Of the total of 2551 cases, 268 (9.3%) were health care workers (Allaranga 2010). Table 1 outlines the affected countries affected by Ebola and Marburg haemorrhagic fevers by year and location.

Outside Africa there have been no major outbreaks. However, imported cynomolgus monkeys (*Macaca fascicularis*) to the United States caused a simian outbreak in a facility in Reston, Virginia, in 1989. The virus was subsequently identified and named *Reston ebolavirus* (Jahrling, Geisbert et al. 1990). Some animal handlers sero-converted, but were asymptomatic. The importation of these monkeys from the same source also introduced the same infection in laboratories in San Antonio, Texas, and Sienna in Italy. The source of this infection was traced to a farm in the Philippines. Accidental needle stick exposures have also been reported among a couple of laboratory workers in Russia and Germany (Emond, Evans et al. 1977).

1.1.2 Marburg haemorrhagic fever

The Marburg virus is similar to and related to Ebola virus and both belong to the same Filoviridae family. The first documented cases of Filovirus infection occurred in Marburg, Germany in 1967 (Kissling, Robinson et al. 1968; Kissling, Robinson et al. 1968; Sanchez 2007). The workers were infected after handling green monkeys from Uganda (Smith, Simpson et al. 1967). Seven laboratory workers out of the 31 who were infected died. The causative agent was named "Marburg virus" corresponding to the location. In 1975, 3 more cases were reported in Johannesburg. A tourist, his companion and the attending nurse were infected. The tourist was the index case and infected the companion who also transmitted the infection to the attending nurse. The tourist had visited Zimbabwe and became ill on return to South Africa (Gear, Cassel et al. 1975). In 1980 another tourist and his physician contracted Marburg disease in Kenya. In 1987, 1 more case was reported in Kenya (Johnson, Johnson et al. 1996) Both incidents were associated with visits to the Kitum caves in western Kenya. The index cases both died (Smith, Johnson et al. 1982; Johnson, Johnson et al. 1996). The first major Marburg epidemic occurred in the Durba/ Watsa area of DR Congo in 1998 in a gold mining community. Out of the 149 cases reported, 83% died.

TABLE 1. KNOWN OUTBREAKS OF EBOLA AND MARBURG BY LOCATION AND YEAR, 1967-2012.

Virus	Year	Country	Location	Cases	Case fatality %
<i>Marburgvirus</i>	1967	Germany; Yugoslavia	Marburg and Frankfurt; Belgrade	32	23
	1975	South Africa	Johannesburg	3	33
	1980	Kenya	Nairobi	2	50
	1987	Kenya	Nairobi	1	100
	1998- 2000	DR Congo	Durba /Watsa	154	83
	2005	Angola	Uige	252	90
	2007	Uganda	Kamwenge	4	25
	2007	Uganda	Kamwenge	1	0
	2008	Uganda	Kasese	1	100
<i>Zaire ebolavirus</i>	1976	DR Congo	Yambuku	318	88
	1977	DR Congo	Tandala	1	100
	1994	Gabon	Mekouka	49	65
	1995	DR Congo	Kikwit	315	88
	1996	Gabon	Mayibout	37	57
	1996	Gabon	Booue	60	75
	2001	Gabon	Libreville	65	79
	2001	Rep. of Congo	Not specified	57	79
	2002	DR Congo	Mbomo	143	90
	2003	DR Congo	Mbomo	35	83
	2005	DR Congo		11	75
	2007	DR Congo	Luebo	264	71
	2008	DR Congo	Luebo	32	47
<i>Sudan ebolavirus</i>	1976	Sudan	Nzara	284	53
	1979	Sudan	Nzara	34	65
	2000	Uganda	Gulu	425	53
	2004	Sudan	Yambio	17	42
	2011	Uganda	Luwero	1	100
<i>Cote d'Ivoire ebolavirus</i> <i>Bundibugyo ebolavirus</i>	1994	Ivory Coast	Tai forest	1	0
	2007	Uganda	Bundibugyo	116	34
		DR Congo (Eastern)	Kampugwu	103	35

Source: Adapted from Leroy, Gonzalez et al, 2011; WHO 2012;;

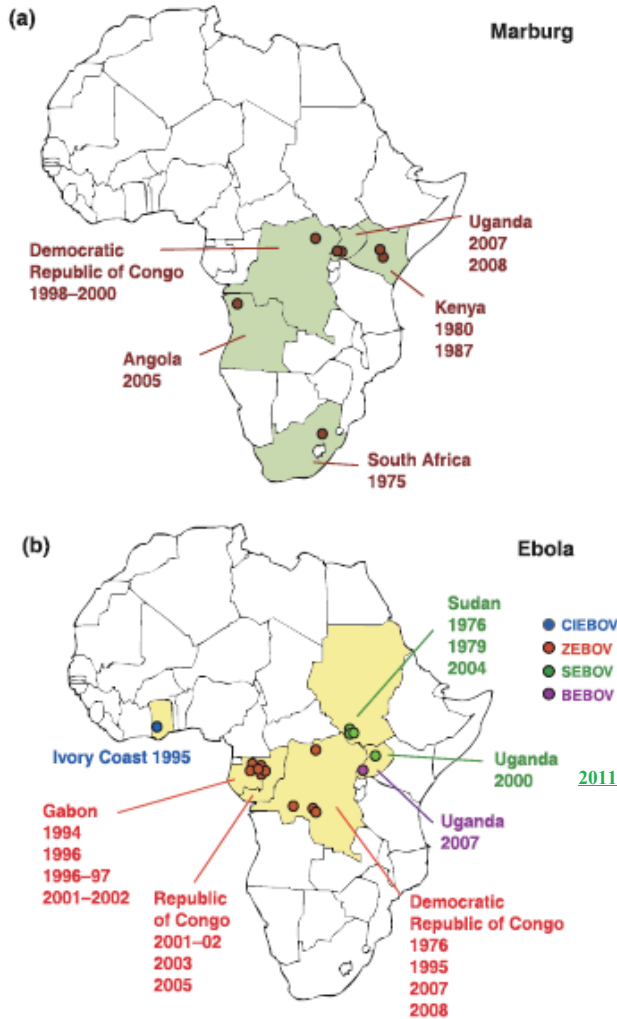


FIGURE 1 REPORTED OUTBREAKS OF MARBURG AND EBOLA IN AFRICA, 1967-2011

(SOURCE: ADAPTED FROM LEROY, GONZALEZ ET AL, 2011; WWW.CDC.GOV, 2012)

In 2004, a large outbreak occurred in northern Angola and of the 374 cases reported, 88% died (Bausch, Nichol et al. 2006). In 2007, an outbreak occurred in the remote Kitaka mines in Kamwenge district in western Uganda (Towner, Amman et al. 2009). One out of the 4 cases died. The cause of the epidemic was confirmed to be the *Marburg virus* Uganda (Towner, Amman et al. 2009).

1.2 Ebola virus

The Ebola virus belongs to the *Filoviridae* family of the RNA viruses, composed of the *Ebolavirus* genus and the *Marburgvirus* genus. So far only two species namely the *Ebolavirus* (EBOV) and the *Marburgvirus* (MARV) (Sanchez 2007) have been identified to be pathogenic. Both species are very virulent and cause very high mortality. Five distinct species of the genus *Ebolavirus* have already been identified. Only one species of the *Marburgvirus* genus is known so far (Table 2). The *Marburgvirus* and three Ebola viruses have caused severe outbreaks of haemorrhagic fever in humans.

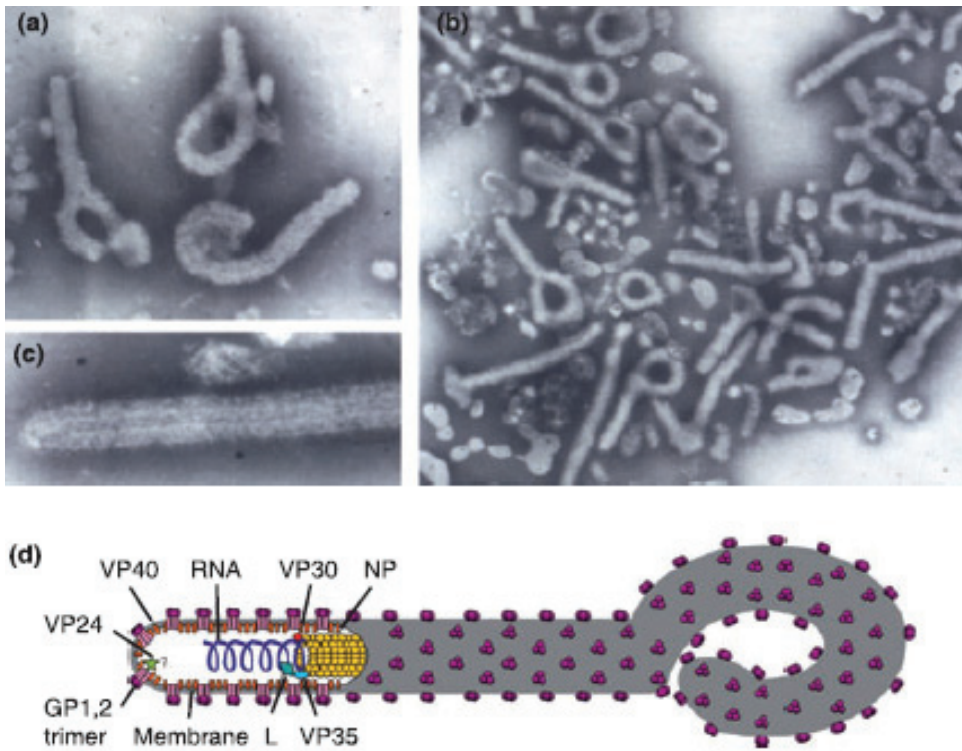
TABLE 2. THE KNOWN GENERA AND SPECIES OF THE FILOVIRIDAE^A

	Common abbreviation	New abbreviation	First discovered	Country
Genus Marburgvirus				
<i>Marburg virus</i>	MARV	MARV	1967	Germany
Genus Ebolavirus				
<i>Zaire ebolavirus</i>	ZEBOV	EBOV	1976	DR Congo ^b
<i>Sudan ebolavirus</i>	SEBOV	SUDV	1976	South Sudan ^c
<i>Reston ebolavirus</i>	REBOV	RESTV	1989	USA, Italy
<i>Cote d'Ivoire ebolavirus</i> <i>or Tai Forest ebolavirus</i>	CIEBOV	TAFV	1994	Ivory Coast
<i>Bundibugyo ebolavirus</i>	BEBOV	BDBV	2007	Uganda

^a By ICTV, the International Committee for the Taxonomy of Viruses; ^b formerly Zaire;

^c formerly Sudan

The Filoviruses are new emerging pathogens. They are RNA viruses. Both the Marburg and the Ebola particles have a unique filamentous non segmented shape (Figure 2) which is often used to distinguish them from other viruses and classify them. (Bowen, Platt et al. 1980; McCormick, Bauer et al. 1983). Particles may be worm-like, branched, circular or U-shaped. Some often assume hair pin shapes. The long filamentous particles are approximately 14000 nm long and 80 nm in diameter. Particles of 665 nm for Marburg and 805nm for Ebola are highly infective (Leroy, Gonzalez et al. 2011). The virions have a core of ribonucleoprotein (RNP) made up of a single strand of RNA and surrounded by lipid membrane envelope. The genome is made up of seven genes: the 3' leader, the nucleoprotein, the virion protein (VP) 35, VP40, and membrane associated glycoprotein VP30, VP24 and the L (large or polymerase) protein 5' trailer. The glycoprotein forms characteristic spikes on the virion surface. The spikes are 7 nm long and spaced (Kiley 1980).



Leroy
098

Source:(Kiley 1980);
EM et al 2011; MED
(RF)



MED098 [RF] © www.visualphotos.com

FIGURE 2 STRUCTURE OF THE FILOVIRUS (A,B,C,DAND E) AND SGHEMATIC REPRESENTATIONOF A FILOVIRUS (D).

www.visaulphotos.com)

(e)

Five distinct species of Ebola have been identified so far (Table 2), of which 3 have caused disease outbreaks in humans (Bowen, Platt et al. 1980; Sanchez 2007). The virulence of Ebola depends on the species of the virus (McCormick, Bauer et al. 1983) (Bowen, Platt et al. 1980; Sanchez 2007). The 5 different species of the *Ebola virus* have different levels of case fatality. Infections with the *Zaire ebolavirus* have the highest case fatality (90%) (McCormick, Bauer et al. 1983) while the *Sudan ebolavirus* is medium at 50-55% (Sanchez 2007). The *Bundibugyo ebolavirus* case fatality rate is lower at 34%. The *Reston ebolavirus* is non-pathogenic to man (Sanchez 2007). The *Cote d'Ivoire ebolavirus* has been reported in only a single case who survived (Formenty, Hatz et al. 1999).

1.2.1 Reservoir

Ebola is believed to be a zoonosis. The *ebolavirus* has been detected in carcasses of chimpanzees and some known primary human cases in Africa have been associated with contact with the meat of killed or dead primates (Georges-Courbot, Sanchez et al. 1997; Formenty, Boesch et al. 1999), (Formenty, Hatz et al. 1999). Humans and higher primates may be the end hosts for Ebola virus. For instance, a large epizootic among a chimpanzee colony has been reported in the Tai forest of Cote d'Ivoire and about 25% of them died (Formenty, Boesch et al. 1999). The green monkeys imported from Uganda were initially suspected to be the reservoir in the Marburg outbreak. However, when experimentally infected with the Marburg virus, the monkeys died from the disease, suggesting that they had not been the natural reservoirs of the virus (Simpson 1969). Bats have been implicated to be important reservoirs of the filoviruses (Figure 3). Both the Marburg and Ebola viruses have been demonstrated to be present in the Gabonese bat population. A high seroprevalence of both viruses was found especially in the *Rousettus aegyptiacus* species (Pourrut, Souris et al. 2009). Three other species of fruit bats (*Hypsignathus monstrosus*, *Epomops frangueti* and *Myonycteris torquata*) could also be reservoirs of the *Zaire ebolavirus* (Leroy, Epelboin et al. 2009), (Pourrut, Souris et al. 2009). Antibody studies in Gabon revealed that 4% of similar bats were positive for the *Zaire ebolavirus* immunoglobulin G (IgG). In western Uganda, genetically diverse Marburg viruses were isolated in 5% of cave dwelling fruit bats, *Rousettus aegyptiacus* (Towner, Amman et al. 2009) during a Marburg disease outbreak in 2007.

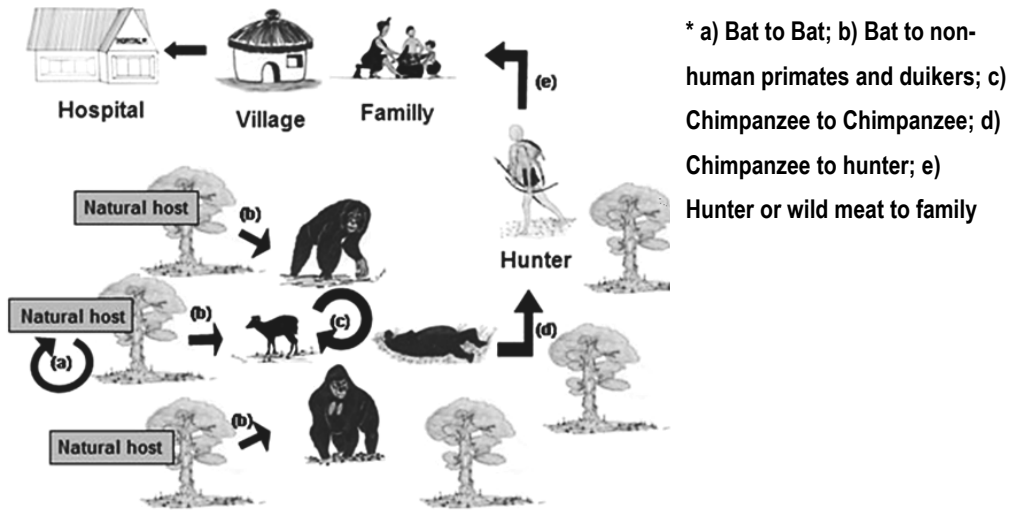


FIGURE 3 THE PRESUMED LIFE CYCLE OF THE EBOLA VIRUS*

Bats are hunted for food in some parts of Africa and may play a role in the transmission. The Ebola infection may be persisting asymptomatically in bats, being activated sporadically by stimuli, such as stress, co-infection, and pregnancy, as shown *in vivo* and *in vitro* experiments (Gupta, Mahanty et al. 2004). In 1998, an outbreak of the Marburg virus disease occurred in the gold mining town of Durba in the DR Congo. Of the 154 cases reported, 82%, mostly miners, died. The outbreak stopped when the mine flooded. A study revealed at least 9 diverse strains of the Marburg virus among the affected population, implying that the natural reservoirs could have been different in or around the caves, or in nearby locations (Bausch, Nichol et al. 2006). The Ebola virus has also been isolated in asymptomatic pigs in the Philippines suggesting that these animals may be reservoirs of the *Reston ebolavirus* (Barrette, Metwally et al. 2009). Sero-surveys have also revealed a sero prevalence as high as 15.3% for the *Zaire ebolavirus* IgG antibodies in some rural populations especially among the pygmies in Gabon and DR Congo (Bausch, Nichol et al. 2006). This may suggest that some rural communities too in Africa have already been exposed to various strains of these viruses. Thus the virus may be evolving and outcomes are unpredictable, as demonstrated by the Bundibugyo Ebola outbreak. Filoviruses may have a wider circulation than indicated by the localised outbreaks that have been reported.

New strains of Ebola continue to emerge in Africa and other endemic areas (McCormick, Bauer et al. 1983) (Bowen, Platt et al. 1980). There are also recent reports that the *reston ebolavirus*, is becoming more pathogenic, and has caused outbreaks of haemorrhagic disease with lung symptoms among previously asymptomatic pigs from the Philippines (Barrette, Metwally et al. 2009). Animal studies have shown that after a few passages of the *Zaire* or *Sudan ebolaviruses* in animal-to-animal transfer, it is possible to progressively increase the virulence to fatal disease in guinea pigs (Connolly, Steele et al. 1999). Ebola related virus has also been reported to have caused a fatal outbreak of Ebola-like haemorrhagic disease in captive macaques in the Philippines (Hayes, Burans et al. 1992). None of the monkey handlers fell sick but some of them sero-converted. There is therefore a

remote possibility that the Ebola virus transmission could occur through the pork and food chains (Barrette, Metwally et al. 2009). The potential use of haemorrhagic viruses as biological weapons has attracted international interest (Leroy, Gonzalez et al. 2011).

1.2.2 Susceptibility and transmission

Virulence and fatality appears to be Ebola species dependant (McCormick, Bauer et al. 1983). Factors related to susceptibility to infection may be related to the route of infection, inoculums or acquired natural immunity. For instance, the mean incubation period for cases of *Zaire ebolavirus* through injection was 3-6 days compared with 5-9 days for contact exposures (Bremam, Piot et al. 1978). A study on 85 cases demonstrated that case fatality associated with the injection route was 85/85 (100%), compared to cases of known contact exposure, which was 80% (119 out of 149 cases) (Bremam, Piot et al. 1978). Other studies have shown that the *Zaire ebolavirus* is highly lethal when given orally to Rhesus macaques (Jaax, Davis et al. 1996). Studies in animal models have demonstrated that exposure by intramuscular or intraperitoneal route, led to a faster disease outcome compared to exposure of animals to aerosol droplets. The aerosol route is believed to be rare in outbreaks (Geisbert, Daddario-Dicaprio et al. 2008). This observation is relevant to the management of cases coughing in overcrowded places. In Africa, non-human primates and bats are often hunted for food. If infected, such animals contain large amounts of the virus particles in the liver and spleen. If the meat, particularly the liver and spleen from these animals is ingested half-cooked, it may lead to infection (Geisbert, Hensley et al. 2003).

The Ebola virus is contagious and enters the body through contact with broken skin and mucosal surfaces of infected persons, dead or alive (WHO 1978; Sanchez 2007). This is supported by an observation of household contacts investigated in Zaire. Of the 173 contacts, 16% of close family contacts developed the disease (Baron, McCormick et al. 1983; Dowell, Mukunu et al. 1999). None of the 78 household members who never had physical contact with cases developed the disease (Dowell, Mukunu et al. 1999). Contact with infected wild mammals has also been believed to be the primary source of infection. The slaughtering of primates for food was associated with outbreaks of *Zaire ebolavirus* in Gabon (Georges-Courbot, Sanchez et al. 1997). Contact with freshly killed bats was also linked to an outbreak of Ebola in DR Congo (Leroy, Epelboin et al. 2009). Infectious Ebola particles have also been isolated from skin, body fluids, and nasal secretions of experimentally infected non-human primates (Jahrling, Geisbert et al. 1990). In poor healthcare settings, contaminated needles and syringes are a frequent source of infection for the healthcare workers and patients. Re-use of needles, for instance, played a key role in escalating the epidemics in Sudan and DR Congo in 1976 (WHO 1978). Needle stick accidental exposure has also been reported among some laboratory workers in Russia and Germany but is rare (Emond, Evans et al. 1977). Transmission takes place after onset of illness (fever). No infection before the onset of symptoms or until the detection of viral antigens (CDC 1988) has been reported. Sexual transmission is rare but has been reported seven weeks after clinical recovery and Ebola has been isolated from semen 61 days after onset of illness (Ksiazek, West et al. 1999).

1.3 Pathogenesis

The Ebola virus affects a wide range of cells but the lymphoid tissues are the primary site of Ebola infection. The liver, spleen, thymus, and lymph nodes and macrophage rich lymphoid tissue, seem to be important targets for the Filoviruses. Generalised lymphoid tissue necrosis as well as damage to liver, testis, ovaries and kidneys is typical and there is usually very little inflammatory response. Monocytes, macrophages and dendrite cells are the preferred replication sites, and they also support the dissemination of these viruses through the lymphatic system and to liver and spleen through the blood (Geisbert et al. 2003). This is probably why hepatocellular necrosis in infected human and non-human primates is common (Sanchez et al. 2007). This leads to haemorrhagic tendencies resulting from decreased synthesis of coagulation and other plasma protein factors because of the severe liver damage. Liver damage leads to decreased production of clotting factors and impairment of coagulation as manifested by bleeding tendencies (petechiae, ecchymosis and mucosal haemorrhages). Coagulopathy is associated with thrombocytopenia, degradation of fibrin and reduction of anticoagulant proteins. Viral antigens are usually present in many organs particularly the liver, thymus and adrenal glands (Geisbert et al 2003). The consequences of organ damage are a series of metabolic dysfunctions. The epithelium appears to remain structurally intact, but it has been suggested that infection of endothelial cells promote vascular cell structural damage. Bleeding may be caused directly by virus replication in endothelial cells (Yang et al. 2000). Similar findings have been found in experiments with non-human primates (Geisbert et al. 2003). The adrenal gland maintains blood pressure homeostasis. Its damage leads to reduced production of steroids, sodium loss and hypovolaemia. These are key features in Ebola haemorrhagic fever which lead to shock in the late stages. The spleen, thymus and the lymph nodes are also affected, leading to depletion of the lymphoid tissue and necrosis often noted in these organs in patients with fatal diseases. This leads to weakening and deregulation of the immune host system. Diffuse encephalitis is seen as in many viral infections. Laboratory parameters are usually nonspecific, but leucopaenia, lymphopaenia, and thrombocytopenia are often observed. Serum amylase aminotransferases are also usually elevated. In later stages the patient develops severe metabolic disturbances and goes into shock. Convulsions and diffuse coagulopathy develop in the terminal stages (Sanchez 2007). Haematological symptoms occur during the peak of the illness, during which the patients develop petechiae, ecchymosis, bleeding and oozing of blood from orifices and mucosa. Post-mortem often shows visceral effusions. Antibody response demonstrates a strong inflammatory response but its role in protection is not clear. (Ksiazek, Rollin et al. 1999).

1.4 Prevention against infection

1.4.1 Protecting the individual and the environment

The individual and the environment should be protected at all times as recommended by the principles of the protection of the health care worker and infection. The standards focus on three critical areas namely: i) disinfection and sterilisation, ii) isolation and safe management of cases iii) ensuring accurate diagnostics for early detection, with personal protection being enhanced by cleaning, disinfection and sterilisation (CDC 1988; WHO 1998.; WHO 2000; CDC 2005; WHO 2008.). Identification of vulnerable groups such as health care workers, bedside relatives must be carried out and the risk assessed. Appropriate

personal protective equipment should be selected before engaging or performing any manipulations on suspected patients. These standard infection control procedures include wearing of surgical gloves, face masks, procedure masks, to prevent soiling of the body by infectious fluids from encounters with the patients. Hand hygiene policy must strictly be implemented at all units. All health care workers must be aware of the danger. The availability of hand washing facilities with detergent and running water will reduce risk of contamination. Hand washing products (soap, alcohol based hand rub) must be available to minimise risks. Education of the health care workers, patients and the visitors mitigates such risks. Needle stick injury must be avoided at all costs. The environment can further be rendered safe by disinfecting and cleaning the surfaces and hospital linen with appropriate detergents (CDC 2009).

Filoviruses are quite stable and infectious at ambient temperature (~25 degrees centigrade; (CDC 2009). The Ebola virus is inactivated at 60 degrees centigrade, but the Marburg virus has been reported to resist desiccation (CDC 2009). Manipulative laboratory investigation on these viruses should only be carried out in a tertiary biosafety laboratory level 4 (BSL 4) facilities. Acceptable standard procedures must be followed strictly (WHO 2000; CDC 2005; WHO 2008.). The Filoviruses can be inactivated and killed by high doses of ultra-violet light, gamma radiation, lipid solvents or commercial hypochlorite. A commercial preparation of hypochlorite commonly used in Uganda was the *JIK* detergent. Hospital infection control and waste management policy need to be updated and rehearsed by all hospital staff, and be followed by regular drills. The recommended standard infection guidelines and procedures must be strictly be adhered to (WHO 1998.; CDC 2005). The hospital environment in low resource settings is potentially dangerous. Sensitisation about the dangers of the new reemerging infections is essential. Operational equipment must be sterilised. The hospital environment must be cleaned, disinfected and with detergents. Isolation of patients from non-case patients should be maintained at all times. Collaboration at national, regional, and international levels is required to ensure quality assurance of the screening and confirmation tests.

1.4.2 Capacity for laboratory diagnosis

A properly functional laboratory is essential for the detecting infection and monitoring the disease process. Upgrading the laboratories to Biosafety level 4 (BSL 4) is essential. It is critical that the virus must be inactivated first before the analysis of any samples (WHO 2000; CDC 2009). The available laboratory tests for diagnosing Filoviruses are outlined in Table 3. The procedure for testing these viruses at the UVRI is outlined in Appendix 7. Figure 4 shows the critical timelines between infection, infectiousness, and the corresponding antibody and antigen laboratory parameters. The IgM antibody is associated with the early stages of the infection (two days post onset) but quickly declines and disappears within about one month. The IgG specific antibodies develop between 6-18 days after onset of illness and remains so for many months. The viral antigen is detectable in blood from the third day up to 7-16 days after onset of symptoms (Kiley 1980; Ksiazek, Rollin et al. 1999; Ksiazek, West et al. 1999; Sanchez 2007). These tests are useful for determining admission to isolation wards and managing patients (Appendix 8). The ideal laboratory capacity may not be available in low resource settings. Collaboration and partnerships with regional and international laboratory networks provides the back-up alternative.

Immunological Response to Ebola virus infection

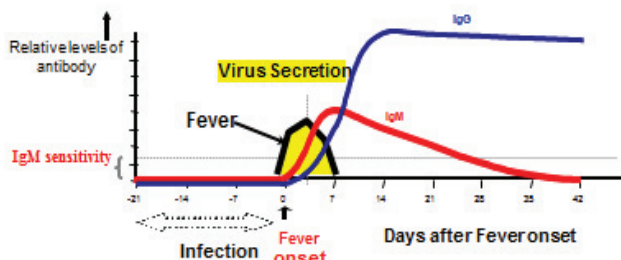


FIGURE 4 IMMUNOLOGICAL RESPONSES TO EBOLA VIRUS INFECTION

The tests outlined below are the commonly used for case detection and routine management of cases (Table 3).

TABLE 3 LABORATORY TESTS FOR FILOVIRUSES

Test	Target	Sample	Remarks
Enzyme linked immunosorbent assay (ELISA)*			Rapid, simple, sensitive, specific
IgM	Viral antibodies	Serum	
IgG	Viral antibodies	Serum	
Antigen detection ELISA*	Viral antigen	Blood, serum, tissues	Rapid, simple, special equipment required
Real time polymerase chain reaction (RT-PCR)*	Viral nucleic acid	Blood, serum, tissues	Rapid, sensitive, special equipment required
Indirect Immuno-fluorescence assay (IFA)	Viral antibodies	Serum	Prone to non-specific positives
Immunoblot	Viral antibodies	Serum	Protein specific
Immunohistochemistry (IHC)	Viral antigens	Tissues	Slow
Fluorescence assay	Viral antigens	Tissues	Rapid but interpretation subjective
Electron microscope	Viral particle	Blood, tissues	Special equipment required, costly
Isolation of virus	Viral particle	Blood, tissues	Slow

* In use in Uganda

Source: (Kiley 1980; Ksiazek, West et al. 1999; CDC 2005)

1.5. Uganda

Uganda is a landlocked country on the Equator. It had an estimated population of 33 million in 2011 with an annual population growth of 3.2%. Some 4.9 million (17%) live in urban areas and over 80% of the population live in rural areas. The life expectancy is 48 years for men and 52 years for women (UBOS 2011). The average income per capita in 2011 was about 370 US dollars and the mean health expenditure per capita was 19 USD. The average literacy rate has improved to 71%, with males at 81% and women lagging behind at just 61% (UBOS 2002; UBOS 2010). Table 4 compares the key indicators between Uganda and Norway. Despite current efforts, poverty levels are still high although proportion of people living below the poverty line, that is less than one dollar a day, has decreased from 52% in 1992 to 31% in 2005 and to 25% in 2010. Poverty has a direct impact on common communicable illnesses like malaria, malnutrition and diarrhoea.

TABLE 4: COMPARISON OF HEALTH INDICATORS BETWEEN UGANDA AND NORWAY, 2011

Health indicator	Country	
	Uganda	Norway
Population (million)	33.7	5.0
Population growth per year %	3.6	0.9
Life expectancy, years	49	81
Infant mortality rate per 1000 live births	68	3
Under 5 child mortality per 1000 children	187	3
Maternal mortality ratio, per 100,000 live births	437	7
Total fertility	6.3	1.9
Proportion of births attended by skilled staff %	42	100
HIV Prevalence %	6.5	0.1
Doctor : patient ratio	1: 25 000	1 : 250
Nurse : patient ratio	1: 4000	1 : 60
Income per capita (USD)	480	86440

Source: (UBOS 2010); UNICEF, State of the World's Children 2011 (www.unicef.org/sowc2011).

1.5.1 National health care system

The national health care delivery in the country is carried out through a decentralised system based on districts (LC5) and local councils. The district administration has other layers of administrative governance exists. These levels are composed of: the county (LC 4), the sub-county (LC 3), the parish (LC 2), and the villages (LC 1) with corresponding populations of

TABLE 5: ORGANISATION OF PUBLIC HEALTH CARE DELIVERY, UGANDA

Health facility	Services available / structure	Level/ location/ Population
Health centre I	Village Health Team, no physical premises	Village Population 2,000
Health centre II	Outpatients clinic only; immunisations; operated by one nursing assistant	Parish Population 5,000-6000
Health centre III	Outpatient clinic; inpatient clinic with 10-20 beds for maternity, general wards; supported by laboratory; clinical officer and nurse	Sub county Population 25,000
Health centre IV	Outpatient clinic; inpatients with 20 beds for maternity, general wards and laboratory, blood transfusion support; caesarean done; medical officer in charge; staff 20	County Population 100,000
General hospital	Hospital, beds 200, staff 100 laboratory, x-ray; medical superintendent	District Population 500,000
Regional referral hospital	Consultants and tertiary services, beds 400, staff 200	Region (10-15 districts) Population 1,000,000-3,000,000
National referral hospital	Advanced tertiary services and super specialists	National (3 hospitals) Population over 10,000,000

The health care system in Uganda, 2000-2015. Adapted from the Government of Uganda, Health Sector Strategic and Investment Plan, 2010-2015). ** The informal sector includes traditional healers, outcome and private practitioners.*

100,000, 20,000, 5,000 and 1,000 respectively. The average population in each district varies but is ~500, 000. A health care referral system are organised alongside these council tiers, starting from health centre I (village) through health centres II (parish), health centre III (sub county), health centre IV (county) and the district general hospital (Table 5). The regional referral hospital receives patients from the general hospitals. Three national hospitals provide tertiary consultations and care. Medical and nurses training schools often operate within the national and regional hospitals. The public health system is complemented by religious organisations (about one third) and by private and community

based NGOs, which also provides about a third of the service. In remote and hard to reach areas, informal health care is provided by traditional healers. Surveillance system exists in each region and district. Reports are regularly sent to Ministry of Health by the district health officer on a monthly basis.

Competing priorities in the health sector and Ebola

The capacity of the health sector is low and has deteriorated over time. Ebola is an additional burden which could divert the meagre resources from essential public health programs. About 75% of the disease burden is due to common preventable maternal and child health conditions and communicable diseases (MoH 2008) (MOH 2000; MoH 2008; MoH 2010). The major causes of the burden of disease are: perinatal and maternal conditions (20.4%), malaria (15.4%), respiratory infections (10.5%), AIDS (9.1%) and tuberculosis, diarrhoea and measles. Tropical parasitic diseases like trypanosomiasis are still highly endemic (MoH 2008; Uganda 2009; UBOS 2011). Child survival in Uganda is carried out through public health programmes directed at the control of infectious diseases, promotion of maternal health (MoH 2008; Minister 2010). Maternal mortality reduced from 527 to 438 per 100,000 live births between 1995 and 2010. Infant mortality decreased from 81 to 76 per 1000 live births (UBOS 2010; UBOS 2011). Under-5 mortality decreased from 156 to 137 per 1000 live births. Underweight has reduced moderately from 23 to 16%, while stunted growth reduced from 41 to 38.5% over the same period (UBOS 2010; UBOS 2011). About 70% of the child mortality is due to preventable diseases namely: malaria (32%), pneumonia (8%), meningitis (10%), HIV/AIDS (5.6%), and malnutrition (4.6%) (UBOS 2011). The children of mothers who have completed 12 years of formal education had only 50% malnutrition compared with those whose parents never went to school.

The infrastructure is inadequate and has deteriorated in some areas (Uganda 2009). Only 28% of the existing 154 Health Centre IVs are fully operational. Human resources for health are grossly inadequate. Only 51% of the approved job positions at national level were filled at the government units (UCMB 2007; MoH 2010). Migration of workers is leading to a massive brain drain out of the country (MoH 2008). Epidemic emergency preparedness was poor as only 52% of suspected disease outbreaks were investigated within 48 hours of notification in 2010 (MOH 2013). With regard to medicines, only 28% of health facilities have constant medicines and supplies all year round. This is not surprising since only 50% of the essential supplies required for providing the essential health package is provided in the budget (Uganda 2009; MoH 2010). Out of the projected USD 4.06 per capita on health, only USD 2.39 was provided for in 2007. Nearly 90% of the medicines are imported. Household out-of-pocket expenditures on health are high compared to their very low incomes (Xu 2007). Of the estimated USD 41.2 dollars per capita budgeted to provide the minimum requirement for the basic health package for the financial year 2008/2009, only half was available (MoH 2010).

2.0 AIM AND STUDY OBJECTIVES

2.1 Aim

The general aim of this thesis is to describe the three Ebola outbreaks in Uganda from 2000 to 2011.

The specific objectives were:

- 1) To describe outbreaks of *Ebolavirus* hemorrhagic fever in Uganda from 2000-2011
- 2) To establish the risk factors associated with the *Bundibugyo ebolavirus* outbreak in Uganda (*Paper III*).
- 3) To estimate the case fatality rate associated with the *Bundibugyo ebolavirus* outbreak in Uganda, (*Paper IV*).

2.2 Rationale of the study

Few countries have experienced large outbreaks of Ebola like Uganda. Despite the known constraints and challenges in the low resource settings the outbreaks were eventually contained. To make improvements we need to share knowledge and experiences about the disease and the challenges in the management.

3. STUDY SUBJECTS AND METHODS

3.1 Study setting

The thesis work was carried out from August 2000 to December 2011, during which the three outbreaks of Ebola hemorrhagic fever occurred in the districts of Gulu, 2000; Bundibugyo, 2007 and Luwero, 2011. The Gulu outbreak also associated with limited outbreaks in the districts of Masindi and Mbarara (Figure 5)

3.2. Study sites and populations

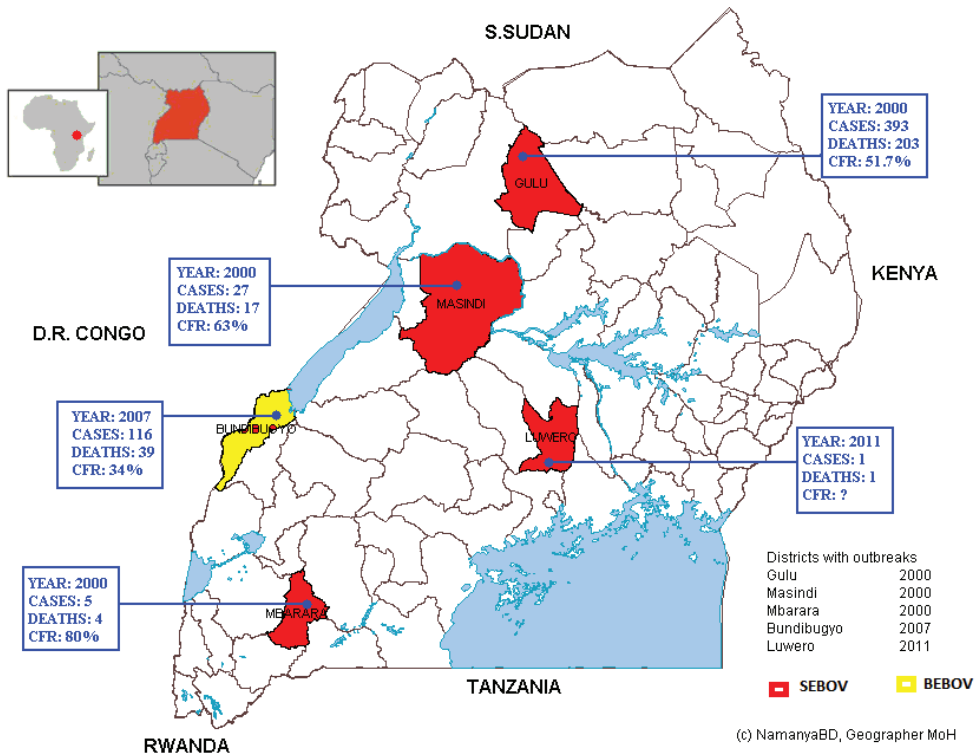


FIGURE 5 MAP OF STUDY SITES BY DISTRICT, UGANDA

The affected population was about close to 2,500,000, which is about 8% of the total inhabitants in Uganda. The district populations (Table 6) associated with each outbreak were distributed as follows: Gulu - 1,911,000; Bundibugyo – 267, 000; Luwero- 341,000 (UBOS 1991; UBOS 2002).

TABLE 6: POPULATION IN THE EBOLA AFFECTED districts, 2000-2011, UGANDA*

Year	District	Population (estimate)	Ebola species	Remarks
2000	Gulu	404,000	<i>Sudan ebolavirus</i>	Gulu epidemic, largest
	Masindi	314,000	<i>Sudan ebolavirus</i>	Related to Gulu outbreak
	Mbarara	1,193,000	<i>Sudan ebolavirus</i>	Related to Gulu
2007	Bundibugyo	267,000	<i>Bundibugyo ebolavirus</i>	New Ebola species
2011	Luwero	341,000	<i>Sudan ebolavirus</i>	Smallest- 1 case only

* UBOS 1991, 2002

Gulu district

Gulu lies approximately 500 km north of Kampala and borders South Sudan. The district had 5 counties, 23 sub counties, 116 parishes and 377 villages (Figure 6). It is served by a 400 bed regional hospital in Gulu. Nearby, Lacor missionary hospital also provides consultant services. A training school for nurses is attached to the facility. The prolonged conflict between the Lord's Resistance Army (LRA) rebels and government troops had greatly affected the delivery of health and other social services in the region. Approximately 2.3 million people were affected and up to 1.7 million inhabitants lived in camps for the Internally Displaced Persons (IDP) in the district. The camps lacked adequate sanitation, water and shelter. An estimated 200,000 refugees from Sudan also resided in northern Uganda (WHO 2005). Nearly 80% of the IDPs were women and children. Homeless and trying to survive, many were subjected to different risks arising from the conflict. Children were particularly vulnerable; the LRA had abducted 12,000 children (WHO 2005). An additional 44,000 children travelled to towns from outlying areas every night to escape abduction and attack by the rebels.

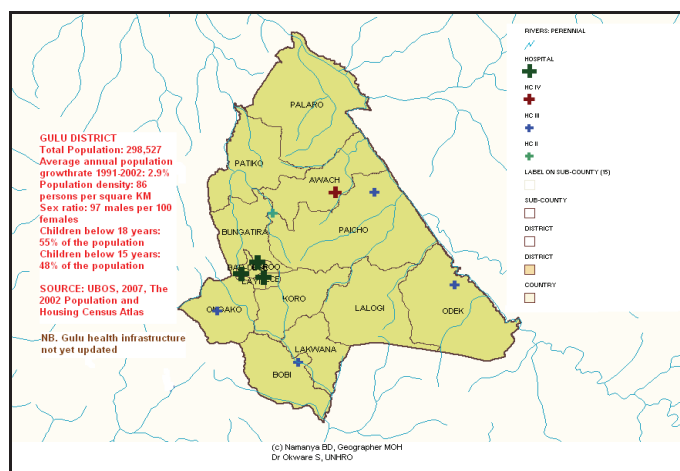


FIGURE 6 MAP OF GULU DISTRICT, UGANDA

Bundibugyo district

Bundibugyo is a remote district (about 450 km) west of Kampala on the border with DR Congo (Figure 7). It is a mountainous area and 60% of the district is a game reserve. The area is heavily forested and its beautiful volcanic hot springs attract tourists. People live either in the valleys or on the mountain slopes (Figure 8, 9) and communication is difficult.

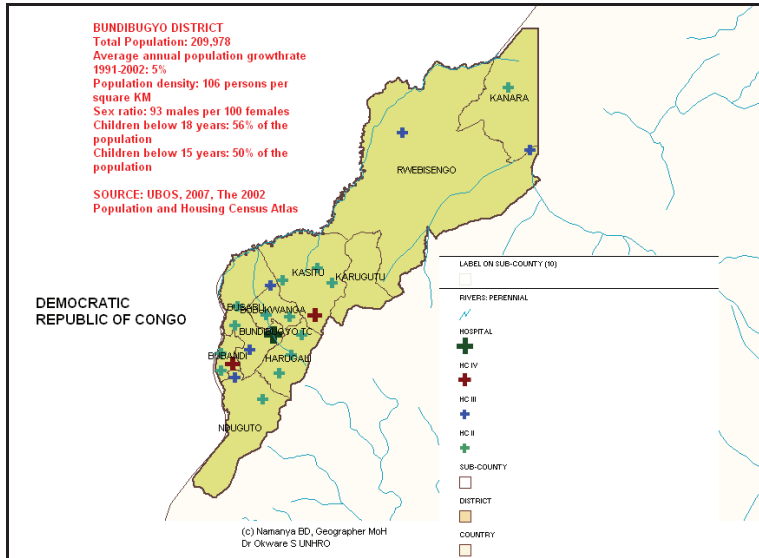


FIGURE 7 MAP OF BUNDIBUGYO DISTRICT, UGANDA



FIGURE 8 RWENZORI MOUNTAIN SLOPES, DIFFICULT TERRAIN, BUNDIBUGYO, UGANDA

Travel including access to healthcare is on foot down or up the mountains. The population is scattered in small homesteads. The population engages in subsistence farming on the slopes and foothills of the Ruwenzori Mountains. They grow bananas and potatoes for home consumption and export excess produce to nearby DR Congo across the border. Hunting of game including monkeys, supplements their diet. The pygmy ethnic groups live in relative isolation in the forests. The district was the centre of an insurgency five years earlier. The district is served by a 100-bed rural hospital (Figure 10) and 26 health centres.



FIGURE 9 PEOPLE IN BUNDIBUGYO TOWN, AT NARROW VALLEYS OF RWENZORI MOUNTAINS, BUNDIBUGYO, UGANDA



FIGURE 10 BUNDIBUGYO HOSPITAL, UGANDA

Luwero district

Luwero district (Figure 11) lies just 40 km north of the capital city of Kampala. It has a population of about 341,000 (UBOS 2002) who live in rural homesteads and engage in subsistence farming. Coffee is the main cash crop, while banana is the traditional staple diet for the indigenous Baganda inhabitants. Literacy is quite high and the people have on average higher incomes than people in other rural districts. Communication by road is good and motorized. Mobile cellphones access and media coverage is almost universal.

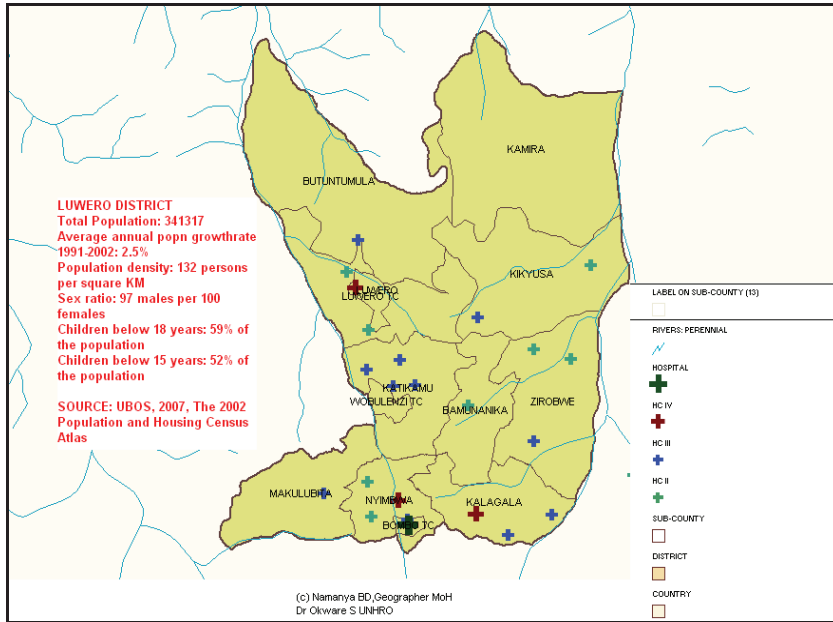


FIGURE 11 MAP OF LUWERO DISTRICT, UGANDA

Most of Luwero is covered by tropical forests rich in wildlife, including primates of various kinds. There is one public 100-bed hospital, 2 private hospitals and a medium-sized military hospital in Bombo town. Several trading centres sell merchandise, household items and services similar to those in the capital. The district suffered a serious disruption of social services due to a civil strife and insurgency in the 1980's. This civil war also led to massive displacement of large populations in the area.

3.3 Methods

3.3.1 Linkages between the papers

The thesis starts with *Paper I* and *Paper II* that developed the initial tools for the study for documenting the outbreak and the national response. The experience and these early instruments were used to improve procedures and case definition for the management of the subsequent outbreaks. Tools and instruments for data collection were revised and refined

over time. The minimum package for personal protection was updated continuously to enhance safety. Inventories (expertise, laboratory, supplies and logistics) from the past epidemics were brought forward and systematically redeployed when the need arose. The Gulu outbreak became the reference point for knowledge, action, processes and resources as well as a basis for monitoring progress in later action. In 2007, anecdotal evidence indicated that a similar pathogen, later discovered to be the BEBOV was different and less lethal. This was in part was the reason for the detailed studies to establish the risk factors (*Paper III*) and case fatality (*Paper IV*) associated with the new virus. It was therefore necessary to establish the risk factors involved in the transmission in order to minimize the delay in taking action. In addition, by studying and estimating the case fatality, *Paper IV* addressed the speculation that the new BEBOV was a less lethal Ebola strain. The new understanding will help improve the management of future outbreaks cautiously and with an open mind. This is demonstrated by the effective management of subsequent outbreaks. Thus the conceptual framework considers outbreaks with delayed action, and carrying forward the experiences and resources forward and linking them to the subsequent outbreak, thus yielding prompt detection and best scenario in 2011. The conceptual framework and the linkages are outlined in Figure 12.

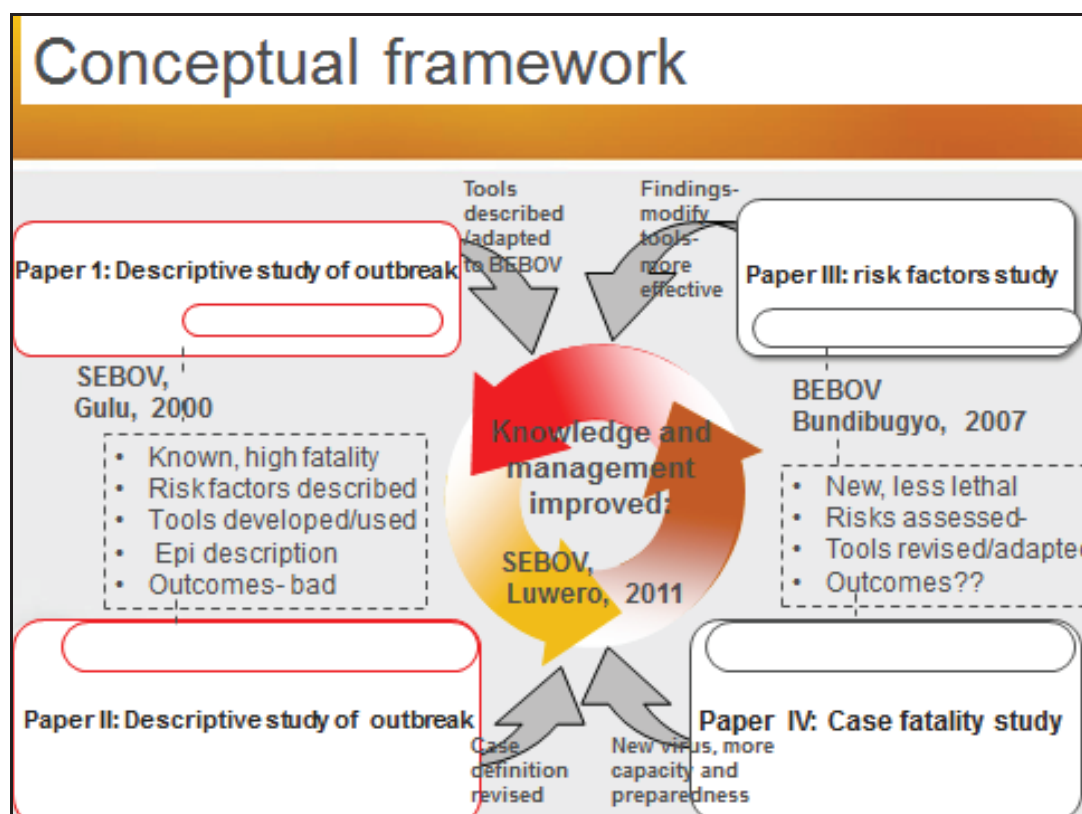


FIGURE 12 CONCEPTUAL FRAMEWORK OF THE STUDY

3.3.2 Study design summary

The summary (Table 7) links the study objectives, designs, methods, data collection and analysis for the three outbreaks. The timelines for the data collection are outlined in Figure 13.

TABLE 7: SUMMARY OF THE OBJECTIVES, DESIGN, METHODOLOGY, DATA COLLECTION AND ANALYSIS

Objective	Design, site, population and method	Data collection	Data analysis
Objective 1: <i>To describe the three outbreaks of Ebola in Uganda</i>	Design <i>Paper I and II:</i> Descriptive study of outbreak by subtype; Site & Population: - Gulu - 404,000 inhab. (SEBOV), - Bundibugyo - 267,000 inhab. (BEBOV) - Luwero - 341,000 inhab. (SEBOV); Method - Serial epidemiological identification and description of cases (Table 8) and contacts (Appendix 1,2 3) and paragraph 3.3.3.1; 3.3.3.2 - Blood tested accordingly (para 3.3.3.3)	- Questionnaires interviews (Appedix 1, 2, 3) used; -collection procedures described in para 3.3.3.2	- -Epi Info software (paragraph 3.2.6) managed and analysed data; also Epimap -descriptive statistics -applied multi logistic regression analysis; also the 2 sample t-test; Chi ² test; Fisher's exact test; p =<0.005
Objective 2: <i>To establish the risk factors associated with the Bundibugyo ebolavirus outbreak</i>	Design <i>Paper III:</i> A case control study between <i>true cases</i> and <i>non-case controls</i> Method - Case control study on cases identified by the community (para 3.3.3.1) - re-assessed as: a) <i>true cases</i> or (b) <i>non-cases (controls)</i> (paragraph 3.3.3.2) - estimated risk factors as in paragraph 3.3.4	- Questionnaires interviews (Appedix 1, 2, 3) used; - collection procedures described in para 3.3.3.2	- Risk factors computed by comparing Odds ratios (OR) between <i>true cases</i> with <i>non-cases</i> -applied bivariate logistic regression analysis (Table 15); Chi x ² test of significance; and p =<0.005.
Objective 3: <i>To estimate the case fatality rate associated with the Bundibugyo ebolavirus outbreak</i>	Design - A descriptive study to estimate case fatality rate of the BEBOV Method - A subset of laboratory confirmed cases were identified on the basis of a positive laboratory test of samples taken during the acute phase only (outlined in Figure 30 and also details in paragraph 3.3.4; 4.2.3); and Appendix 7 - Case fatality calculated	- Questionnaires interviews (Appedix 1, 2, 3) and used data collection procedures described in para 3.3.3.2 -Blood samples from acute and convalescent tested - procedures detailed in paragraph 3.3.4;	- Case fatality rate calculated among the laboratory acute phase patients only -determined whether symptoms, age and gender, were associated with survival -applied the 2 sample t-test; Fisher's exact test ; Chi x ² test of significance and p =<0.005 (Table 16)

Timelines for data collection: 2000-2011

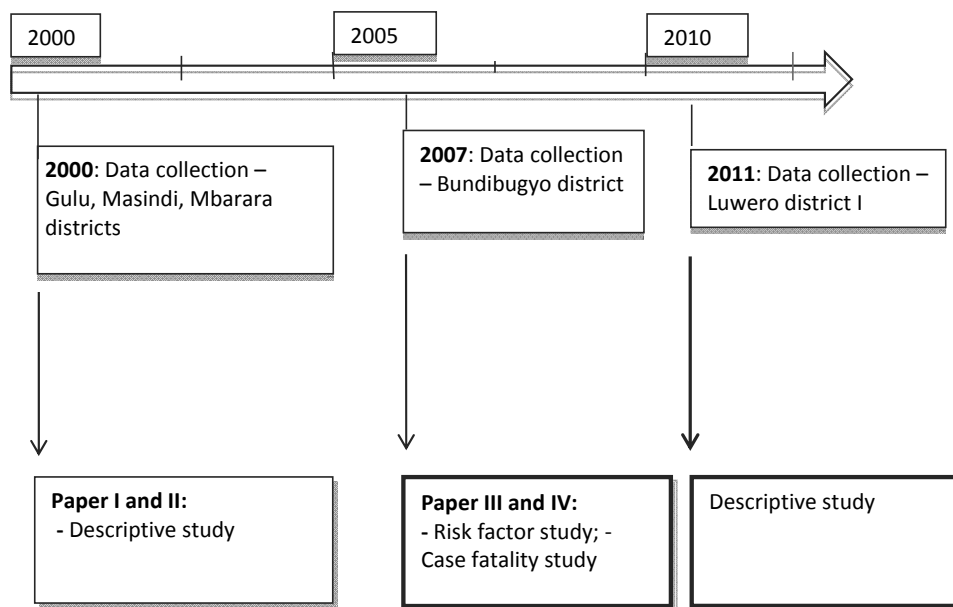


FIGURE 13 TIMELINES FOR DATA COLLECTION 2000-2011

3.3.3 Methodology and tools for data collection

3.3.3.1 Adapting the Case definition

A team from the MOH was sent to conduct initial investigations on a suspected strange disease in Gulu following a communication from the district director of health services. Similar deaths in rural villages were also reported by the media. Nurses were among the victims and several more were admitted to Lacor hospital. The team was comprised of an epidemiologist, a clinician, a laboratory technician, an environmental officer and a virologist from the UVRI. The team adapted standard tools for epidemiological investigation (including person, place, and time) to generate check lists of cases and deaths. Interviews were primarily conducted on the households with cases and contacts. Interviews also targeted the district health personnel, administrators, and community leaders. Hospitals and other health facilities were also targeted for review of cases, and contacts as well as conduct of verbal autopsy on any deaths. Clinical assessment of cases was conducted and clinical records of patients in hospitals and other health facilities were reviewed. Blood specimen was taken for advanced analysis abroad as a viral haemorrhagic disease was highly suspected. A national cases definition for Ebola (Table 8) was adapted from the World Health Organisation guidelines (WHO 1998; WHO 2006). Three distinct categories

based on clinical symptoms namely: “suspected”, “probable” and “confirmed” were adopted as the case detection. The “*alert*” category was integrated to the case definition in the Gulu outbreak. The team also made an assessment of the resources available at the district as well as stockouts of essential supplies and logistics. The team prepared a report which was discussed further and on the basis of which adapted tools (Appendix 1, 2, 3) for case management, contact tracing and surveillance and education were developed. No validation of the case definition was conducted.

TABLE 8: ADAPTED CASE DEFINITION FOR EBOLA HAEMORRHAGIC FEVER ADAPTED UGANDA, 2007

<i>Suspected cases*</i>	Sudden onset of fever and at least 4** of the following symptoms in a resident of or visitor to the affected areas in the district: <i>vomiting, diarrhoea, abdominal pain, conjunctivitis, skin rash, unexplained bleeding from any body part, muscle pain, intense fatigue, difficulty swallowing, difficulty breathing, hiccups, or headache</i> since suspected onset, 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.
<i>Probable case</i>	Suspected EHF in any person (dead or alive) with at least 3 of the following symptoms: <i>vomiting, diarrhoea, or unexplained bleeding from any site, conjunctivitis, or skin rash</i> ; AND either 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.
<i>Confirmed case</i>	Laboratory confirmation of infection by isolation of virus from any body fluid or tissue, OR detection of <i>viral antigen</i> in any body fluid or tissue by antigen-detection ELISA, reverse transcription-PCR, or immuno-histochemistry, OR demonstration of serum ebola virus-specific IgG antibodies by ELISA, with or without IgM, in any person with suspected or probable EHF.
<i>Contact</i>	A person who had slept in the same household and/or had direct physical contact with a person (dead or alive) with suspected, probable, or confirmed EHF, and/or had been exposed to an infected person or to an infected person's secretions, excretions, tissues, or linen within 3 weeks after that person's onset of illness.

* The “Alert ” category was used only in Gulu and dropped in subsequent epidemics and merged with suspected “category” This category presented with sudden onset of fever, sudden death or any evidence of hemorrhage in a person (dead or alive).

** Only 3 not 4 symptoms were required for the “suspected” category in the Gulu outbreak

Community based teams of volunteers were facilitated to identify cases and refer them to hospital (paragraph 3.3.5). The data and information on the outbreak was subsequently sent to the surveillance officer at the communications centre (Figure 13). The timelines and the procedures for data collection for each of the outbreaks and studies is outlined in the paragraphs (3.3.3.1, 3.3.3.2, 3.3.3.3) below.

3.3.3.2 Procedures for data collection

Gulu outbreak

Tools and questionnaires adapted from the WHO verbal autopsy guidelines and related sources (Anker 1999; Georges, Leroy et al. 1999; Roels 1999; WHO 2000; WHO 2003) were used to record and collect data from the cases and contacts (Appendix 1,2,3,). Physicians developed the algorithms and monitored the application of the criteria. Epidemiological links with clinical diagnosis guided the interviewers. The tools were developed in English and then translated to local dialects. The tools were administered with sensitivity taking into account the local cultural norms. Educated interviewers were recruited and trained on clinical skills and coding. Completed questionnaires were checked and coded by supervisors before being entered into data bases.

Each case detailed clinical and epidemiological information and data was recorded in case report forms (Appendix 1,2,3) When mobile teams identified a suspected case, lists of all possible contacts were recorded using the contact report form. Information and data was systematically collected from cases or parents and the next of kin were interviewed in case the patient had died before the interview. The reports were forwarded daily to the surveillance team coordinator at the district health office. At the hospital, a technician recorded similar information for new cases admitted. An identification code and numbers were serially given to each admitted patient. Each patient was given an identification code. The technician forwarded blood sample to the Ebola specific laboratory while the case report form and the contact forms were sent to the surveillance team, together with the name and identity code of the case. The laboratory results were sent to the surveillance team, with copies to the hospital. The surveillance team checked the symptoms, and the case definition application. This information and data was then entered into an Epi Info database. Progress at the isolation wards was recorded daily. A triage point was created in the outpatient clinics for screening and notification of cases to mobile teams. Line lists of contacts, newly infected villages and other relevant information was given to mobile teams for follow up. Village health teams made door-to-door visits of homes in their villages in search of contacts. The mobile teams used algorithms (Appendix 4) to screen and identify suspected cases. Health records at the health units were also reviewed. Data was shared with several agencies in the field.

Bundibugyo outbreak, 2007

Around mid -August 2007 there were reports of an insidious strange disease affecting people in Bundibugyo district. Earlier in August, the Ministry of Health sent teams from the Uganda Virus Research Institute to investigate 2 cases of diarrhea that had died, but the investigation was inclusive. Subsequent repeated epidemiological investigations during the following 2-3 months yielded no clue. On the 5th of November the Ministry of Health received reports that another 20 deaths had occurred including health care workers. Assistance was sought from the CDC and an epidemiological team arrived in early November 2007 to support the investigation. Epidemiological data was collected and blood samples too were taken accordingly (Appendix 1,2,3). The samples were initially tested at the UVRI using standard procedures (Appendix 7) guided by the CDC (CDC 1998; WHO 2000; WHO 2003; CDC 2005). There were no apparent clues. Parallel aliquots of the blood specimen were shipped to CDC, Atlanta, GA, USA for advanced analysis. On the 27th November 2007, a new novel Ebola virus was isolated by the CDC Special Pathogens Branch unit. The CDC confirmed the new virus to be the *Bundibugyo ebolavirus*. The

national response was launched on the 28th November 2007. The UVRI laboratory facility was upgraded to a BSL4 facility able to test the new novel BEBOV virus routinely using the procedures outlined (Appendix 7). An isolation ward was set up immediately at Bundibugyo hospital and Kikyoo health centre. Health care workers with experience from the Gulu outbreak were redeployed to manage cases in the isolation wards. Case management was supported by teams from MSF and the WHO. From the 29th of November 2007 active case search using the adapted case definition (Table 8) was intensified together with public education in all districts as part of the national response (paragraph 3.3.5). Methods similar to those used in Gulu (Appendix 1,2,3) were applied to collect data and information from patients and contacts.

Methodology for investigation of risk factors (Paper III)

A case control study of the risk factors in the Bundibugyo outbreak (*Paper III*) was designed and conducted. Exposures to suspected risk factors within 3 weeks preceding onset of the disease was ascertained through interviews and a coded adapted questionnaire (Appendix 1, 2,3,). Information and a social history were collected for each of the cases identified by the mobile teams. Reassessment by the surveillance team supervisors further segregated and reclassified the cases into 3 categories namely: *laboratory confirmed*; *remained probable*; were *laboratory negative* and as such re-categorized as *non-cases*. The combined category (suspected, probable, confirmed combined) were reclassified by the supervisors as *true (cases)*. The remaining category who did not meet the criteria was considered *non cases* (controls). The *non-cases* were then regarded as the control reference group. The analysis (Table 15) of the risk factors is discussed under the results section. The case fatality was also calculated on the basis of the 116 *true cases*.

Methodology for estimating case fatality (Paper IV)

During this time a quantitative study was carried out to estimate the case fatality of the BEBOV outbreak. Adapted tools and similar methodology (Appendix 1, 2,3,) were again used in this study. Blood samples from inpatients with acute fever in the isolation units or identified in the community using the adapted case definition were collected and tested for *Bundibugyo ebolavirus*. A subset of 56 laboratory confirmed cases were identified on the basis of a positive test of samples taken either in the acute phase, the convalescent phase or both. Another group of Ebola survivors with no acute phase blood samples available for testing had their convalescent blood samples tested. The tests followed the procedures adapted (Appendix 7) at the Uganda Virus Research Institute between November and December 2007. Virus isolation and other related advanced tests and were carried out at CDC, Atlanta. The case fatality was calculated on the basis of deaths among only positive blood sample taken during the acute phase.

Luwero outbreak, 2011

On the 4th of May 2011, the Ministry of Health received a report from the Luwero district director of health services of a suspected hemorrhagic disease from Nakisamata village, some 50 miles north of Kampala. The report indicated the suspect to be a young girl who was subsequently admitted to Bombo Military hospital on the 6th May 2011. She had a history of fever and bleeding manifestations (epistaxis, conjunctivitis, gingivitis, hematuria,

hematemesis, and vaginal bleeding). The attending physician made a provisional diagnosis of disseminated intra vascular coagulopathy. A differential diagnosis of a viral hemorrhagic fever (VHF) was also considered. The patient was isolated and oxygen administered using an intra-tracheal tube. She died soon after admission. The health care workers were warned to take precautions. Infection control measures were instituted by wearing gowns, gloves, and masks. The body was disinfected, wrapped in a body bag and then taken to the nearby mortuary and transferred to a wooden coffin for burial. Relatives were instructed not to open the coffin or touch the body. A blood sample had been taken for analysis.

A team from the MOH visited the area the following day for verification. On May 13, 2011, another team that included experts from the MOH and CDC visited Bombo hospital and the affected village. Four asymptomatic family members with presumed contact with the case were investigated and blood samples taken. Close contacts of the patient were followed up. On the 15th May 201, two more teams arrived from CDC, Atlanta, GA, USA, and MSF arrived to support laboratory surveillance and patient care respectively. The team travelled to the outbreak site the following day and took samples. Bats were trapped and blood specimen taken. Laboratory investigations on blood samples were carried out using standard ELISA and PCR procedures(Appendix7) for SEBOV and other EHF's (Ksiazek, Rollin et al. 1999; Ksiazek, West et al. 1999; Towner, Sealy et al. 2007). The samples were tested at the Uganda Virus Research Institute Entebbe. Aliquots of the specimen were shipped to CDC Atlanta, USA.

Similar tools and procedures from previous outbreaks (Appendix 1,2, 3,) were used to define cases, undertake contact tracing as well as collect data. Temporary tents were set up to accommodate suspected cases as the main isolation facility was still under construction. Personal protection equipment (PPEs) donated by WHO and CDC were given to Bombo General Military Hospital and Mulago hospital. Public education materials (Appendix 6) used during the 2007 Ebola outbreak were adapted for intensified public education, launched immediately in the district Luwero district.

3.3.3.3 Procedures for laboratory testing

Five milliliters of blood samples were taken from cases for testing in the isolation wards. The necessary guidelines and precautions were applied to minimize nosocomial infection (WHO 1998;; WHO 2000). A portable field screening machine was available at the Gulu isolation unit for immediate testing of blood samples. The skin snip specimen were occasionally taken but had to be shipped abroad for analysis but the results were rarely used as they came late. There was no feedback in a number of cases. The tests carried out included Ebola IgG and IgM antibodies, antigen detection, RT-PCR and elevated liver enzymes using standard procedures (Ksiazek, Rollin et al. 1999; Ksiazek, West et al. 1999; Towner, Sealy et al. 2007). Laboratory work for the subsequent epidemics was carried out at the Uganda Virus Institute laboratory with a biosafety level 4 supported by the CDC and MRC. Appendix 7 gives details of the standard laboratory protocol used at the UVRI. A case was considered laboratory positive if any of the following tests were positive: either the antigen detection or the IgM ELISA test or the RT-PCR or virus isolation (Ksiazek, West et al. 1999).

3.3.3.4 Wildlife Investigations

During the Ebola outbreaks in Bundibugyo and Luwero districts there were reports of death among wild animals including monkeys in the neighboring forests (Mpigi) and also in Semuliki game reserve. A team from the national task force routinely worked together with wild life authorities (Figure 14, Appendix 10). They collected blood samples from the carcasses of dead animals reported and forwarded them for laboratory analysis at the UVRI facility. All districts were urged to report any suspected death among animals to the wildlife authorities who subsequently investigated the cause of death particularly among non-human primates.



FIGURE 14 SPECIMEN COLLECTION FROM DEAD MONKEY BY MOH/UWA, SEMULIKI GAME RESERVE, BUNDIBUGYO, UGANDA, 2008

3.3.4 Methodology for data management and analysis

A summary of the design and methodology is outlined in Table 7. Data collected was edited, cleaned, and checked for completeness, accuracy and consistency. The EPI Info software version 3.4 (CDC 2009) was used to manage information and data collection from the patient and contact forms. Epidemiological statistical data analysis was generated to describe (*Paper I and Paper II*) personal, geographical and temporal parameters of each epidemic. Descriptive statistics were generated by Epi-info, Epimap, excel software as appropriate. Relative Odds of the potential risk factors were computed by comparing the risks in the confirmed group of cases using against the non-cases who were regarded as the reference group (*Paper III*). Multivariate logistic regression application gave odds ratios (OR) between the independent and dependent variables. Statistical significance ($p < 0.05$) was adopted as the level of significance. Multivariate analysis was also used to explore relationships between symptoms and survival. Pearson's chi-square tests, the 2 sample t-test, and the χ^2 test were used during this exercise. The proportion of Ebola patients reporting the symptom against the number of Ebola patients with information about presence of that symptom had the p value determined by the Fisher exact test - a comparison was then made between Ebola patients who survived against those who died to determine whether symptoms were associated with survival (Table 16). Case fatality rates between the two species of virus were computed. The case fatality was determined using two approaches: i) calculating the proportion of death among those diagnosed on the basis of the

case definition from study *Paper III*, and ii) calculating the case fatality based on a subset of those patients (*Paper IV*) with only laboratory confirmed acute phase blood sample.

3.3.5 Planning and implementing the national response

The country was alerted at the highest level by His Excellency the President. He directed the full involvement of the entire cabinet and departments. A separate circular with more details was sent to the districts. A national task force was formed to coordinate and implement the national response. The task force reported to the Office of the Prime Minister, the leader of government business. This linkage provided the political support for the mobilization of national, bilateral and international support and expertise. The task force established the following operational working groups: coordination and resource mobilization; surveillance and laboratory work; case isolation and management; public education, and logistics management. Similar task forces were established at district, county, sub county, parish and village levels. At each district a rapid response team (RRT) was set up. The team at each district was composed of a district surveillance focal person, district health educator and a district laboratory focal person. Their role was to monitor implementation of activities and verify rumours. The contributions of local NGOs such as Uganda Red Cross (URC), World Vision were intergrated into the various task forces at national, district and community levels. One national joint plan was developed to which the various collaborators subscribed. The Government immediately pledged supplementary funding. Additional support came from the international agencies and partners, especially from the WHO, CDC, MRC and MSF (Appendix 11). Joint planning and discussion (Figure 19) helped the interragnation of the national efforts. Keeping inventories (expertise, supplies and logistics) of previous epidemics also supported early action.

Isolating and managing cases

Isolation facilities and infection control barriers nursing were set up to mitigate nosocomial transmission. Protective materials (gowns, masks, gloves, plastic aprons) were provided to the staff. Two isolation wards were set up at Gulu Regional Hospital and Lacor Missionary Hospital. Similar units were set up at Mbarara and Masindi. In Bundibugyo and Luwero districts, isolation wards were set up. The isolation wards were overcrowded and labour intensive (Figure 15). All district hospitals established isolation units and suspended unnecessary surgery, blood transfusion and blood veno-puncture. Blood samples were collected routinely y from cases and contacts and transferred to the approved laboratory. Laboratory screening supported surveillance and case management and deciding on the non-infectious status before discharge (Appendix 8). Training and personal protective equipment were provided.



FIGURE 15 ISOLATION UNIT, KIKYO HEALTH CENTRE, BUNDIBUGYO, UGANDA, 2007



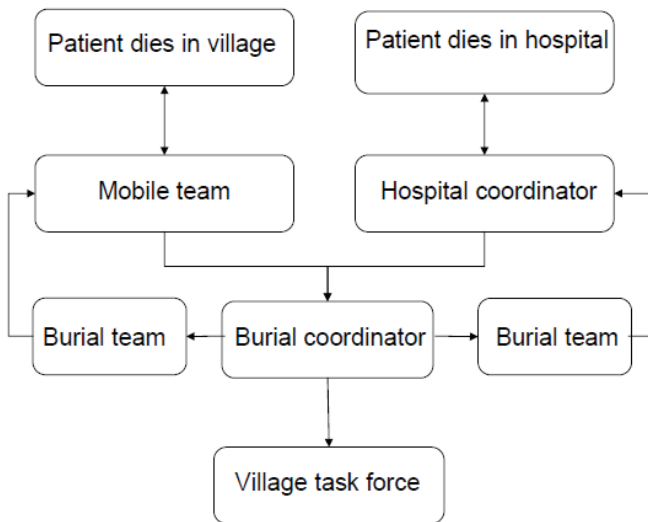
FIGURE 16 NATIONAL TASK FORCE (L TO R) DR SAM OKWARE, HON MINISTER OF HEALTH, STEPHEN MALLINGA, BUNDIBUGYO, UGANDA, 2007

Incentives were paid to motivate health care workers. Allowances of 100 US dollars were paid per day as risk allowance, which amount was twenty times their monthly earnings. The budget for managing a district-led programme was USD 3.6 million for a 6 months operation (Appendix 9). This is equivalent to about 25% of the recurrent nonwage government allocation for the MOH. That year the Occupational Health and Safety Statutes were revised to ensure timely compensation of health workers dying in the course of duty. Regular visits (Figure 16) to the districts by members of the national task force monitored implementation of the national plan.

The patients clothing was disinfected on discharge (Figure 17). The bed side attendant—usually a relative was educated on how to avoid contamination. Trained health care workers provided counselling and psychological support. Follow up centres were set up for discharged patients. Post Ebola Clubs were also set up to provide humanitarian needs during convalescence. Food rations as well were regularly provided to patients discharged.



FIGURE 17 HEALTH CARE WORKER AND ATTENDANT DISINFECTING PATIENTS CLOTHES, BUNDIBUGYO, 2007



(a)

(b)



(c)



(d)



(e)

FIGURE 18 PROCEDURES AND TOOLS FOR BURIALS-DISINFECTION, TRANSPORTATION AND BURIAL, BUNDIBUGYO, 2007.

(a) Flow chart surveillance (b) disinfecting body (c) transporting body (d) lowering body to grave (e) substitute greeting "bonga" adopted by public

Burials had to be done safely. Burial teams in Gulu were made up of 12 Army personnel, 6 police officials, 12 health care workers and 18 health care workers. Burial was restricted to just these teams, trained and well protected (Figure 18) with proper tools. The same teams carried the same tasks in Masindi, and Mbarara. In 2007, the same teams were redeployed to bury all suspected cases in Bundibugyo (2007), Luwero (2011). The same teams buried all bodies of suspected Ebola victims and followed strict burial procedures. Burial places were specified and each corpse was secured in a body bag for safe transfer to the burial ground.

Community mobilization and public education

Social mobilization at community level was started as soon as detection and confirmation was done. This was a systematic process adapted from previous outbreaks and the standard guidelines (WHO 1998). At community level four important institutions existed namely: the public government systems, the schools, the religious organizations and the non-Governmental Organizations. These are key members of the village health teams (Figure 19). They participated fully educating the community by word of mouth. Village teams moved from house to house on foot. Radio messages, discussions, film shows and media coverage alerted the public. Mobile phones were new and very helpful. Handshakes were suspended and substituted with the back of the hand, "*bonga*" (Figure 18e). Large gatherings were suspended. Community based surveillance were intensified. Media briefs were made daily and SITREP reports were shared with stakeholders in an attempt to reduce rumours. A cascade of training for health care workers and journalists was carried out in all the districts. Basic logistics and personal protection materials were also provided to a few of them for use in emergencies. Intersectoral collaboration brought in key security agencies like police and the Army to enforce quarantine and limit unnecessary movements.



FIGURE 19 NTF PLANNING MEETING (LEFT); COMMUNITY WORKERS FROM UGANDA RED CROSS, (RIGHT), 2007

Police handling suspected cases were trained and provided with personal protection. Insurgency in the north at that time required negotiation of a truce with the rebels to enable implementation of activities. Each village had a village scout who led active case search, and public education. The scouts met daily and shared progress. A radio communication linked the village teams to a designated ambulance service and the mobile team (Figure 20). The reports were carried to the next levels for onward transmission to task forces at district and national level.

Surveillance flow chart

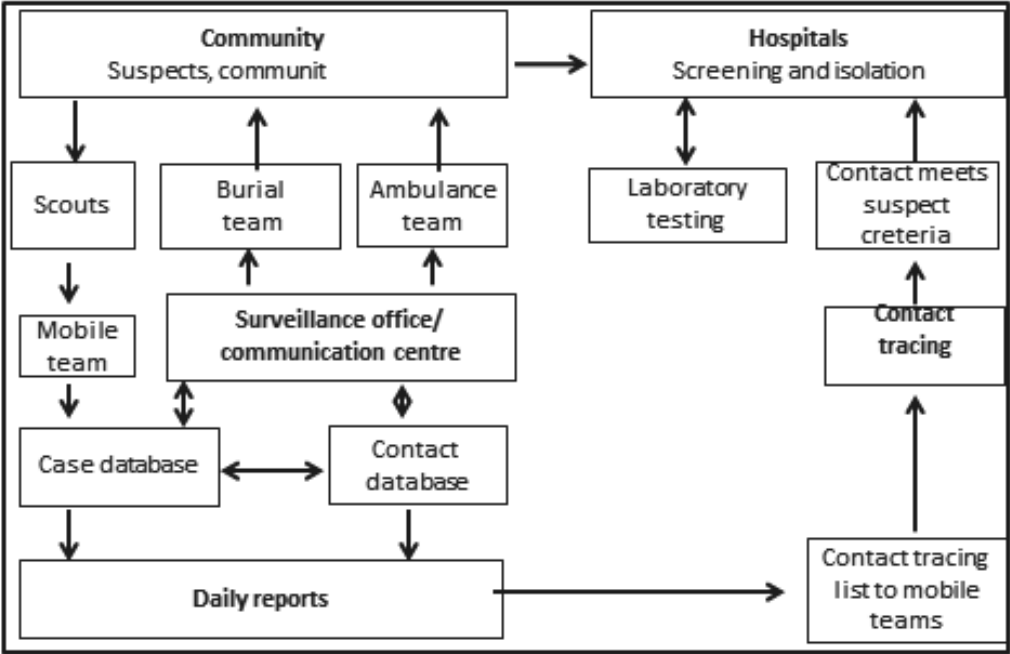


FIGURE 20 SURVEILLANCE FLOW CHART FOR EBOLA

The media were partners involved right from the start and information was shared openly and freely with them. The media reduced panic and hysteria by reporting accurately without dramatisation. The media (Figure 21) responsibly promoted spontaneous participation by the public in the national response.



FIGURE 21 MEDIA SUPPORT AND COVERAGE, UGANDA

3.3.6 Ethical clearance

These studies took place at a time of panic and extreme emergency. The priority was to contain the outbreaks. Formal ethical clearances were obtained from the institutional review committee at the Uganda Virus Institute. Official approval was also obtained from the Uganda National Council of Science and Technology. However, it was not practical to implement strict informed consent guidelines¹ during the crisis. Waiver of informed consent was applied in accordance with the recommended guidelines by the National Council for Science and Technology (UNCST 2007). Another major ethical dilemma was the application of confidentiality and professional secrecy to patients as some patients were openly filmed and paraded to the public by the world media, including satellite/television outlets. The issue of waived consent is discussed further under paragraph 5.5.

¹Sequence of explanation, discussion of benefit and adverse effects, and formal signature

4.0 SUMMARY OF RESULTS

Results summary

Three outbreaks of Ebola have occurred in Uganda from 2000 – 2011. The Gulu district outbreak caused 425 cases and 224 deaths. The Bundibugyo outbreak left 116 cases with 39 deaths. Some 31 health care workers were infected in Gulu and 14 in Bundibugyo. The Luwero outbreak caused a single fatal case. There were delays of up to six weeks in recognition of the earlier outbreaks. The signs and symptoms of the two varieties were similar and consisted of acute high grade fever, severe headache and chest pain, abdominal pain, associated with some bleeding manifestations. Patients deteriorated very quickly and died within days. Bleeding tendency in Gulu was associated with high fatality ($RR=1.8$, $p<0.001$) (Table 12). A high case fatality above 53%; (95% CI = 47.8-57.5) in the case of the Sudan Ebola subtype was observed. The case mortality for the Bundibugyo Ebola subtype was lower, 33.7%; (95% CI =25.0-42.2; $p=0.005$). Apparent asymptomatic infection in the population was demonstrated in Gulu, Luwero and neighbouring Mpigi districts. The three outbreaks occurred between June and December during the rainy and fruit season. The primary cases that started the outbreaks came from rural areas. Large displacement of populations due to insurgency 5-10 years earlier was a common observation in the affected districts of Gulu, Bundibugyo and Luwero. The role of monkeys in these outbreaks is unclear although some monkey blood specimens were found positive for Ebola IgG in Mpigi.

Attack rates were higher among women than men ($RR=1.6$; 95% C.I. = 1.3; 1.9) in the Gulu outbreak. Children between 5-14 years had the lowest attack rate. The risk increased with age and was highest at 60-64 years age group ($RR=16.4$; 95% CI = 9.4; 28.8). This was not the case in the Bundibugyo outbreak. In Bundibugyo participation in funeral rituals was a highly significant risk exposure factor (OR 7.4, 95% CI 2.9-19.3). However, sex (OR 1.3, 95% CI 0.7-2.5) and age (OR 1.3, 95% CI 0.7-2.7) differences did not suggest any significant association. Ethical principles of informed consent and confidentiality were breached and difficult to apply under the circumstances. Informed consent was waived during the study.

4.1 Description of outbreak No 1: the Sudan ebolavirus, Gulu district, 2000

4.1.1 Outbreaks detection and verification: How and where did it all begin?

For over a month, rumors were circulating of unexplained deaths among rural communities north of Gulu town in northern Uganda. These villages were inaccessible to the public because of the ongoing military operations against insurgency in the area. On the 8th October 2000, nearly six weeks late, the Ministry of Health was notified by the district director of health services of the deaths of two student nurses in Lacor hospital. A team was sent the following day. The team sent on the 9th October to support the district in the

investigations. They reported that on the 17th August 2000 a presumed initial death from Ebola occurred in Rwot Obilo village, 14 km north of Gulu town. A couple of deaths were reported in a household in this rural community. There was speculation that the disease could have started in the middle of July 2000 among the rural communities, although this could not be verified. The villagers had suspected poisoning or witchcraft. The village community believed that this was linked to witchcraft because of selective clustering of cases and deaths by household. However, by retrospective estimate the earliest recognized case probably occurred on the 30th August 2000. The clinical features were suggestive of a viral hemorrhagic disease. The first Ebola case was probably admitted to Lacor Hospital on the 27th September 2000. The team found 3 more student nurses critically ill together with 5 other patients from the same community. The team also identified 7 contacts. Blood samples were collected from the 8 admitted suspected cases and the 7 close contacts. The samples were subsequently sent to the South African Institute of Virus Research for analysis. On the 14th October the Ebola *Sudan ebolavirus* was laboratory confirmed. This was the first confirmed outbreak of Ebola in Uganda. A national response (paragraph 3.3.5) was launched immediately to intensify active case search, isolation and care, public education and community mobilization.

4.1.2 Epidemiological description of the outbreak

Cases

A total of 425 cases with 224 deaths were recorded. Of the cases identified only 195² were serologically confirmed. No results were available for the skin biopsies sent abroad. Most cases, 393 (92.5%, 95% CI 89.4 – 94.7) were from Gulu district, 27 (6.4%, 95% CI 4.3-9.2) were from Masindi and only 5 (1.2% 95% CI 0.4-2.9) were from Mbarara district (Table 9). Some 31 health care workers were infected of whom 17 died (54.8% 95% CI 36.3 – 72.2). The last reported case in Gulu district was on the 14th January 2001. She was a grandmother and caretaker to an infant who died of Ebola on the 4th January 2001. The last death in Masindi was on the 21st December 2000. Of the 56 districts then only Masindi and Mbarara imported cases from Gulu. The overall case fatality rate was 52.7% (224/425) [95% CI, 47.8 – 57.5].

TABLE 9: SUMMARY OF CASES³ AND DEATHS BY DISTRICT, UGANDA, 2000

² This figure changed to 216 in later publication (Paper II) after further review and checking and cleaning of the laboratory results

³ Some of the cases were identified retrospectively and were epi-linked

District	Cumulative Totals				
	Cases	Deaths	Survivors		
Gulu	393	203	190	51.7	46.6-56.7
Mbarara	5	4	1	80.0	28.4-99.5
Masindi	27	17	10	63.0	42.5-80.0
Other districts	0	0	0	-	-
County Total	425	224	201	52.7%	47.8 – 57.5

* CI Confidence interval

Time and the epidemiological curve

At least 3 apparent epidemic clusters and peaks occurred in the Gulu outbreak (Figure 22), most obvious at the beginning and towards the end of the outbreak. Clustering of cases was not so obvious by time frame but rather by location of cases. The first, second and third peaks occurred at 18 days, 30 days and 50 days after the presumed onset of the 28th of September. The peaks occurred at intervals of about 13 days. The incubation period was presumed to correspond to the interval between the respective clusters. The mean incubation period was estimated to be 12 days with a range of 2-21 days, (95% CI = 12-14). Most of cases, (74.8%) occurred between September 9th - November 11th 2000.

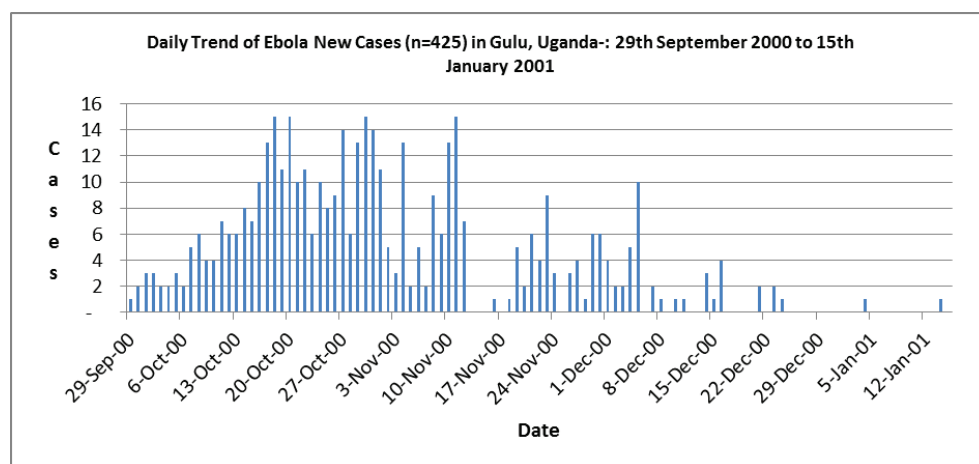


FIGURE 22 DAILY TREND NEW EBOLA CASES, GULU DISTRICT, 2000⁴

The time interval between date of onset of symptoms and death was estimated on the basis of 115 patients with available records. The medium was 7 days; mean 8 days (95% CI 6-10) with range of 1-41 days. For the 82 hospital cases with available data patient average time between onset of symptoms and recovery (that is discharge date) ranged from 2 days to 35 days. The mean was 12 days and medium 13 days (95% CI 10 - 14).

The period of stay at hospital for 83 patients with available data ranged from 1-29 days (mean 10 days) (95% CI 8-11), medium 10 days). The length of stay for those who died in hospital ranged from 1 to 32 days (median 4 days) among the 160 cases whose data was available. Uganda was declared Ebola free on the 27th February after the lapse of 42 days (corresponding to two incubation periods) since the last case.

TABLE 10: CUMULATIVE EBOLA CASES BY MOST AFFECTED PARISH, GULU DISTRICT, UGANDA, 2000

Parish	Sub county	County	No. of cases*
Kasubi	Bardege	Gulu Municipality	40
Kirombe	Layibi	Gulu Municipality	36
Atibaar	Bungatira	Aswa	31
Bardege	Bardege	Municipality	19
Kanyonga	Bardege	Municipality	18
Techo	Layibi	Municipality	17
Ariaga	Laroo	Municipality	17
Pageya	Koro	Omoror	16
Patudat	Layibi	Municipality	15
Vanguara	Pece	Municipality	13
Pabbo Kal	Pabbo	Kilak	13

*No reliable demographic data was available at sub county level to compute attack rates in parishes, due to insurgency and rebel activities. Only absolute numbers used.

Attack rates by residence

The majority of the victims related to this outbreak 393 (93%) were from Gulu district. Another 27 (6%) were from Masindi, and only 5 (1%) were reported from Mbarara. All these cases were linked up to the primary cases in Gulu. Seven relatives from Kenya had attended a funeral at Masindi. They sneaked back to Western Kenya immediately after the

funeral. They were followed up and quarantined, and there were no secondary cases among them. Of the 23 sub counties in Gulu district, 19 were affected. Gulu municipality was the most affected county. The worst affected sub counties in the municipality area were Bardege, Layibi, Pece, and Laroo. The most affected parishes were again in Gulu municipality (Table 10). The primary cases were all from rural areas north of Gulu municipality.

Some demographic data was available by county. The attack rates per 10,000 inhabitants ranged between 1.6 and 23.8 when considered by county. The attack rates were highest (almost 15 fold) in the Gulu Municipality (Table 11).

Persons affected: characteristics and outcomes

Of the 413 cases with recorded and available occupation data, peasants were 112 (27%), housewives 146 (35%), pupils 108 (26%), health care workers 24(6%) and students 23(6%). The age distribution of some cases with information available revealed that 81.1% of the cases were older than 15 years (Table 11, Figure 23). Children less than 10 years were only 13.3 % of the cases. Despite the establishment of isolation units for Ebola patients in Gulu the infection continued to spread among health care workers. The hospital director of Gulu died as well. In Gulu sixty four percentages (64%) of the infections among health personnel occurred after isolation wards were established, clearly showing the gaps in the infection control barriers. Thus nosocomial infection was common despite attempts to implement isolation practices. Those who died included the hospital director the late Dr Matthew Lukwiya, nurses, medical students, an ambulance driver and a support staff. Clustering of cases by family and households was observed, and clearer at the beginning and towards the end of the outbreak.

TABLE 11: ATTACK RATES/10,000 POPULATION AND RELATIVE RISK BY COUNTY, GULU DISTRICT, UGANDA, 2000

County	Population, 2000	Confirmed cases	Attack rates	Relative risk*
Omoro*	111,886	19	1.6	1.0
Aswa	88,450	9	1.0	0.6
Mwoya	45,350	10	2.2	1.4
Gulu Municipality	45,768	109	23.8	14.9
Kilak	105, 995	32	3.0	1.9

* Reference county

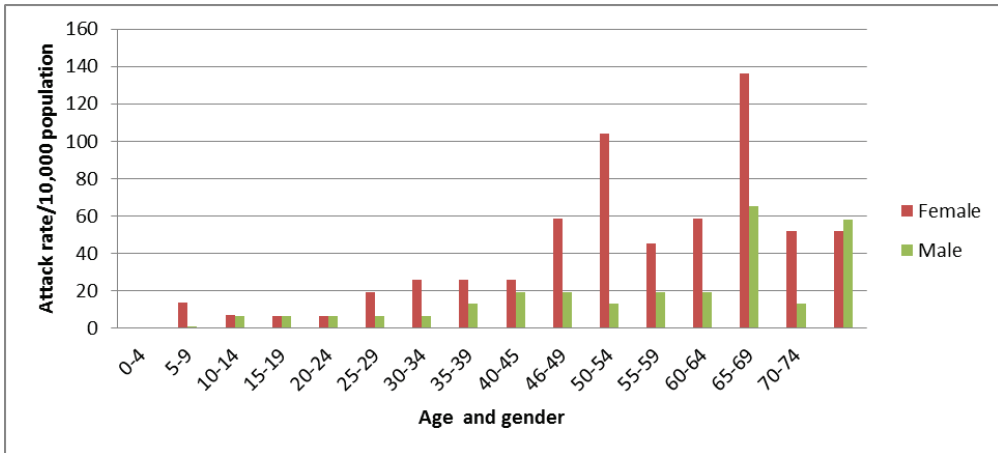


FIGURE 23 ATTACK RATES BY SEX AND AGE, GULU DISTRICT, UGANDA, 2000

In Gulu district, history of contact with a known case was very difficult to establish. Most of the participants did not understand the question or could not recall. Only 31 out of only 56 respondents who had information on this question reported a history of contact. The rest could not answer this question because they had been to too many places and could not recall specific circumstances. Twice as many women than men were affected. The F/M ratio was 1.7 - attack rates were higher among women than men (RR=1.6; 95% C.I. = 1.3; 1.9). The median age of cases was 27 years (range 3 months to 86 years). The mean was 27.5 years with a standard deviation of 17.8. Children between 5-14 years had the lowest attack rate. The risk increased with age (Table 12, Figure 23) and was highest at 60-64 years age group (RR =16.4; 95% CI = 9.4; 28.8). Women between 45 to 50 years also demonstrated a higher risk (RR = 14.0; 95% CI= 7.7; 25.2.).

TABLE 12: DISTRIBUTION OF EBOLA CASES BY AGE GROUP, GULU OUTBREAK, UGANDA, 2000

Age group	No cases	Percentage (%)
Less than 1 year	11	3.7
1-9 years	29	9.6
10-14 years	17	5.6
15 years +	244	81.1
Total	301*	100

*only those with complete verified data on age.

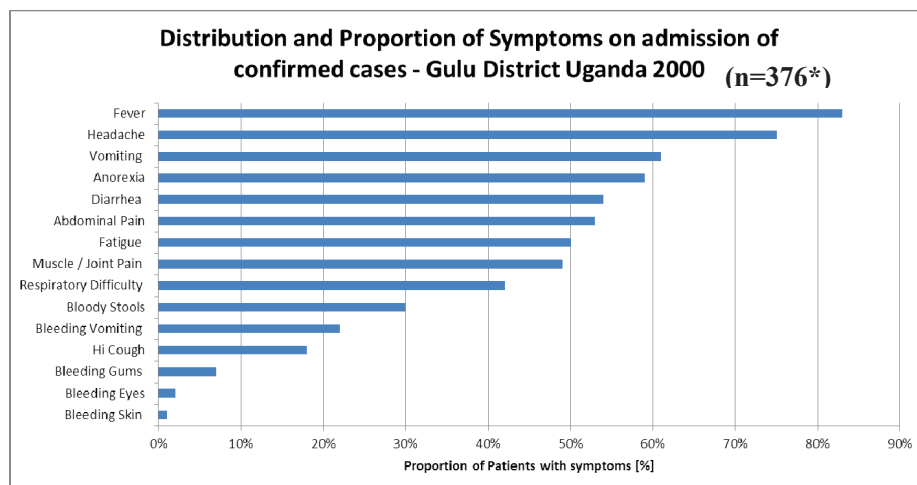


FIGURE 24 DISTRIBUTION OF SYMPTOMS ON ADMISSION, GULU, UGANDA, 2000

n =376 confirmed cases includes *suspected*, *probable* and *laboratory confirmed* categories with information available.

Clinical features on presentation

The majority of patients (85%) presented with a sudden onset of high grade fever above 38⁰ Celsius. This was accompanied by headache, vomiting, anorexia and chest pain. Diarrhea, abdominal pain and inflammation of the eyes, mucosa was among the common signs and symptoms. Bleeding manifestations (from the gums, orifices, and skin petechiae) was

TABLE 13: RELATIVE RISK OF DYING ACCORDING TO CLINICAL CONDITION ON ADMISSION,; RESULTS OF A UNIVARIATE ANALYSIS, GULU DISTRICT, UGANDA, 2000

Symptoms	Relative Risk	P Value
Bleeding skin	1.8	<0.001
Bleeding eyes	1.7	<0.001
Vomiting	1.6	<0.001
Bleeding vomiting	1.5	<0.001
Bleeding nose	1.5	<0.001
Bleeding gums	1.5	<0.001

Diarrhoea	1.3	<0.001
Bloody stools	1.2	0.07
Hiccough	1.2	NA
Respiratory difficulty	1.1	NA
Swallowing difficulty	1.1	NA
Abdominal pain	1.0	NA
Anorexia	0.9	NA
Fatigue	0.8	NA
Muscle/Joint pain	0.7	0.03
Fever	0.7	0.001
Headache	0.7	0.002

NA= not available

observed (about 30%) in some cases (Figure 24). Symptoms were more frequent ($p < 0.05$) among those who died than survivors. Hemorrhagic symptoms were often associated with high risk of death (Table 13). A simultaneous outbreak of measles erupted in internally displaced persons (IDP) camps in Pabbo Sub County, Gulu district, during the Ebola epidemic. The outbreak started in September 2000. A mass vaccination was mounted in the camps on November 27th and 28th 2000. This compounded the application of the case definition. The clinical presentation of measles in children mimicked the Ebola signs and symptoms and this made the application of the case definition difficult as a screening tool.

Case fatality

Although the overall case fatality rate associated with the Gulu outbreak was 53%, the mortality rate during the early days of the epidemic was close to 100%, but declined towards the end to about 10% (Figure 25). Bleeding tendencies and vomiting in general were more associated with death, $p = < 0.001$. The relative risk of death among those with bleeding under the skin and eyes was high ($RR = 1.8$, p value < 0.001) (Table 13).

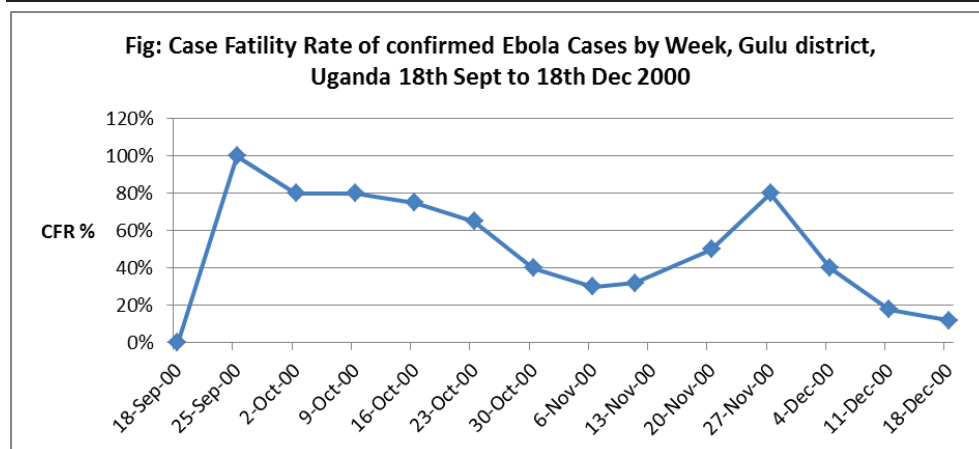


FIGURE 25 CASE FATALITY FROM EBOLA BY WEEK, GULU,2000

4.2 Outbreak No 2: The Bundibugyo ebolavirus outbreak, Bundibugyo district, Uganda, 2007

4.2.1 Description of the epidemic

How did it all start? The index case suspected (D.B)) was a 13 year old male from Bugharamali village, Bundimulagya parish, Bubukwanga sub county. He had an acute abdominal condition for which he was admitted to a general ward in Kikyo Health Centre IV on the 4th September 2007. He died the next day and was buried by close relatives. The first cluster of fifteen suspected cases and another 4 deaths are linked to D.B's family. Altogether 20 cases and deaths were linked to this family. Another cluster of cases was epidemiologically linked to the second suspected case (M.G). She was an 18 year old female from Butolya village from Kisuti sub county, Bundibugyo. On the 3rd of September, she had been admitted to Kikyo Health Centre to deliver a baby. She shared a ward with the index case (D.B.). Three days after delivery of the baby, she developed fever, abdominal pain, vomiting and diarrhea. She died three days later and was buried by close relatives. A second cluster of 9 were those that nursed or visited her – they fell sick within the next 2 weeks. Thus five suspected cases and four deaths were linked to her. A third cluster developed around a clinical officer (K.I), who nursed the above patients at Kityo Health Centre. Subsequently cases developed around their neighborhoods and communities and spread gradually to other sub counties in the district. Blood was taken although there was unwillingness from some participants to cooperate if the blood samples were taken retrospectively. On the 28th November 2007 a report from CDC, confirmed the isolation of a new *Bundibubyo ebolavirus*. By this time the disease had spread to most sub-counties in the district.

A total of 192 cases that met the case definition were identified by mobile teams and referred to the triage centre for testing and final re-validation and re- classification as outlined in paragraph 4.2.2. Of these only 42 (22%) were confirmed as positive for BEBOV virus by laboratory criteria. Another 74 (38%) maintained the probable case status. The

remaining 76 (40%) were laboratory negative and were accordingly considered non-cases. These categories were later (paragraph 4.2.2) used for determining the risk factors and case fatality. After excluding the non-cases 116 probable and confirmed cases remained. Of these 39 died.

Time trends and clustering of cases

Clustering of cases by family and households was observed. The epidemic had three peaks separated by absence of cases (Figure 26). The interval between the peaks was 3-11 days. Each cycle peaked and lasted 6 weeks before a decline. The incubation period estimate based on date of presumed contact to onset was 7 days (range 2-20 days). The cases peaked maximally 3 months after the presumed onset of the disease. The largest cluster of secondary cases were associated with the death of a single community leader. However, the occurrence of this cluster coincided with confirmation of the outbreak and launching of the national response. Only 2% of tertiary cases developed from this cluster. The last case occurred on the 16th January 2007

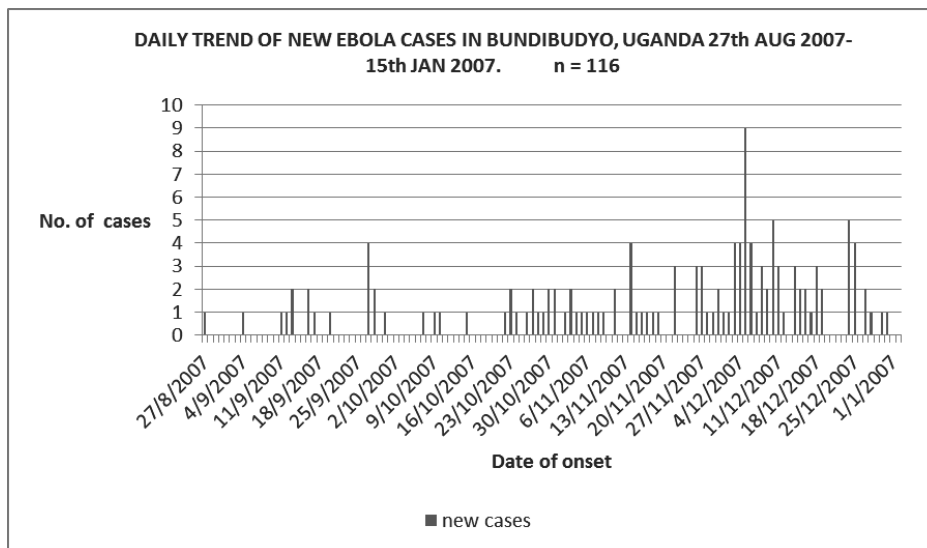


FIGURE 26 EPIDEMIOLOGICAL CURVE OF NEW EBOLA CASES, BUNDIBUGYO, UGANDA, 2007

Attack rates by location and person

A total of 116 cases with 39 deaths were confirmed. The patient's ages ranged from 3 weeks to 70 years, with a mean of 34 years and a median of 35 years. The majority of the cases 36% (40/116) were subsistence farmers. Some 14 out of 116 cases (12%) were healthcare workers. Unlike in Gulu, the health care workers here were infected before strict isolation procedures were initiated. The hospital director of Bundibugyo hospital and the head of nursing at the facility both died. The initial case in Kikyo Health Centre was suspected to

have originated from the maternity ward. This showed gaps in procedures for infection control.

Bundibugyo district had 10 sub counties but over 97% of the cases were reported in just the 4 sub counties of Kasitu, Bubukwanga, Busaru and Bundibugyo town council. Kasitu sub county alone had over 54% of the cases. The district attack rate was 43 cases per 100,000 population; 95% CI (79-118) Kasitu (Table 14) had the highest attack rate of 185 per 100,000 inhabitants, followed by Bundibugyo town council (142 per 100,000 inhabitants) and Bubukwanga (73 per 100,000 inhabitants). The outbreak never spread beyond Bundibugyo district.

TABLE 14: DISTRIBUTION OF CASES BY SUBCOUNTY, BUNDIBUGYO DISTRICT, UGANDA, 2007

Sub county	Population	Number cases	Number deaths	Case fatality rate, %	Attack rate*	95% CI Attack Rate*
Kasitu	33,968	63	18	29	185	142-237
Bundibugyo town council	17,590	25	8	32	142	92-210
Bubukwanga	23,398	17	7	41	73	44-119
Busaru	40,547	8	3	38	20	9-39
Harugali	29,162	1	1	100	3	0.09-19
Karugutu	19,384	1	1	100	5	0.13-29
Bubandi	22,063	1	1	100	5	0.11-25
Other sub counties	81,879	0	0	0	0	
Total	267,991	116	39	34	43	36-52

per 100,000 population

Clinical features *Bundibugyo ebolavirus*

The symptoms occurred 7 days (range 2-20days) after contact with a case. The onset was sudden and characterized by high fever (over 38°C), muscle aches and severe headache. Diarrhea and vomiting, chest and respiratory symptoms soon followed. Bleeding manifestations from the eyes, skin, and vagina orifices were common. Within a week the patients developed shock and multi organ failure and died. The median interval from onset

to recovery was 10 days (range 2-26 days). The median time from onset to death was 10 days (range 3-21days). The distribution of cases by symptoms is shown in Figure 27.

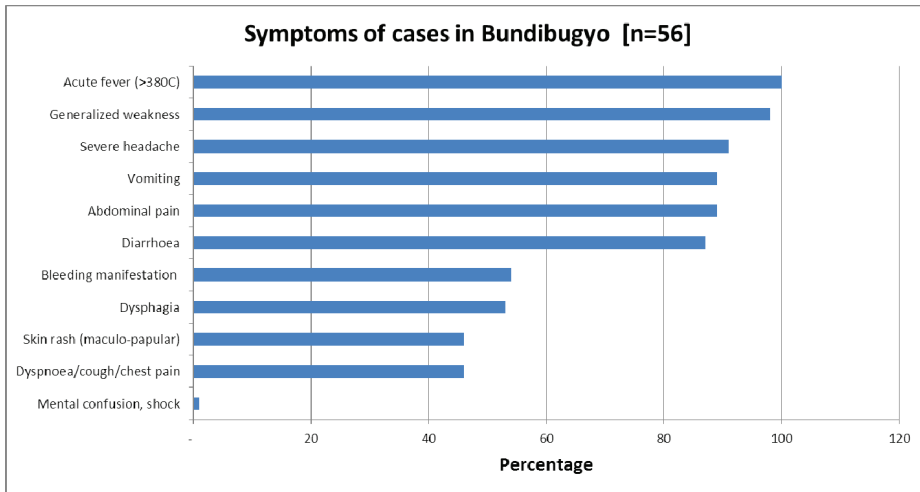
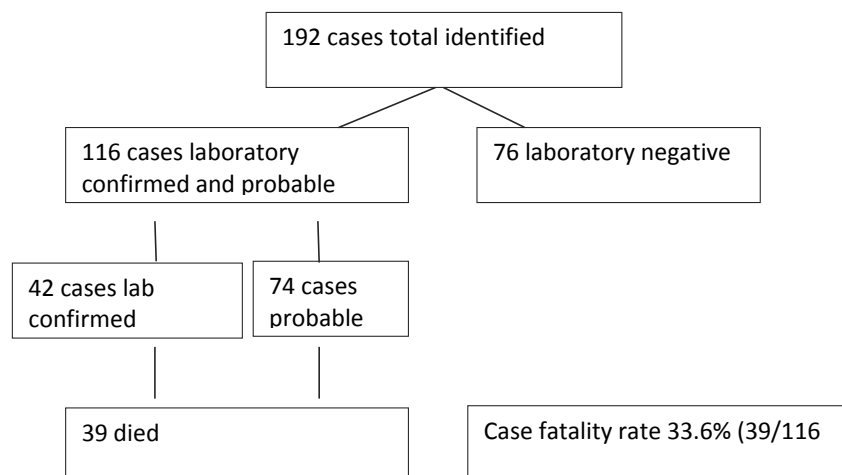


FIGURE 27 DISTRIBUTION OF CASES BY SYMPTOMS, BUNDIBUGYO, 2007

4.2.2 Risk factors investigation and analysis (Paper III)

From the 192 cases identified by mobile teams (paragraph 4.2.1) on the basis of the case definition, a combined category (suspected, probable, confirmed combined) totaling 116 cases was re-validated by the supervisors as *true (cases)*. The remaining 76 non-cases were then regarded as the control reference group. The scheme below summarizes the outcomes.



Critical risk factors identified were analyzed for associations using bivariate analysis and other methods discussed above (paragraph 3.2.5). The results of the bivariate analysis of selected potentially high risks for Ebola in Bundibugyo district is shown in Table 15.

Risk analysis

The risk factors data of each of the selected exposures was analysed by calculating the relative risks between the cases group with reference to the non-case (control) group. A multivariate analysis was performed using binary logistic regression to control for confounding. Table 15 shows the outcome of the analysis for the risk factors that were studied. Participation in funeral rituals was a highly significant risk exposure factor. The patient who participated in the funeral rituals 3 weeks earlier was more likely to get sick. When considered by category, the “probable” Ebola patients (OR 3.2, 95% CI 1.6-6.6) and “confirmed” Ebola patients groups (OR 7.4, 95% CI 2.9-19.3) each were significantly more likely to have participated in burial rituals than the reference group. The third category, that is, the “probable” and “confirmed” combined, considered as a group was significantly more likely than the reference group to have participated in the funeral rituals, within 3 weeks before onset of illness (OR 4.2, 95% CI 2.2-8.2). Visiting a hospital or a hospitalized patient, 3 weeks before onset of illness was a significant risk factor. The laboratory “confirmed” Ebola cases (OR 8.71, 95% CI 3.03-26.30), and the combined “probable” and

TABLE 15: BIVARIATE ANALYSIS OF RISK FACTORS FOR EBOLA, BUNDIBUGYO, UGANDA, 2007

Potential risk factor	Probable case n=74	Confirmed case n=42	Probable/ confirmed case, n=116	Non-case n=76 (ref)
Hospitalized/visited hospital, number (%) OR (95% CI)	38 (51.40) 1.5 (0.8-3.1)	36 (85.70) 8.7 (3.0-26.3)	74 (63.79) 2.6(1.4-4.9)	31 (40.80) 1
Consulted traditional healer, number (%) OR (95% CI)	1 (1.4) 0.25 (0.01-2.4)	0 Undefined	1 (0.9) 0.2 (0.01-1.5)	4 (5.3) 1
Participated in funeral rituals, number (%) OR (95% CI)	43 (58.1) 3.2 (1.6-6.6)	32 (76.2) 7.4 (2.9-19.3)	75 (64.7) 4.22 (2.2-8.2)	23 (30.2) 1
Traveled before illness, number (%) OR (95% CI)	29 (39.20) 2.62 (1.2-5.8)	11 (26.20) 1.4 (0.5-3.8)	40 (34.50) 2.1 (1.0-4.5)	15 (19.74) 1
Had contact with person with known suspected case, no. (%) OR (95% CI)	48 (64.90) 1.4 (0.7-2.9)	42 (100.00) Undefined	90 (77.60) 2.7 (1.35-5.24)	43 (56.58) 1
Had contact with wildlife number % OR (95% CI)	1(1.4) 1.0 (0.0-38)	0 Undefined	1 (0.9) 0.7 (0.02-24)	1 (1.3) 1
Male sex, number (%) OR (95% CI)	40 (54.00) 1.2 (0.6-2.5)	25 (59.52) 1.6 (0.7-3.6)	65 (56.00) 1.3 (0.7-2.5)	37 (29.31) 1
Age 41-60 years, number (%) OR (95% CI)	18(24.30) 1.0 (0.5-2.3)	16 (38.10) 2.0 (0.8-4.9)	34 (0.90) 1.3 (0.7-2.7)	18 (1.32) 1

“confirmed” cases (OR 2.6, 95% CI 1.4-4.9) were more likely to have visited a hospital or hospitalized. Contact with “suspected” or “confirmed” Ebola case within 3 weeks before onset of their illness, was a significant risk factor as demonstrated in the combined “probable” and “confirmed” group being more likely to have had such contact (OR 2.76,

95% CI 1.35-5.24). Consulting a traditional healer 3 weeks prior to illness was not significantly associated with having Ebola (OR 0.2, 95% CI 0.01-1.5) for the “probable” and “confirmed” category. Similarly having had contact with wildlife was not significant in the “probable” and “confirmed” group (OR 0.7, 95% CI 0.02-24.3). Sex (OR 1.3, 95% CI 0.7-2.5) and age (OR 1.3, 95% CI 0.7-2.7) differences were not significant.

4.2.3 Case fatality rate estimation

Estimation of case fatality by case definition

The case fatality was determined using two approaches based on the case definition on the one hand or the laboratory confirmed positive acute phase blood sample on the other. Basing on the case definition, there were 192 individuals (*Paper III*), who met the case definition (paragraph 4.2.1). There were 39 deaths among the 116 that were considered *true cases*. Therefore the proportion of death among the cases identified by the case definition was (39/116) 33.7%, CI 25.8- 54.6.

Estimation of case fatality by laboratory confirmation (*Paper IV*)

This study (*Paper IV*) on case fatality was prompted by anecdotal speculation that this new virus was less lethal. The low estimate of case fatality based on case definition was also surprisingly low. Another quantitative descriptive design studied a subset of the above patients on the basis of positive acute blood samples only as outlined in the study design. The schematic chart below (Figure 28) summarizes the results.

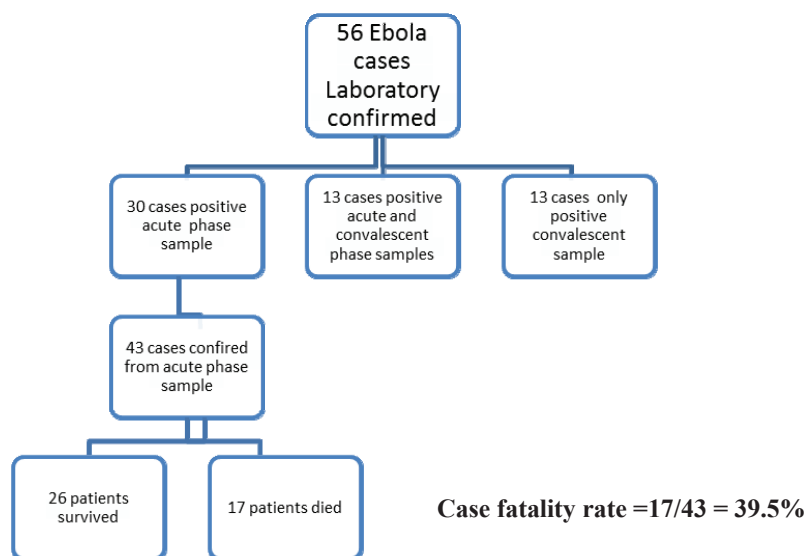


FIGURE 28 LABORATORY CONFIRMED CASES BY CRETARIA, BUNDIBUGYO, UGANDA, 2007

Analysis of the results

Of the 56 “confirmed” cases the following three categories (Figure 28) were identified under these criteria: 30 positive acute phase samples; 13 positive acute and convalescent phase samples; and 13 only positive convalescent phase samples. Thus 43 cases had blood samples confirmed from the acute phase samples. The 13 cases with positive convalescent

blood samples only were discarded because as survivors they were a biased sample – their status at the acute phase was unknown. Of the 43 cases with confirmed test during the acute phase only, 26 patients survived and 17 patients died. The case fatality rate recalculated on the basis of patients with confirmed positive blood samples only from the acute phase is 39.5% (17/43) during the outbreak. The case fatality was calculated on the basis of cases with a laboratory positive acute phase sample only. The association between the demographic characteristics by symptom and survival or death for the 56 cases identified as laboratory positive for Ebola was further analyzed by the 2 sample t-test and the χ^2 test as well as the Fisher exact test (Table 16). Older age appeared to be a risk factor for death – the older age group more likely to die than the young (p value 0.039). The mean age among the deaths was 42 years (range 20-70 years). This was significantly higher than among survivors whose mean age was 33 years (range 12-50 years), p value 0.039. No gender difference was observed between survivors and those who died (p value 0.100). The mean incubation period for the *Bundibugyo ebolavirus* was 6.3 days estimated from among only the 24 laboratory confirmed cases with complete data. There were no differences in days of incubation period between survivors (5.7; CI 95% 4.4-7.0) p >0.100) and those who died (7.4; CI 95% (5.4-9.3) p >0.100).

TABLE 16: ANALYSIS OF THE DERMOGRAPHIC AND THE CLINICAL CHARACTERISTICS OF THE LABORATORY CONFIRMED CASES BY SURVIVAL OR DEATH OUTCOME, BUNDIBUGYO DISTRICT, UGANDA, 2007

Characteristic	Ebola patients confirmed by acute-phase sample, n= 43			Total no. confirmed, n=56
	No survived, n= 26	No. died, n=17	p value	
Mean age, y (range)	33 (12-50)	42(20-70)	0.039 ^b	37.4 (11-70)
Male sex (%)	16 (62)	8 (47)	>0.100 ^c	30 (54)
Mean incubation period (95% CI) ^a	5.7 (4.4-7.0)	7.4 (5.4-9.3)	>0.100 ^b	6.3 (5.2-7.3)
Signs and symptoms, no reporting/no available(%) ^d				
Fever	26/26 (100)	16/16 (100)	>0.100	55/55 (100)
Fatigue	22/23 (96)	14/14 (100)	>0.100	49/50 (98)
Headache	21/25 (84)	14/15 (93)	>0.100	48/53 (91)
Nausea and vomiting	24/26 (92)	13/15 (87)	>0.100	48/54 (89)
Abdominal pain	23/26 (88)	13/14 (93)	>0.100	47/53 (89)
Muscle/ joint pain	19/23 (83)	12/14 (86)	>0.100	44/50 (88)
Diarrhea	24/26 (92)	13/15 (87)	>0.100	47/54 (87)
Anorexia	19/23 (83)	12/15 (80)	>0.100	43/51 (84)
Difficulty in swallowing	10/23 (43)	6/15 (60)	>0.100	27/51 (53)
Rash	9/26 (35)	5/15 (33)	>0.100	25/54 (46)
Difficulty in breathing	6/23 (26)	8/14 (57)	0.085	23/50 (46)
Hiccups	4/23 (17)	6/15 (40)	>0.100	16/51 (31)
Bleeding tendencies	11/26 (42)	9/17 (53)	>0.100	30/56 (54)

CI = Confidence interval; ^a for Ebola patients reporting contact with a confirmed Ebola patient;

^b by 2 sample t-test; ^c by χ^2 test; ^d no. of Ebola patients reporting the symptom/no Ebola patients with information about presence of that symptom, the p value was determined by the Fisher exact test - a comparison was made between Ebola patients who survived against those who died.

4.3 Outbreak No.3: Outbreak of Sudan Ebolavirus, Luwero district, Uganda, 2011

4.3.1 Outbreak Description and containment

The investigations confirmed the death from Ebola in a 12 year old girl from Nakisamata village, Luwero district. She died on the 6th of May 2011. There was no history of travel outside the village or any funeral contact preceding the onset of illness. There was no exposure to any sick or dead animal. Investigations further established the date of onset of illness to be around 1st May 2011 (Figure 27). It all started with headache followed two days later with high grade fever and vomiting. Nasal bleeding and excessive body weakness developed on the 6th May. Her grandmother took her to a local clinic where she was treated with quinine, vitamin K and adrenaline nasal packs to stop bleeding. She deteriorated and developed vaginal bleeding and hematemesis. She was transferred on a motorcycle taxi to Bombo Military Hospital, in company of her grandmother and father. She was admitted to Bombo hospital and died three hours later after an attempted resuscitation. The laboratory results confirmed the case to be infection with *Ebolavirus Sudan ebolavirus* (SEBOV) on the basis of a positive RT-PCR and antigen-detection ELISA. However, the ELISA IgM against Ebola viruses was negative. The tests for the Marburg virus were negative. Further tests on the patient's specimen isolated and confirmed SEBOV⁴. The phylogenetic analysis demonstrated the isolate (Nakisamata isolate, JN638998) to be related (99.3% identical) to the Gulu outbreak SEBOV strain of 2000. Infection control measures had been instituted in the hospital pending confirmation of the diagnosis. This was the only and last case- an ideal scenario and outcome compared with the earlier observations.

4.3.2 Investigation of contacts and bats

Twenty five close contacts were followed up and included 13 who had physical contact with the case at home and 12 hospital staff associated with the patient. Two of the contacts had performed the tracheal intubation and another two had prepared the body for burial. Some 24 other sick suspected patients were also identified and tested. The tests for all these contacts were negative for the viral hemorrhagic fever. However, one asymptomatic household contact tested positive for the Ebola out of the four members. A positive IgG result was registered in a juvenile relative, but, the IgM was negative Ebola tests for the 3 remaining family members were negative. It was not possible to determine any epidemiological link between the case and the relative with the positive test. Contact with wild animals was denied but several bats were found in the abandoned houses and classrooms in Nakisamata primary school where the case had been attending classes. The bats were provisionary identified as belonging to the genera *Chaerophon*, *Epomophorus*, *Hipposideros*, and *Pipistrellus*. Sixty-four bats collected were tested for the Ebola virus. The tests showed none to have the Ebola virus infection. A national standard response

⁴ The confirmation and isolation was done from blood on Vero E6 cells at the Viral Special Pathogens Branch, CDC, Atlanta, GA, USA.

(paragraph 3.3.5) was applied and the anti-Ebola campaign quickly contained the outbreak- yielding the best scenario of just a single fatality recorded. The timelines for the outbreak management and investigations carried out in Luwero are outlined in Figure 29.

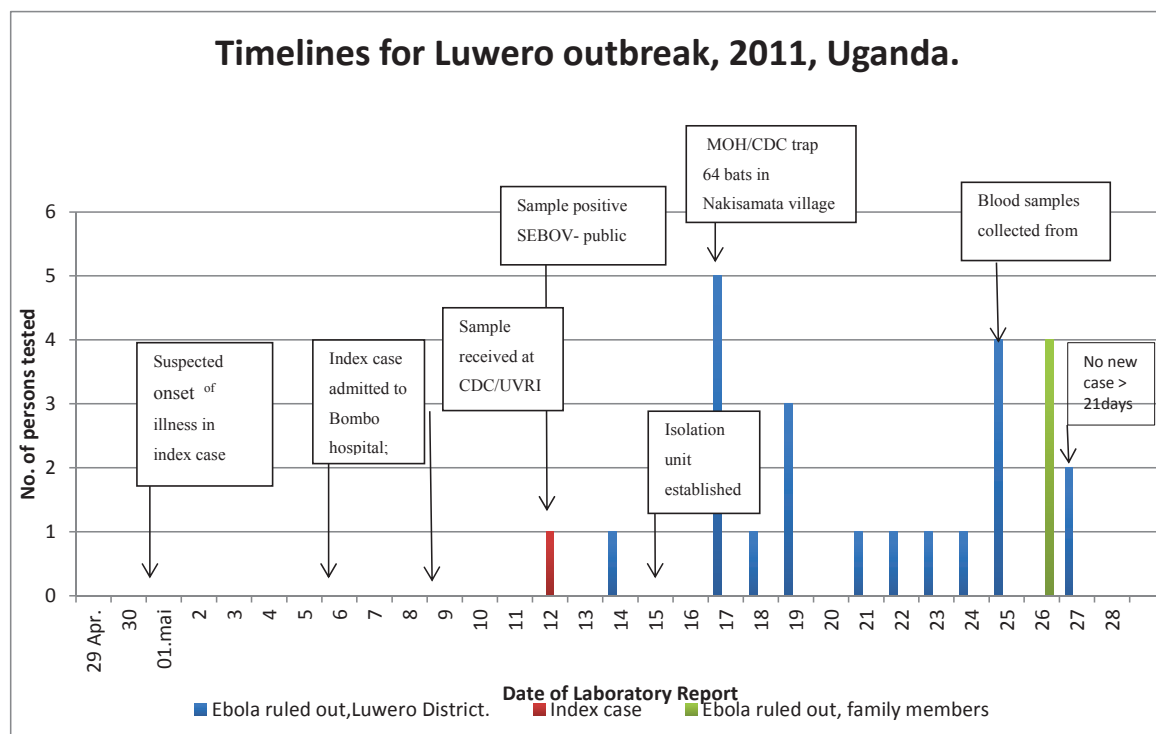


FIGURE 29 TIMELINES FOR THE EBOLA OUTBREAK, LUWERO, UGANDA, 2011

4.4 Laboratory results

Results validity

In the Gulu outbreak, only 195/425 (45.9%) whose data was available were serologically confirmed as the SEBOV. Some five asymptomatic cases also tested positive for the IgG antibody. The proportion of cases confirmed by the laboratory in his outbreak were also few - 21.9% (42/192) compared with the cases identified by case definition (Table 17). This is a major weakness with this study, especially as the sensitivity, specificity and the positive predictive values for the case definition and the laboratory tests were not known. The results from the skin specimen were not readily available and were somehow ignored.

TABLE 17: LABORATORY RESULTS OF SUSPECTED EBOLA CASES⁵, BUNDIBUGYO, UGANDA, 2007

Laboratory status	Number	Proportion %
Laboratory positive	42	21.9
Laboratory negative	76	39.5
Laboratory negative but probable	74	38.5
Total tested	192	100
Total lab positive and probable combined (42+76)/192	116	60.4

Among the suspected cases identified by the mobile teams, many were found false positive by the supervisors and the concurrence was low (Table 17). The table shows the proportional concurrence and the positive predictive value of the screening test (i.e. case definition) in the outbreaks. Table 18 shows a low concurrence values in the districts of Gulu (50.1%) and Bundibugyo (60.4%). The screening test has a low specificity.

TABLE 18: PROPORTION OF SCREENED CASES REVALIDATED BY SUPERVISORS, GULU DISTRICT, UGANDA, 2000

District	Identified by mobile teams	Revalidated by supervisors as cases	Regarded by supervisors as non-cases	Positive predictive value %	95% CI
Gulu	1069*	536	533*	50.1%	47.1 – 53.2
Bundibugyo	192	116	76	60.4%	53.1 – 67.3
Luwero	5	1	4	20.0%	***

*Reclassified later to 425 cases only; ** a subset of only those with all information available; *** Numbers small;

Wildlife investigations

In 2008 joint MOH/UWA team investigated the purported deaths of monkeys in Bundibugyo and Luwero districts. The team was unable to verify the claim as the carcasses had been buried. However, one sickly monkey was found at Golola parish. The farmers also reported several monkey deaths in Gombe County, Mpenja Sub County, Golola parish, Golola village. Another monkey had been killed by one of the villagers and thrown into nearby forest. Another dead monkey was found in nearby Kyanika forest. There was anxiety in the village. Blood samples were taken by UVRI from the respective villagers at GPS location N: 00 18537 and E 032 04609 at elevation 1226 metres above sea level. (Appendix 10) but the samples were negative for Ebola. However out of four asymptomatic villagers (residents of Kyanika parish, Maddu Sub County, Mpigi district) whose blood samples were taken for screening, two were positive for Ebola for the SEBOV 1g G antibodies on ELISA

⁵ Laboratory “confirmed” cases plus “probable” cases combined

test (MOH 2008). Also blood specimen was taken from a fresh monkey carcass (Figure 30) at the GPS location N: 00 21893 and E 03149495. The UVRI confirmed as positive for Ebola SEBOV IgG the samples from the carcass (MOH 2008). This suggested a possible link with the asymptomatic but seropositive resident villagers screened. Bats in Luwero (paragraph 4.3) were also tested for the virus following the death of the 12 year old from the disease in 2011. Blood specimens from bats were negative.



FIGURE 30 DEAD MONKEY IN FOREST IN MPIGI DISTRICT, UGANDA, 2008

4.5. Delays in case detection and the community mobilisation

TABLE 19: LEVEL AND TIMELINES OF DELAYS HINDERING DETECTION AND ACTION, UGANDA, 2000-2011

District	Gulu, 2000		Bundibugyo, 2007		Luwero, 2011	
Level	Date	Days since onset	Date	Days since onset	Date	Days since onset
Onset of strange disease in community	19/09/2000	0	02/08/2007	0	01/05/2011	0
Report to Ministry Health	9/10/2000	20	27/09//2007	56	06/05/2011	6
Investigation: Blood sampled	12/10/2000	24	29/09/2007	59	06/05/2011	6
Blood confirmation Ebola	14/10/2000	26	28/11/2007	117	09/05/2011	9
Declaration national action	15/10/2000	27	29/11/2007	118	09/05/2011	9
Last Case	14/01/2001	117	08/01/2008	159	06/05/2011	0
Total days epidemic lasted**		117		159		6
From laboratory confirmation to last case		91		42		-6

** Figures are approximate

Table 19 above shows the approximate timelines for key actions from onset of illness to the last case. The greatest delays were at the community level. This observation facilitated the spread of the disease and undermined containment. It took 20 days for the disease to be reported to MOH in the Gulu Ebola epidemic in 2000. The longest outbreak was in Bundibugyo which lasted 159 days from onset to the last case. The Gulu outbreak took 117 days to be contained. Even after being detected, the disease took longer (91 days in Gulu and 42 days in Bundibugyo) to report the last case. Delays in laboratory confirmation detection of the first case were observed (Table 19) in Gulu (26 days) and in Bundibugyo (3 months). In contrast, the Luwero 2011 outbreak was reported to the Ministry of Health within 2 days. Once the diagnosis was made, the outbreak was contained quickly within only 4 days, a most desirable outcome

5.0 DISCUSSION

5.1. Challenges in early detection and isolation

Early detection of Ebola was difficult as the common symptoms were nonspecific and mimicked several tropical illnesses. These clinical characteristics were similar (Figure 24, 27) in all the three outbreaks: a sudden acute onset of high grade fever, severe headache and chest pain, abdominal pain, associated often with some bleeding tendencies. Misdiagnosis can also occur during a concurrent measles outbreak (Onyango 2004) and this was the case in Gulu. The index cases were not identified for sure under the circumstances. In Bundibugyo a pregnancy related condition mistaken as abortion, may have obscured the Ebola diagnosis, thereby probably starting the person to person transmission

Ebola was a great risk to the health care workers. It is a serious nosocomial disease which required strict infection control procedures. Contact with body fluids of a case is probably the major mode of spread (paragraph 5.2). In Bundibugyo all the 14 health care workers were infected before the isolation procedures were instituted. However, despite instituting isolation procedures, 64% of the 31 health care workers in Gulu were infected after the isolation units were established, thus showing gaps in routine procedures for infection control. Complacency in the general wards and the false sense of security created by isolation units may have contributed to the continued spread. Practical drills and daily training and sensitisation of hospital workers are essential. A detailed assessment should be carried out to determine the gaps in the apparent ineffectiveness of the isolation facilities.

Variability of Ebola strains was demonstrated as two sub types of Ebola were involved. The two distinct types of Ebola species: the *Sudan ebolavirus* and the new *Bundibugyo ebolavirus* caused these outbreaks, which had similar but different case fatality. Morphologically, this new species is distinct from the from the other four known Ebola species- and by about 32% nucleotide difference from even its closest relative, the *Cote d'Ivoire ebolavirus*. The surface glycoprotein which is responsible for pathogenicity differs from the *Cote d'Ivoire ebolavirus* by 27% and from the *Zaire ebolavirus* by 35% at the amino acid level (Towner, Sealy et al. 2008). These divergences are likely to be reflected in the antigenic and pathogenic properties of these viruses. These differences also may have implications in future screening tools and the application of potential antiviral remedies. Further studies in non-human primates may be required to study and compare the pathogenicity of the various Ebola species.

In Bundibugyo, the incubation period estimate was established from the individual patient interview, and represented the interval from contact with a known case to onset of symptoms. Although the date of the last contact with a known Ebola case and onset of symptoms was regarded as the most appropriate incubation interval, infectiousness starts when the patient actually becomes symptomatic or when the patient actually develops detectable Ebola antigen (CDC 1988; Jahrling, Geisbert et al. 1996; CDC 2005; CDC 2009). In addition convalescent males can sexually transmit the infection for up to 61 days

(Ksiazek TG, et al 1999). Therefore, the concept of incubation period needs to be reconsidered with caution in future outbreaks.

A major weakness in this study is that of the cases identified by the community and mobile teams, only a small proportion (less than 50%) was confirmed laboratory positive (Table 17, 18). The reagents for the assay in the Gulu epidemic were developed from the early SEBOV strain. The test's sensitivity, specificity and the positive predictive value were not known. A separate study should have been done concurrently to validate the case definition and the laboratory tests. This was not done. Some false laboratory positives and false negatives were also observed in Ebola cases that met the case definition. Serial repeat testing and relating the results to clinical features usually supported in reassessing indeterminate laboratory outcomes. The few asymptomatic IgG false positives detected in Gulu district and Mpigi raised the possibility of past mild infection or cross-reacting unknown strains that may quietly be endemic in the area. Lack of laboratory capacity and technical skills at local level may also have affected quality of some laboratory results. The need to build laboratory capacity and skills at the national level is essential for proper and timely outbreak management.

The delays in initial case detection impacted negatively on outcomes and occurred at various levels (Figure 31, Table 19), but especially at the community level. This delay facilitated person to person transmission in the community and so expanding the epidemic. Thus by involving individual communities in local surveillance, it is hoped that this could enhance individual health seeking behavior and confidence. The reported cultural perception that the disease was caused by witchcraft, due to selective clustering of cases and deaths by households also may have delayed action. Community participation in the process was supportive, and hence the need to strengthen community based surveillance. The very effective containment of the Luwero outbreak demonstrates that prompt detection with immediate communication produced the best outcomes.

Challenges

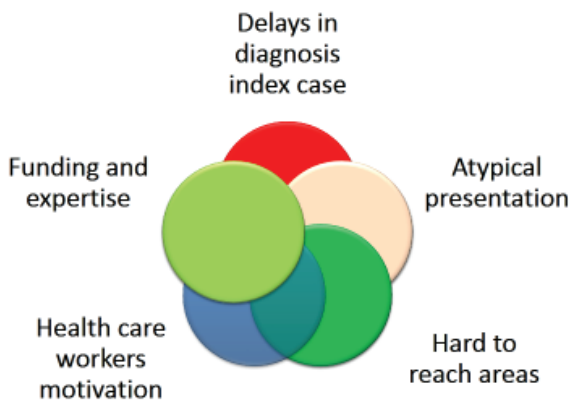


FIGURE 31 PROBABLE CAUSES OF DELAY IN DETECTION AND ACTION, UGANDA

5.2 Seasonality⁶ and zoonotic connection

There are two seasons in Uganda: a rainy season between end March and end of December and a dry season which starts in January and ends in March. Seasonality of the outbreaks along these seasons has also been demonstrated as all the outbreaks started between May and December during the rainy and fruit season. It is an interesting coincidence that these epidemics occurred between May – December of each year and this corresponds to rainy season during which fruits are abundant for both human and non-human primates. It is also a planting season when villagers spend considerable time in their gardens in the forests. No outbreaks have started during the dry season. Deforestation has resulted from more forests being cleared for agriculture. The monkeys are very closer to homesteads now than ever before. Such encroachment promotes animal to person contact directly by hunting or indirectly through fruits partially eaten by monkeys. It was a common observation to see fruits that drop down being picked and eaten by the villagers. Such fruits if partially eaten by fruit bats or non-human primate suspect could easily transmit the infection. Also fruit eating bats in Uganda have been demonstrated to have evidence of viral hemorrhagic viruses (Towner, Pourrut et al. 2007; Towner, Amman et al. 2009). This may also explain why the outbreaks only occur during the rainy season when fruits are abundant. A zoonotic connection was also apparent when there were so many unexplained deaths among monkeys in the area - a monkey specimen from the Mpigi forest near Luwero was found positive for the Ebola Sudan subtype IgG antibodies; two asymptomatic resident villagers also concurrently tested positive for the SUDEV IgG during the same period. However, there is also the possibility of other cross reacting endemic strains (Macneil, Reed et al. 2011). Working with and through the wildlife authorities is a vital collaboration in improving surveillance and early detection.

5.3. Susceptibility and risk factors

More women (Figure 23) were attacked in Gulu than Bundibugyo, $p=0.005$. Children between 5-14 years had the lowest attack rate. The risk increased steadily with age, and rose 14 fold in the 45-50 years age group and 16 fold in women in the age group 60-64 years compared with children. However, the massive population movement in the areas and the small numbers among the elderly should be taken into account in the interpretation of this observation. The cultural practices at least in Gulu may have promoted the escalation of spread. For instance, women are heavily involved in funeral rituals which involve cleansing and manipulation of the body before burial (Francesconi, Yoti et al. 2003). A retrospective study followed 24 case patients and their 65 identified contacts. The study demonstrated that contact with patients body fluids was associated with the highest risk of getting infected (adjusted prevalence proportion ratio =4.61%; (95% confidence interval =1.73-12.24) (Francesconi, Yoti et al. 2003). However, only 56% of the cases (*Paper 1*) in Gulu acknowledged contact with a known case, suggesting probable other indirect modes of spread. Close contact while caring for an infected person was probably the major route of transmission in this and previous EHF outbreaks, but the virus may also have been

⁶ Rainy season: End March to end December; Dry season: January- end March (approximate)

transmitted indirectly by touch, droplet, airborne particle, or fomite - expansion of the use of barrier techniques to include casual contacts (Figure 18e) might prevent or mitigate future epidemics.

In Bundibugyo a higher risk was associated with direct contact with a known case especially participation at funeral rituals, attending to patients and visiting an unsafe hospital environment (Table 15). Contact with a known case was included in the case definition and this is a weakness in the risk study. . More men were affected in Bundibugyo than women, $p=0.005$ (Table 20). Men in Bundibugyo are generally hunters of game and monkeys are hunted for food. However, having had contact with wildlife (Table 15) was not significant in the risk analysis (OR 0.7, 95% CI 0.02-24.3; $p=0.005$). This was surprising because hunting is common in this community as spears and other hunting gear was seen in most homes. The respondents may have feared the repercussions of saying the truth because it is illegal to hunt in the game reserve.

5.4. Differences in severity and fatality

The case fatality rate and the attack rates can be a reflection of severity. The case fatality for the *Sudan ebolavirus* was very high (over 53%) and similar to previously reported epidemics. The later was associated with a lower case fatality of between 34-40%. These differences in case fatality rates were significant, being higher in SEBOV in Gulu (52.7%; 95% CI= 47.8- 57.5 ;) district than in BEBOV in Bundibugyo district (33.7%; 95% CI= 25.0-42.2); $p=0.005$. The attack rates are also lower for the Bundibugyo virus than that in Gulu, $p=0.001$ (Table 20). The weakness about this contrast is that these were two different and separate studies.

TABLE 20: ATTACK RATES AND CASE FATALITY IN GULU AND BUNDIBUGYO DISTRICTS

	Ebola cases by case definition		
	Gulu, n= 324	Bundibugyo , n=116	p value
% Male cases (95% CI)	37 (32.0-42.4)	53 (43.5-61.7)	0.005
% Women cases (95% CI)	63 (57.7-68.2)	47 (38.3-56.5)	0.005
Attack rates/ 100,000 population (95% CI)	97(79-118)	43 (31-58)	0.001
Case fatality rate % (95% CI)	53.1 (47.7-58.5)	33.6 (25.0-42.2)	0.005

These differences in mortality could be due to severity of condition on admission, increased vigilance, or improved supportive care. Differences in antigenic properties of the new virus can also produce different outcomes. Some symptoms such as bleeding manifestations were also associated with severity and poor prognosis (Table 13). Although the bleeding manifestation was less in the SEBOV outbreak, but where it occurred, it carried the poorest prognosis (RR 1.8 $P=0.001$). In the Bundibugyo epidemic, none of the symptoms influenced survival or death (Table 16) except difficulty in breathing, $p=0.085$ which was moderately associated with increased mortality. Skin rash was commoner in the Bundibugyo subtype than the Gulu type, $p < 0.001$.

5.5. Limitations on data collection

Adherence to ideal research protocols is often difficult in emergency situations because of the overriding priority to prevent further spread of infection. The studies were not comparable and took place in different parts of the country with different demographic and social characteristics and at different times. Each group studied had different baseline characteristics of age, ethnicity, and socioeconomic status. The study groups were not similar in vital parameters (baseline, time, demography). Cultural and ethical obstacles were assumed to be same in the different groups although this was not the case. Unreported community deaths in rural communities cannot be ruled out completely. The census projections were based on the extrapolated national census data (UBOS 1991; UBOS 2002). However, the civil wars and insurgency in these districts had displaced many people especially men. It was also assumed that each individual remembered their date of birth accurately, which was unlikely to be the case. It was also assumed the respondents were unlikely to lie or exaggerated their history or social profile. Earlier knowledge and exposure to the outbreak management could also impact on the responses.

5.6. Challenges in the application of tools for data collection

The adapted case definition and tools were helpful in community based contact tracking and surveillance, but little is known about the sensitivity and specificity of the case definition in different settings. The tools used for data collection had not been systematically validated. The case definition was also modified from outbreak to outbreak. A comprehensive validation study of the tools should have been done to distinguish between deaths due to Ebola and those due to other causes. There were also other shortcomings associated with data collection. Lack of ethnographic studies for the community made it difficult to exercise sensitivity during information gathering. The tools should have been developed in local language and then translated into English, but the reverse was the case. The question on next of kin was likely to be ambiguous. The response to the choice of (next of kin) respondents and interpretation of lineage was not uniformly understood by the community. Often all the male and female offspring of uncles were considered “brothers” and sisters respectively. The interpretation of lineage was understood differently by the various respondents. Other threats to internal validity of the study arose in part from selection and information bias, as well as confounding.

Selection bias

The respondents were not randomly selected. Self-selection of participants was evident - only those who self-reported were picked to be included in the studies. Others could have

died quietly in the community for fear of being buried away from their ancestral places. Some of the participants were unwilling to participate in the retrospective interviews and sample collection because of the perceived unclear purpose. Some questions were not answered especially in the Gulu study where only 56 cases out of 425 responded to having had contact with a case. The rest of the respondents by not participating rendered themselves different from the respondents, thereby introducing a non-respondent selection bias. Losses on follow-up too affected selection of participants, since by not participating they were different from the participants. Loss of information (respondents) on several questions especially in Gulu by not answering led to selection bias.

The application of different detection criteria in each outbreak (incubation period and case definition) may have introduced selection bias in the measurement of outcomes and interpretation of surveillance data. The concurrence between cases identified by case definition compared with those cases determined by laboratory confirmation was low (Table 17,). In the Gulu outbreak, a few laboratory measurements indicated some false positive asymptomatic cases. These shortcomings were likely to contribute to selection bias of the respondents.

Information bias

Information bias could have resulted from the differences in observation, classification and the measurement criteria used during the various outbreaks. In principle the collection tools were similar in the three outbreaks, but in reality persons involved in data collection were different. Information bias may have been introduced as data was collected so differently, by different persons at different times. For instance, information bias was likely to occur during the recall of dates of birth, onset of illness and subsequent estimation of incubation period. Rural participants hardly remember their dates of birth. Information bias from interviewers may have arisen as well. Ideally interviewers should not have been aware of each participant's status (controls or cases) to minimize information bias. This was not true in the case control study (*Paper III*) as controls and cases were known in advance. Also the data was likely to have been collected by the local community with some subjectivity and self-interest because of perceived incentives. Information gathered by the different groups was likely to lead to diagnostic bias and therefore could obscure real differences. There were complaints that some questions were ambiguous. It is possible that some ambiguity was created during the translation of questionnaires from English to the local dialect by the different individual interviewers could be another possible source of errors. For instance, there was ambiguity in the interpretation of some questions such as history of last contact, for which unexpected answers were given. In Gulu only 56/425 (13%) of the cases were able to satisfactorily respond to this question. In future some of these identified ambiguities may be minimized by developing the primary questions in the local dialect and then translating them into English. Recall bias may also not provide the entire clinical spectrum for each Ebola case.

Confounding

Attempts were made to minimize confounding by restriction and exclusion criteria. For instance in *Paper IV*, non-cases were identified and excluded from the calculation of case fatality rate by using laboratory criteria only. Matching and pairing of cases and controls would have also reduced confounding, but this was not possible. However, stratification of cases by sex, age, laboratory outcome and calculating the results separately minimized

confounding. In the risk analysis study (*Paper III*) use of multivariate logistic regression model controlled confounding (Table 15). The lack of randomness and the uniqueness of the study populations are non-representative and undermine external validity. The data and information collected was fair measure of variables, but the generalization of findings should be done cautiously.

Other challenges existed when collecting data. The attack rates were based on earlier census demographic data (UBOS, 1991, 2002). This demographic data unfortunately was no longer accurate after the insurgency displaced large populations during fighting. This was the case in the Gulu, Bundibugyo and the Luwero data. The very high attack rates in the Gulu Municipality (Table 11) could have resulted from the underestimation of populations. The military conflicts in the area could also change the sex ratios because men died or ran away. This could in turn over estimate the attack rates in women. Initially information flow was not streamlined and integrated. Multiple sources (hospital, mobile teams, and laboratory) collected data in different formats before it was streamlined. There was some hesitancy by the health units to share clinical records with the data collectors. There was also some reluctance to share clinical records by some agencies, especially during the Bundibugyo outbreak.

5.7 Ethics and contentious issues

Ethics and confidentiality were breached and informed consent was waived as well during the study. Ideally, securing informed consent assures individual participants perception of risks and benefits and protects the respondents autonomy (Helsinki Declaration 1964). The waiver of professional ethics and informed consent in the study breached individual autonomy and human rights. Several alternatives to the standard informed consent were considered namely: deferred, prospective, surrogate consent s or waiver of consent. Deferred consent would involve the participants assenting to the study first through surrogates with a view to getting informed consent later to continue (Fost and Robertson 1980). However this practice as a substitute for informed consent takes the patient's continued participation for granted. It should also be questioned because the subjects had already been exposed to the risks. In this study the families were devastated and patients very ill. Some of the information was collected retrospectively further complicating the application of this alternative. In addition this approach was not culturally sensitive and was therefore not used. The prospective consent approval in advance was not feasible as the outbreaks were not predictable. Some authorities including the Food and Drug Administration allow waiver in some special circumstances (Biros, Lewis et al. 1995; Brody, Katz et al. 1997; Canada 2001; Gray 2001). Ebola is a very rare disease for which opportunities for research are very limited. In these circumstances it was reasonable to bend the traditional standard procedures while reasonably assuring some personal autonomy and human dignity. Under these circumstances the waiver of consent was a plausible option and was applied during the study.

6.0 LESSONS LEARNT AND CONCLUSION

The following lessons have been learnt from the study:

1. *Ebola is a highly fatal nosocomial infection. Two large outbreaks of the Sudan ebolavirus and the Bundibugyo ebolavirus occurred in Uganda in 2000 and 2007 respectively, followed later by a single case outbreak. Bleeding manifestations in patients with the Ebola Sudan subtype was associated with a two fold increase in mortality (RR=1.8, p value <0.001).*
2. *Established that the Sudan subtype was associated with a high mortality rate of >53%, while the Bundibugyo subtype fatality rate was lower at <40%.*
3. *Clustering of cases associated in a highly fatal febrile condition with evidence of nosocomial and person to person spread and bleeding tendencies is suggestive of viral haemorrhagic disease and should be treated cautiously.*
4. *Late recognition often led to extensive tragic nosocomial spread of infection in health care settings. Prompt detection and communication was demonstrated to be effective in containing the Luwero outbreak of 2011.*
5. *Despite instituting isolation procedures, 64% of the 31 health care workers in Gulu were infected after the isolation units were established, thus showing gaps in procedures and culture for infection control. A detailed assessment should be carried out to determine the gaps in the apparent ineffectiveness of the isolation facilities.*
6. *The case definition helped in screening suspected cases but the major weakness of this approach was that it had a low specificity, and less than 50% of suspected cases identified by the community were confirmed as true Ebola patients. A concurrent measles epidemic and an instance of septic abortion were indistinguishable from Ebola and undermined the usefulness of the case definition under these circumstances. Studies (concurrent and post outbreak) should have been undertaken to assess sensitivity, specificity and positive predictive value of the case definition..*
7. *Variability of Ebola strains was demonstrated as two different strains: the Sudan ebolavirus and the newly discovered less lethal Bundibugyo ebolavirus sub types were both involved.*
8. *Susceptibility to Bundibugyo ebolavirus was universal irrespective of sex or age group but higher (3 to 7 fold) risks were associated respectively with attending to patients or visiting an unsafe hospital environment and having direct contact with a known case especially participation at funeral rituals. The role of cultural practices which expose women to excessive risks in Gulu need further study.*
9. *Although close contact while caring for an infected person was probably the major route of transmission in these and previous EHF outbreaks, only 56% of the cases in Gulu acknowledged contact with a known case, suggesting that there may be other indirect methods of spread. Research is required to elucidate other potential modes of transmission including casual contact to mitigate future epidemics.*
10. *Apparent asymptomatic infection was demonstrated in some close contacts and also among some village residents, suggesting past unrecognised exposure or cross reacting antibodies.*
11. *Seasonality of onset was observed as all the outbreaks occurred between May and December in the rainy and fruit season during which period there could have been potential risk and contact with fruits partially eaten by monkeys.*

- 12. A zoonotic connection was apparent by the demonstration of Ebola IgG in a monkey carcass and a concurrent asymptomatic infection among village residents during an investigation into unexplained monkey deaths in nearby forests. All the primary cases that started the outbreaks came from rural remote areas and the need to work with and through the wildlife authorities is therefore crucial.*
- 13. Involving local communities and the media in outbreak control activities supported community based surveillance and timely identification of cases, in areas without health care workers.*
- 14. Strengthening laboratory capacity and surveillance at national level and enhancing collaborative networks at regional and international levels is crucial for effective outbreak management.*
- 15. Ad hoc incentives and allowances improved staff commitment, demonstrating that better remuneration of health care workers may contribute to better performance. Inadequate and demotivated health care workers, poor infrastructure, insufficient supplies and logistics and limited funding undermined the national response.*
- 16. The epidemic diverted resources from other critically essential primary health care programmes since no emergency preparedness plan was in place. An emergency preparedness plan should always be an integral part of the national health budget.*
- 17. Ethical principles were breached and waiver of informed consent was considered a practical option under these life threatening circumstances, but the arguments remain contentious and a subject for continued debate.*

The experiences and challenges from the three Ebola outbreaks in Uganda have been described. Attempts were also made to establish the risk factors and severity. Not all was one hundred percentage perfect, but the Ministry of Health working alongside its partners and the community contained the outbreaks against the constraints of the low resource settings, sometime with delays but once promptly and effectively done.

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Appendix 1: Case investigation form

Case Investigation Form

ID Case

Date of reception:

Date of detection of the case ____/____/____

This Case was notified by *(tick off the right answer and specified)*

Mobile team, # _____ Health Centre _____

Hospital _____ Others: _____

Form filled by (first name and surname)

Information given by (first name and surname)

Family link with the patient _____

Identity of the patient Nickname _____

First name _____ Surname _____

For the babies, son/daughter of (name of father)

Birth date ____/____/____ Age (years)____ Sex M F

Permanent address: Head of Household (first name and surname)

Village/Suburb _____ Country _____

GPS lat _____ long _____

Nationality _____ Ethnic group _____

Profession of the patient *(tick off the right answer)*

Miner House wife Hunter/trading game meat Children

Pupil/ Student Farmers Health staff, details: Healthcare centre _____

service _____ qualification _____

Others _____

Status of the patient

Status of the patient at detection Alive Death

If dead, please specify date of death ____/____/____

Place of death: Community, name village _____

Country _____

Hospital, name and service _____ Country _____

Place of the funerals, name village _____ Country _____

History of the disease

Date of onset of symptoms ____/____/____

Name of the village where the patient get ill _____

Country _____

Did the patient travel during illness Yes No DNK

If Yes, indicate the places and the country :

Village _____ Health Centers _____ Country _____

_____ Health Centers _____ Country _____

Did the patient have fever? Yes No DNK

If Yes, date of onset for the fever: ____/____/____

Does or did the patient have the following symptoms

(tick off when apply)

ID Case

Date of reception:

Headache	Yes		
Vomiting/Nausea	Yes	No	DNK
Anorexia/Loss of Appetite	Yes	No	DNK
Diarrhoea	Yes	No	DNK
Intense Fatigue	Yes	No	DNK
Abdominal Pain	Yes	No	DNK
Muscle or Joint Pain	Yes	No	DNK
Difficulty swallowing	Yes	No	DNK
Difficulty breathing	Yes	No	DNK
Hiccoughs	Yes	No	DNK
Skin Rash	Yes	No	DNK
Bleeding from injection sites	Yes	No	DNK
Bleeding gums	Yes	No	DNK
Bleeding into eyes (red eyes)	Yes	No	DNK
Black or bloody stool	Yes	No	DNK
Blood in vomitus	Yes	No	DNK
Bleeding from nose	Yes	No	DNK
Bleeding from vagina other than menstruation	Yes	No	DNK

Exposition Risks

Was the patient hospitalized or did he visit anyone in the **hospital** anytime in the three weeks before becoming ill?

Yes

No

DNK

If Yes, where _____ between (dates) ____/____/____ and ____/____/____

Three ebola outbreaks in Uganda

Did the patient have visit/consult a **traditional healer** during the three weeks before becoming ill or during illness? Yes No DNK

If Yes, name of the traditional healer _____ Village _____ Country _____

When and where did the contact take place? Place _____ date : ____/____/____

Did the patient receive traditional medicine ? Yes No DNK

If Yes, explain which kind : _____

Did the patient attend **funeral ceremonies** during anytime in the 3 weeks before becoming ill?

Yes No DNK

Did the patient **travel** anytime in the three weeks before becoming ill?

Yes No DNK

If Yes, where _____ between (dates) ____/____/____ and ____/____/____

Did the patient have a contact with a **known suspect case** anytime in the 3 weeks before becoming ill? Yes No DNK

If Yes, Surname _____ First name _____ ID Case

During the contact, the suspect case was Alive Dead date of death ____/____/____

Date of last contact with the suspect case ____/____/____

Did the patient have contact with a **wild animal** (non-human primate or others), that was found dead or sick in the bush, or animal behaving abnormally anytime in the 3 weeks before the illness?

Yes No DNK

If Yes, kind of animal _____ Location _____ date ____/____/____

A sample have been collected ? Yes No DNK

Si Yes, date ____/____/____ Blood sampling Urine Saliva Skin Biopsy

The patient was send to an hospital ? Yes No

The patient was admitted in the isolation ward ? Yes No

If Yes, name of Hospital _____ No. de hospital _____ Hospitalization date ____/____/____

Update on the Hospital information

ID Case _____ Reception date: ____/____/____ Country: _____

Member of family helping the patient _____ Name and Surname _____

Date of discharge ____/____/____ **OR** **Date of death** ____/____/____

Laboratory

A specimen was collected _____ before the death _____ after the death

Date sample ____/____/____ Date results ____/____/____ IDLab _____

Sample	blood	blood with anti-coagulants
	skin biopsy	other

Results	PCR	pos	neg	NA	date	___/___/___	
	Antigen detection	pos	neg	NA	date	___/___/___	
	Antibodies IgM	pos	neg	NA	date	___/___/___	
	Antibodies IgG		pos	neg	NA	date	___/___/___
	Immuno Histochemistry		pos	neg	NA	date	___/___/___

Outcome

(Verified 4 weeks after the onset of symptoms)

Alive Dead

If dead, date of death ____/____/____

Case Classification

Alert Case Suspect Probable Confirmed Not a case

Appendix 2: Contacts recording sheet

CONTACTS RECORDING SHEET

Contacts Recording Sheet filled in by

Case name Case number (if assigned)

Case's Village LC1 Chairman

Sub - County County

Hospitalised / Found in the community If Hospitalised, Hospital Date of Admission:.....

Surname	Other Name	Relationship with the case	Age (yrs)	Sex (M/F)	Head of Household	Village	LC1 chairman	Sub-County	Type of Contact (1, 2 or 3, list all)	Date of last contact	Last date for follow-up	1 st Visit	Outcome

Contacts = 1 - sleeping in the same household with a suspected or a case within 3 weeks

2 - direct physical contacts with the case (dead or alive)

3 - has touched his / her linens or body fluids

4 - Has eaten or touched a dead animal (monkeys)

Appendix 3: Contact tracing form (follow up)

CONTACT TRACING FORM (FOLLOW-UP)

Contact Tracing Form – by Village Team Volunteer's name

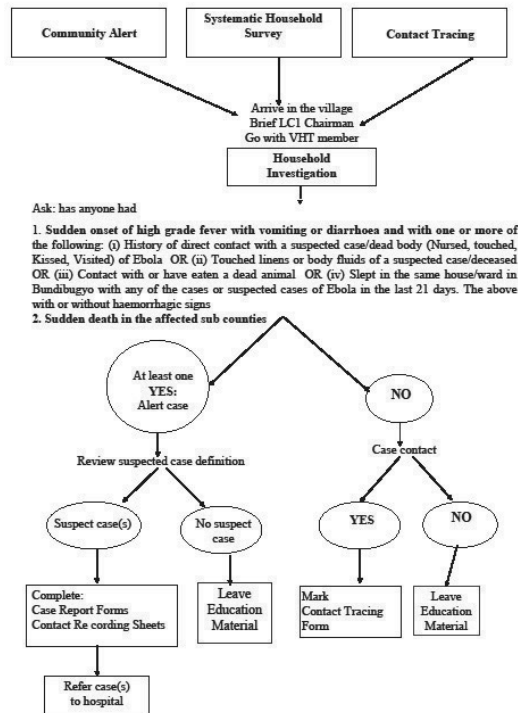
Village LC1 Chairman

SUB-COUNTY COUNTY

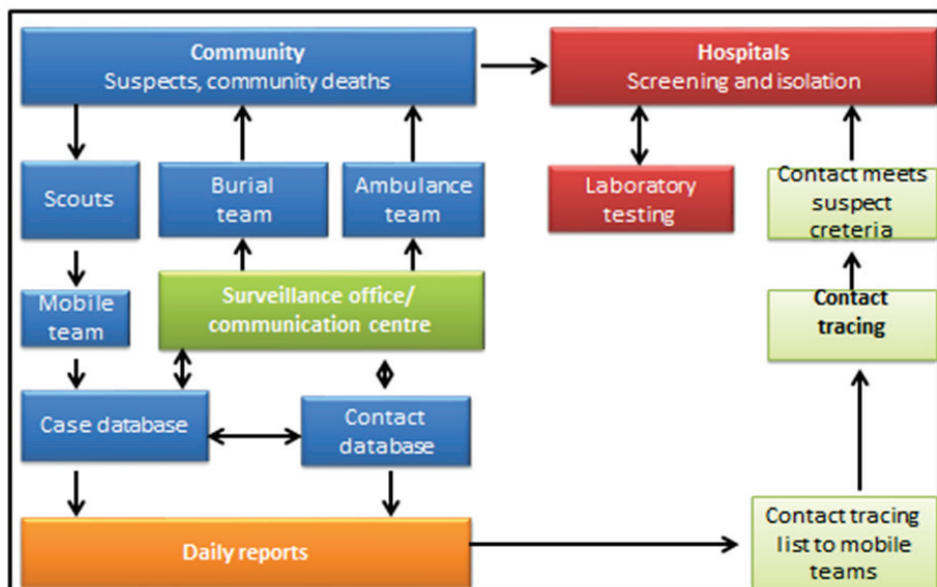
CN	Family	First	Age	Sex	Date of last conta ct	DAY OF FOLLOW-UP																				
	Name	Name				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21

Tick "0" if the contact has not developed fever or bleeding. Tick "X" if the contact has died or developed fever and/or bleeding (complete Case Investigation Form and, if alive, refer to the hospital)

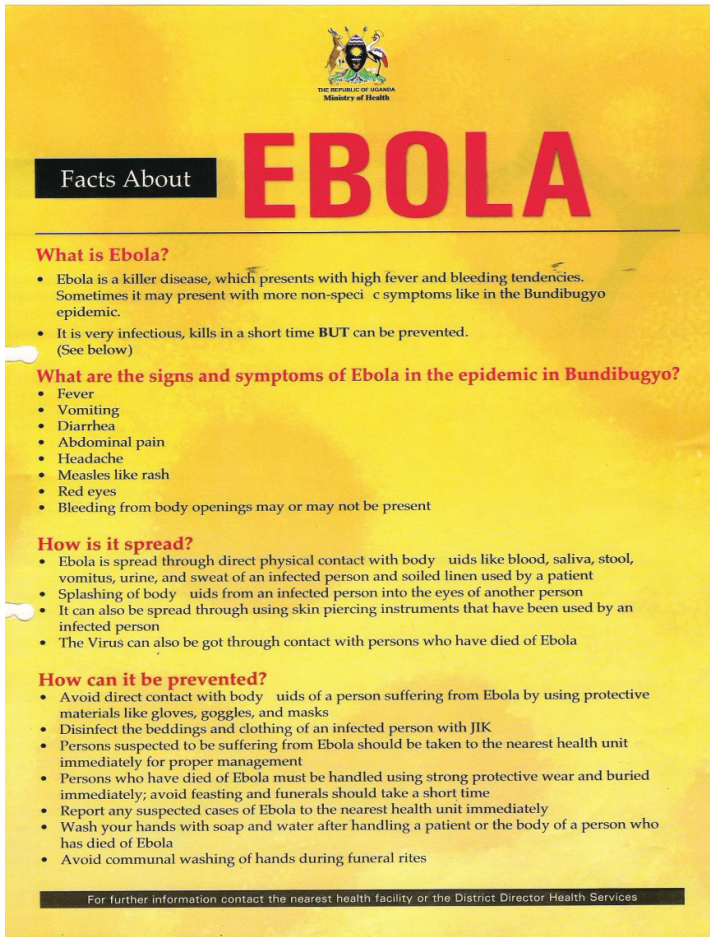
Appendix 4: Algorithm for mobile team



Surveillance flow chart



Appendix 6: Educational Facts Sheet



The factsheet is titled "Facts About EBOLA" and is issued by the Ministry of Health of the Republic of Uganda. It provides information on what Ebola is, its signs and symptoms, how it spreads, and how it can be prevented. The text is presented in a clear, bullet-pointed format on a yellow background.

THE REPUBLIC OF UGANDA
Ministry of Health

Facts About EBOLA

What is Ebola?

- Ebola is a killer disease, which presents with high fever and bleeding tendencies. Sometimes it may present with more non-specific symptoms like in the Bundibugyo epidemic.
- It is very infectious, kills in a short time **BUT** can be prevented. (See below)

What are the signs and symptoms of Ebola in the epidemic in Bundibugyo?

- Fever
- Vomiting
- Diarrhea
- Abdominal pain
- Headache
- Measles like rash
- Red eyes
- Bleeding from body openings may or may not be present

How is it spread?

- Ebola is spread through direct physical contact with body fluids like blood, saliva, stool, vomitus, urine, and sweat of an infected person and soiled linen used by a patient
- Splashing of body fluids from an infected person into the eyes of another person
- It can also be spread through using skin piercing instruments that have been used by an infected person
- The Virus can also be got through contact with persons who have died of Ebola

How can it be prevented?

- Avoid direct contact with body fluids of a person suffering from Ebola by using protective materials like gloves, goggles, and masks
- Disinfect the beddings and clothing of an infected person with JIK
- Persons suspected to be suffering from Ebola should be taken to the nearest health unit immediately for proper management
- Persons who have died of Ebola must be handled using strong protective wear and buried immediately; avoid feasting and funerals should take a short time
- Report any suspected cases of Ebola to the nearest health unit immediately
- Wash your hands with soap and water after handling a patient or the body of a person who has died of Ebola
- Avoid communal washing of hands during funeral rites

For further information contact the nearest health facility or the District Director Health Services

Appendix 7: Procedures for testing Ebola at the UVRI laboratory

A. Plate Set-up

Each column of the plate is for one sample. For example, sample 1 will be in column 1, sample 2 in column 2, etc. Therefore, each 96-well plate can hold 10 samples, 1 positive control, and 1 negative control. Each sample is diluted 4 times with the initial dilution being 1:100 (rows A and E), followed by 1:400 (rows B and F), 1:1600 (rows C and G), and 1:6400 (rows D and H).

The top half of the plate (Rows A-D) will contain the samples and the positive antigen. The bottom half of the plate (Rows E-H) are controls and will receive control (EBOS negative) antigen.

B. Procedure

The day prior to running the assay:

Add 21 µl of patient sample to 500 µl of Masterplate Diluent in a masterplate tube (this is a 1:25 dilution).

NOTE— 42 µl of patient sample in 1000 µl of Masterplate Diluent can also be made.

WARNING: The samples may contain infectious virus at this step and should be handled using appropriate PPE.

Place the tubes in the masterplate rack and cover the tubes with parafilm and the masterplate rack lid. Make sure the masterplate rack has holes in it for the water to enter.

Place the samples into a preheated 56 °C water bath for 30 minutes.

Keep the samples at 4 °C overnight.

While the samples are being inactivated, add 100 µl of EBOS Antigen diluted 1:2000 in PBS (**no tween**) to each well in rows A-D.

Add 100 µl of Control Antigen diluted 1:2000 in PBS (**no tween**) to each well in rows E-H.

Cover the plate with parafilm or plastic wrap to prevent evaporation.

NOTE— If multiple plates have been prepared they can be stacked and wrapped in foil or plastic wrap. Plates can be used for up to 5 days after being coated. Keep plates at 4°C.

Incubate overnight at 4°C (preferred) or alternatively 2 hours at 37°C in a humidity controlled environment (emergency cases).

The day of the assay:

Wash the wells of the coated 96-well plate three times with Wash Buffer. After washing, invert the plate and tap on dry absorbent paper.

NOTE — Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into each well. After aspiration, fill the wells to capacity without overflowing (usually 200-300 µl) with wash buffer. Let soak for 10 to 20 seconds, and then aspirate the liquid. After washing, the plate is inverted and tapped dry on absorbent paper.

Add 100 µl Serum Diluent to each well of the ELISA plate.

Add 33 µl of heat inactivated sample (diluted 1:25 in Masterplate Diluent) and positive (diluted 1:25 in Masterplate Diluent) and negative controls (diluted 1:25 in Masterplate Diluent) to the appropriate well of row E. If you have a multichannel, it can be used here. Pipette up and down at least 10 times. Remove 33 µl from row E and add to row F. Pipette up and down at least 10 times. Remove 33 µl from row F and add to row G. Pipette up and down at least 10 times. Remove 33 µl from row G and add to row H. Pipette up and down at least 10 times. Remove 33 µl from row H and discard (all wells should have 100 µl).

Change tips.

Add 33 µl of heat inactivated sample (diluted 1:25 in Masterplate Diluent) and positive (diluted 1:25 in Masterplate Diluent) and negative sera (diluted 1:25 in Masterplate Diluent) to the appropriate well of row A. If you have a multichannel, it can be used here. Pipette up and down at least 10 times. Remove 33 µl from row A and add to row B. Pipette up and down at least 10 times. Remove 33 µl from row B and add to row C. Pipette up and down at least 10 times. Remove 33 µl from row C and add to row D. Pipette up and down at least 10 times. Remove 33 µl from row D and discard (all wells should have 100 µl).

Place the plate in a container with a lid that contains absorbent material wet with water (such as a paper towel or cotton balls) to create humidity.

Incubate at 37°C for 60 minutes.

Wash the wells three times with Wash Buffer. After washing, invert the plate and tap on dry absorbent paper.

Add 100 µl of anti-human IgG diluted 1:4000 in Serum Diluent to each well.

Place the plate in a container with a lid that contains absorbent material wet with water (such as a paper towel or cotton balls) to create humidity.

Incubate at 37°C for 60 minutes.

Wash the wells three times with Wash Buffer. After washing, invert the plate and tap on dry absorbent paper.

Add 100 µl of pre-mixed Substrate into each well.

Place the plate in a container with a lid that contains absorbent material wet with water (such as a paper towel or cotton balls) to create humidity.

Incubate at 37°C for 30 minutes.

The reaction can be stopped by adding 100 µl 1% SDS (optional).

Read on plate reader at 410 nm or 414 nm.

INTERPRETATION OF RESULTS

Criteria for determining positives (cut-off value): A standard control antigen has been provided and will be run in a standard dilution series. This, in effect, provides a standard curve which will determine the limits of detection of the assay. There is a possibility of these reagents cross-reacting with other strains of EBOV.

First, the background of the assay is subtracted from the samples giving you the adjusted OD. To do this, the ODs from rows E-H are subtracted from rows A-D. For example, in column 1, the OD of E is subtracted from the OD of A, the OD of F is subtracted from row B, etc. An excel spreadsheet can be created to do this for you.

The sum OD is created by adding the 4 adjusted ODs for each column.

A sample is considered **positive when the following criteria are met:**

The adjusted OD of either the 1:400, 1:1600, or 1:6400 dilution must be greater than 0.2.

AND

The sum OD for that column must be greater than 0.95.

A sample is considered **negative when the positive criteria are not met.** If only one of the 2 criteria is met, another sample should be requested and tested (for example, if the 1:1600 adjusted OD is greater than 0.2, but the sum OD is below or at 0.95). Depending on the timing, both the IgM and IgG ELISAs can be run.

COAT PLATE WITH EBOS & CONTROL ANTIGEN

HEAT INACTIVATE SAMPLES (56°C, 30 minutes)

4°C overnight



Wash 3X



ADD SAMPLES & CONTROLS

37°C, 60 minutes



Wash 3X



ADD CONJUGATE: ANTI-HUMAN IgG

37°C, 60 minutes



Wash 3X



ADD SUBSTRATE

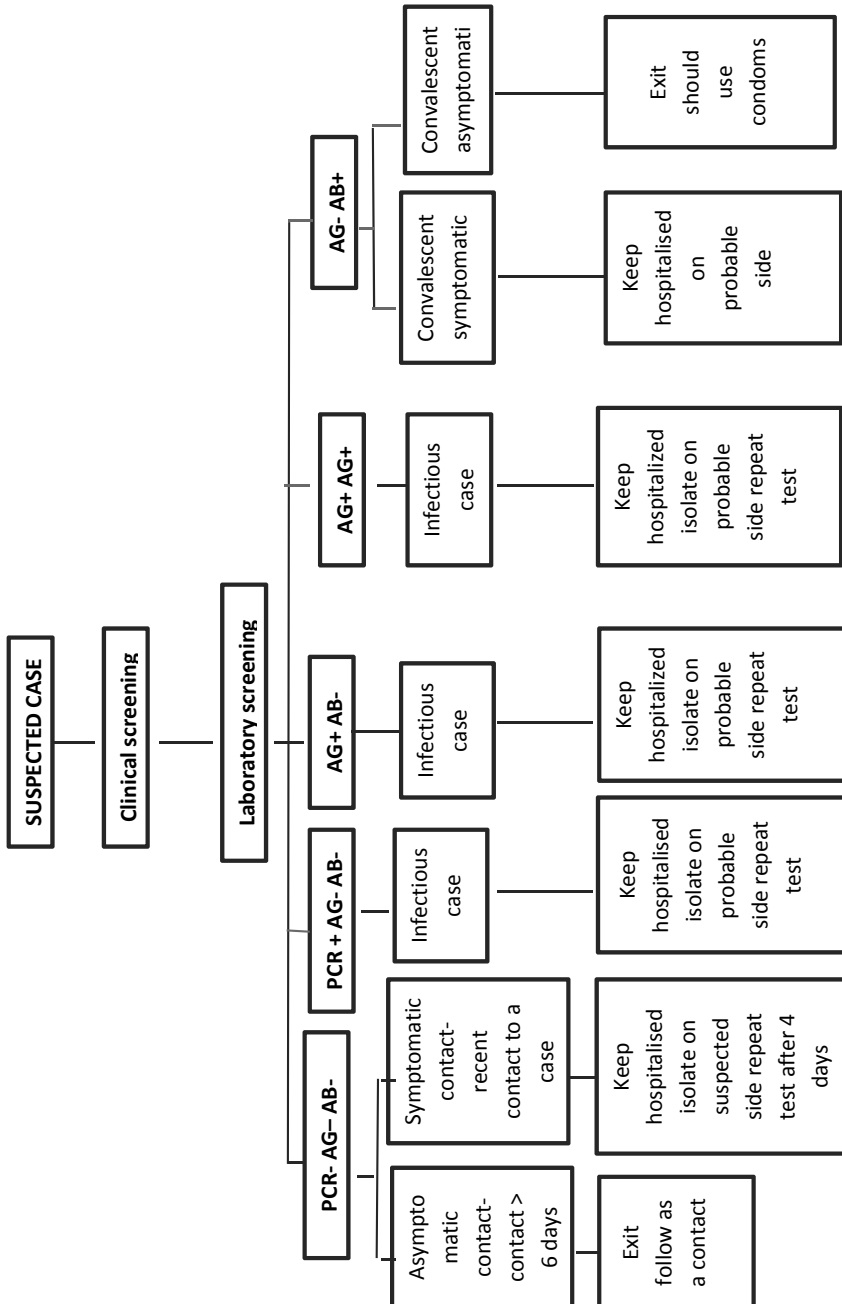
37°C, 30 minutes



READ PLATE

405, 410, or 414 nm

Appendix 8: Algorithm for discharge



Appendix 9: Estimated cost of a district work plan against Ebola

BUDGET IN UGANDA SHILLINGS (UGX) FOR EBOLA OUTBREAK CONTROL ACTIVITIES, Luvuvu district, May - August 2011								
No.	OBJECTIVE	ACTIVITY	Budget in US dollars				Comment	
			National Level	Luvuvu district	5 Neighbouring Districts	Other Districts		Grand Total
1.0	To strengthen early detection, reporting and referral of suspected cases through active surveillance and extended outreach investigations	1.1 Adapt surveillance protocol, mass production and distribution to all health facilities	60,000	0	-	-	60,000	National level activity
		1.2 Conduct orientation of health workers on surveillance guidelines and tools & data for epidemic response	130,000				130,000	
		1.3 Support rapid response teams to verify suspected cases/ contacts	180,000	30,000			180,000	
							20,000	
		1.4 Support daily reporting from health facilities to district and HC levels	0	5,000	15,000			
		1.5 Conduct active case finding, contact tracing and contact follow up by the mobile team and VCHs	0	60,000	15,000	-	95,000	
		Investigation	1.6 Support timely specimen collection and referral for field	30,000	0	30,000		40,000
	Sub-total		378,000	123,000	40,000	-	541,000	
2.0	To initiate prompt and effective management of all suspected cases	2.1 Conduct training of health workers on case management and infection control practices and use of PPEs	130,000	-	-	-	130,000	Control local activity - to be done together with surveillance training
		2.2 Provide allowances for health workers in isolation facilities	100,000	100,000	50,000	50,000	300,000	
		2.3 Procure emergency medicines, medical and infection control	300,000	-	-	-	300,000	
		2.4 Support ambulance services to facilitate referral of suspected cases/ health services	30,000	20,000	-	-	50,000	
		2.5 Conduct support supervision to strengthen infection control practices/ establish triage	45,000	15,000	25,000	-	85,000	
		2.6 Support Epidemiologists to provide psychosocial support services/counselling	40,000	-	-	-	40,000	
		2.7 Support training of Epidemiological support team/counselling	50,000	25,000	-	-	75,000	
		2.8 Support compensation of destroyed items/ reintegration into community	50,000	-	-	-	50,000	
		Sub-total		745,000	200,000	75,000	50,000	1,070,000
3.0	To create public awareness about Ebola the risk factors for its transmission, its prevention and control among the people	3.1 Social mobilization using the film van (community film/talk shows)	100,000	30,000	100,000	-	230,000	
		3.2 Social Mobilization by the national / district leadership	50,000		-	-	50,000	
		3.3 Conduct community sensitization through IEC materials and radio	200,000	50,000	60,000	-	310,000	
							30,000	
		3.4 Sensitization of VCHs to enhance early detection & referral of new cases to CHW	0	30,000	-	-	30,000	
		3.5 Development and translation of health education messages, and radio spots	15,000	5,000	-	-	20,000	
			3.6 Mass production of IEC materials	300,000	0	-	-	300,000
	Sub-total		665,000	115,000	100,000	0	960,000	
4.0	To ensure effective coordination of the	4.1 Support national and district coordination structures	200,000	50,000	90,000	-	340,000	
		4.2 Provide technical assistance/ support supervision	200,000	0	-	-	200,000	
		Sub-total		400,000	50,000	90,000	0	540,000
							1,000,000	
	GRAND TOTAL						3,611,000	
Note: US Dollar = 2000 Uganda Shillings (UGX)								



Dr. Kaboyo, Dr. Atimnedi and Mr. Balinandi wrap the monkey carcass in a bio-hazard bag ready for shipment to UVRI Lab, Entebbe.

- 4.7 Collected two human blood samples from Mr. Emmanuel Kawesa, 54 yr old man and Mr. Frank Nuwenyine 12 yrs old boy.



Mr. Balinadi takes blood samples from two of the residents of Kyai parish, Gomba sub-county.

Appendix 11: Acknowledgement and list of partners at national and international level

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African Medical and Research Foundation (AMREF-Flying Doctors), Emergency for Coordination in Research and Development (ACORD-UK), Catholic Relief Services, Department of Foreign and International Development (DFID), UK, DANIDA, International Committee of the Red Cross (ICRC), International Federation of Red Cross and Red Crescent through the Ugandan Red Cross Society, Italian Cooperation, Rotary International, Save the Children Fund (Denmark), UNHCR, UNICEF, United Kingdom Department for International Development (DFID), US Embassy and USAID, World Food Program (Nairobi), World Vision Uganda, Swedish International Development Agency (SIDA).