

Molecular detection of viral pathogens from suspected viral hemorrhagic fever patients in Ghana

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Abstract

Background: Viral hemorrhagic fevers (VHFs) are infectious illnesses that can cause serious morbidity and mortality to infected persons. During the 2014 Ebola virus disease outbreak in some West African countries, Ghana revamped its surveillance system across the country to prepare, effectively respond and pre-empt any public health concerns

Objective: We report on suspected VHF clinical specimens submitted to the Noguchi Memorial Institute for Medical Research (NMIMR) from health facilities across the country for diagnosis within the period under review. This was partly to provide rapid response and to alert the health system to prevent outbreaks and its spread.

Methods: From January 2017 to December 2018 clinical specimens of blood from 149 cases of suspected VHFs were collected at health facilities across the country and sent to NMIMR. Patient specimens were tested for viral pathogens including Lassa fever, Yellow fever, Dengue fever, Chikungunya, Zika, Ebola and Marburg by real-time reverse transcription-polymerase chain reaction. A case was however tested for influenza as the patient exhibited respiratory distress symptoms as well. Demographic and clinical information collected on a structured case-based forms were analyzed for each patient

Results: Out of the 149 clinical specimens tested, three (3) were found to be positive, with two (2) being Dengue and one (1) seasonal Influenza A H1N1. Analysis of the case-based forms revealed shortcomings with regards to standard case definitions used to enroll suspected cases.

Conclusion: Our results buttress the need for a routine surveillance activity for VHFs to minimize spread and possibly forestall outbreaks. Moreover, febrile illnesses can be caused by a host of pathogens hence there is a need for enhanced diagnosis to help in patient management.

Keywords: Ghana, Reverse transcription PCR, viral haemorrhagic fever

INTRODUCTION

Viral haemorrhagic fevers (VHFs) are a group of viral infections that can cause severe life-threatening illnesses [1]. They may be caused by four distinct families of RNA viruses including *Arenaviridae*, *Filoviridae*, *Bunyaviridae*, and *Flaviviridae* [1]. They mostly occur in the tropical areas of the world with initial signs of high fever ($\geq 38^{\circ}\text{C}$), fatigue, muscle, bone or joint aches and weakness. Severe cases of some types of VHFs may cause bleeding. Most hemorrhagic fever viruses are zoonoses and generally infect both sexes and all ages mainly by occupational exposure.

Transmission to humans is by a bite of an infected tick or mosquito or through aerosol from infected rodent hosts [1]. In the wake of the 2014 Ebola outbreak in some West African countries that accounted for more than 11,000 lives [2], the health authorities in Ghana in their response set up a surveillance system and sensitize health staff across the country on case definitions as part of a preparedness plan. It was to monitor, detect and rapidly respond to cases and rumors of cases that presented with symptoms consistent with VHFs. Clinical specimens of blood processed into serum or plasma were submitted to the country-designated laboratory at the Noguchi Memorial Institute for Medical Research (NMIMR) for testing of VHFs. In order to determine a conclusive diagnosis, a testing algorithm that involved investigating for the known endemic VHFs in the sub-region was adopted. Laboratory investigations were performed for Ebola, Marburg, Yellow fever, Lassa fever,

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West Nile and Dengue fever. While a definitive viral diagnosis could not be established for the majority of the suspected cases, two were found to be positive for Dengue virus and one for seasonal Influenza A H1N1, a case that exhibited respiratory symptoms in addition to the defined symptoms for VHF and hence tested for Influenza. Apart from the intended goal of this surveillance activity which is to prepare for and respond rapidly to outbreaks and rumors of it, the laboratory investigations of suspected VHFs also provide an early warning system for the emergence or re-emergence of viral pathogens of public health concern. Here we write to give details of data collected between January 2017 and December 2018.

MATERIALS AND METHODS

Patients were assigned laboratory identification numbers once specimens are taken for processing and their laboratory test results were not anonymized as these were required for routine patients care. All documents were kept confidential and only researchers involved in the study had access to the data.

Sample collection

Whole blood processed into serum or plasma, and cerebrospinal fluid (CSF) from eligible patients suspected of VHFs were collected from health facilities across the country by trained health staff (Figure 1). The standardized set of uniform criteria used to identify cases for laboratory investigations were based on guidelines of detailed clinical assessment, epidemiological linkage and history of exposure [3]. These guidelines involve a case definition of a sudden onset of fever (temperature of 38°C or above) accompanied by any of the following symptoms:

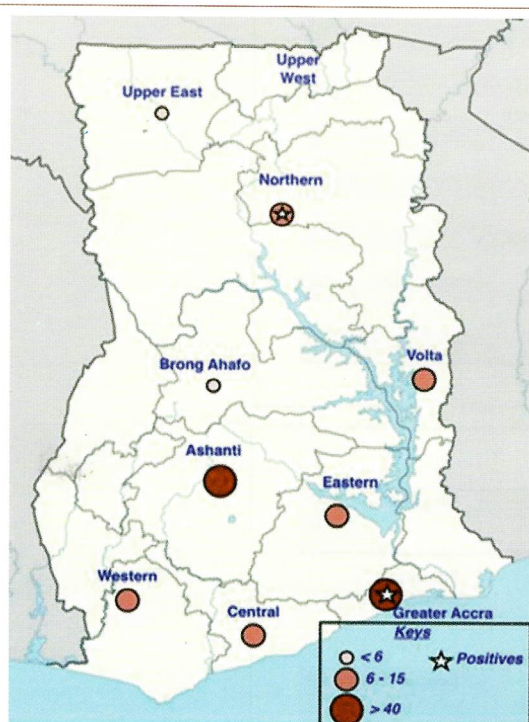


Figure 1: Map of Ghana showing regions where specimens were collected, and positives recorded. The size of the figures in the map define the number of samples as in the legend and figures with the symbol, star indicates the location where positives were detected

Table 1: Assays and viral targets

Viral Pathogen	Reagents	Target gene
Ebola	RealStar Filovirus RT-PCR kit 1.0	L-gene
Marburg	RealStar Filovirus RT-PCR kit 1.0	L-gene
Lassa fever	1. Superscript One Step RT-PCR	S-gene
	2. Real star Lassa virus RT-PCR	L-gene
Dengue	AgPath-ID One Step RT-PCR	5'-Untranslated regions
Chikungunya	AgPath-ID One Step RT-PCR	Non-Structural Protein 1
Zika	AgPath-ID One Step RT-PCR	Envelope gene
Yellow fever	AgPath-ID One Step RT-PCR	5'- noncoding region

headache, diarrhea, difficult breathing, loss of appetite, vomiting, hiccup, lethargy, stomach pains, difficulty swallowing, unexplained bleeding or any sudden unexplained death. Demographic and clinical data for the patients were captured on case-based forms that accompanied specimen transported to NMIMR for laboratory investigations. A total of 149 clinical specimens were received from all the ten regions of Ghana except the Upper West Region from January 2017 to December 2018 (Figure 2). Suspected clinical specimens were also received from neighboring countries of Togo and Benin. They were all tested by real-time reverse transcription polymerase chain reaction (rRT-PCR) for Ebola, Marburg, Lassa fever, Yellow fever, Dengue fever and Zika viral infections (Table 1). As a surveillance activity under the auspices of the Ghana Health Service, and in conjunction with NMIMR, consent for approval for a manuscript write-up was sought from the former. All the clinical specimens used were anonymized to protect patient confidentiality before authors were given access to data.

Extraction and amplification of viral nucleic acids

The viral targets for the VHFs all fall under the category of RNA viruses. From the clinical specimens received, viral RNA was extracted and purified from 140 µL volumes of each with the use of the QIAmp Viral RNA mini Kit (Qiagen, Hilden, Germany). The manufacturer's instructions were followed throughout the process and ended with a total of 60 µL purified nucleic acid.

Reverse transcriptase PCR

Amplification of nucleic acid was undertaken using different assays based on the various pathogens of interest. All rRT-PCR assays were conducted in a volume of 25 mL with 2.5 mL or 5 mL purified nucleic acid extract as a template. The reagents, cycle amplification numbers, primer sequences, target region, and amplicon length are shown in Table 1.

RESULTS

Regional distribution of specimens collected

No specimen was received from Upper West Region during the period under review. The Ashanti Region however recorded the highest number of cases (50), followed by Greater Accra (47) (Figure 2). In 2017, forty-seven (47) specimens were received

and 102 the following year. March 2018 reported the highest number of specimen (42) received followed by 33 specimens in December 2017, with no specimen received in 2017 (May and August), and July 2018. Other samples were received from Togo (Figure 2).

Clinical and demographic features of patients

Out of the 149 specimens received nearly 50% of the patients enrolled reported without the key known symptoms for VHFs as described on the case investigation forms (Table 2). A total of 57 (38%) had their temperatures measured or reported of history of

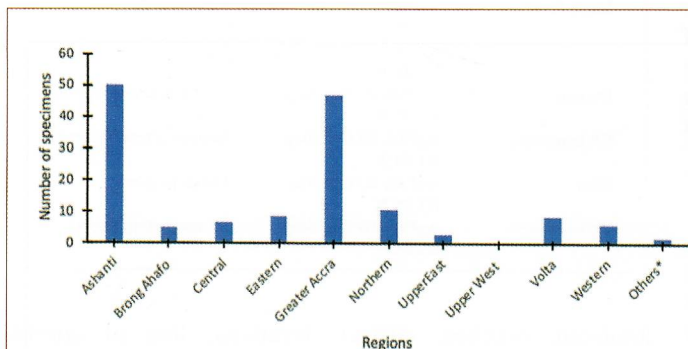


Figure 2: Regional Distribution of suspected VHF specimen received in Ghana from 2017 to 2018

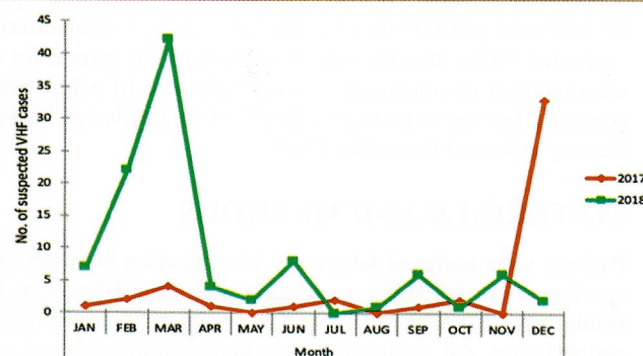


Figure 3: Monthly Distribution of suspected VHF specimen received in Ghana from 2017 to 2018

Table 2: Clinical Characteristics of suspected VHF specimens received in 2017-2018

Symptom	Proportion with symptom n (%)	Proportion without symptom n (%)	Proportion with blank n (%)
Fever	57 (38)	86 (58)	6 (4)
Headache	55 (37)	83 (56)	11 (7)
Vomiting and Nausea	56 (38)	86 (58)	7 (5)
Anorexia	56 (38)	85 (57)	8 (5)
Diarrhoea	56 (38)	86 (58)	7 (5)
Abdominal Pain	55 (37)	85 (57)	9 (6)
Muscle pain	57 (38)	83 (56)	9 (6)
Difficulty in breathing	57 (38)	86 (58)	6 (4)
Difficulty in swallowing	57 (38)	85 (57)	7 (5)
Hiccups and Cough	11 (7)	0 (0)	138 (93)
Skin rashes	7 (5)	0 (0)	142 (95)
Bleeding from gum	57 (38)	86 (58)	6 (4)
Bleeding from nose	57 (38)	86 (58)	6 (4)
Bleeding from stool	54 (36)	86 (58)	9 (6)
Blood in vomitus	57 (38)	85 (57)	7 (5)
Bleeding from vagina	1 (1)	0 (0)	148 (99)
Outcome			
Dead	31 (21)		
Alive	112 (75)		
Status Unknown	6 (4)		

*VHF, viral haemorrhagic fever.

Table 3: Demographics and Clinical Characteristics of Positive Specimens

Patient Lab ID	Sex	Age in yr.	Outcome	Region	Clinical Characteristics	Diagnosis
VHF-18-007	Male	24	Dead	Greater-Accra	Fever, Headache, Sore throat, Generalized weakness, Blurred vision, blood in sputum	Influenza (pandemic H1N1)
VHF-18-050	Female	4	Alive	Northern	Headache, vomiting, diarrhea, bleeding gums, bloody vomits, bleeding from the nose and vagina	Dengue- 2
VHF-18-084	Male	35	Alive	Greater-Accra	Blank	Dengue-2

*ID, identification.

fever (38°C and above) and the following symptoms were recorded from 56(37%) patients. Recorded symptoms were headaches, vomiting, anorexia, diarrhea, abdominal and muscular pains, difficulty in breathing and swallowing and bleeding from the nose, gum and in stools. No patient was recorded to have hiccups, and skin rashes (Table 2). Within the period under review, records indicated that 31(21%) of the suspected cases ended with mortality.

rRT-PCR

Two (2) of the specimens tested positive for Dengue fever virus type -2 (DENV-2) and one (1) was found positive for seasonal Influenza A (H1N1) virus after the laboratory testing by rRT-PCR assays for the familiar VHF and other viral pathogens in the sub-region. The patient's specimen that tested positive for seasonal Influenza A (H1N1) virus was specifically queried for Influenza virus with a second specimen of an oropharyngeal swab after the initial VHF testing was found to be negative. All the other patients' specimens have tested negative by RT-PCR for VHF.

DISCUSSION

The detection of active Dengue fever cases through our routine hospital-based febrile surveillance for VHF suggests the possible circulation of "pre-outbreak" strains of Dengue fever virus. The detected Dengue virus were type-characterized and genetically identified as Dengue virus serotype 2 with close homology with recent outbreak strains from Burkina Faso [6]. Appropriate measures were taken to contain and prevent spread. This corroborates the set objective of our surveillance activity which serves to provide an alert system to ward off possible outbreaks of known VHF and other emerging viruses. Additionally, febrile illnesses can be caused by a host of pathogens and this makes it evident that improved surveillance across the country and the sub-region with enhanced diagnosis is needed to aid in patient management. Captured patient information, both clinical, demographic and epidemiologic are relevant in clinical determination and proper case management especially in the case of VHF. Nearly 50% of the case investigation forms received along with clinical specimens either had missing information or clinical details recorded did not meet the case definition. This observation supports our finding in an earlier study where we catalogued the effects of information gaps in our surveillance data from suspected cases on the recent West African Ebola virus disease outbreak [7].

The middle part of the country the Ashanti region, recorded highest number of suspected cases received during the period under review (Figure 3). This was due to an outbreak which the aetiology turned out to be respiratory viruses but was cryptic at the onset [8]. Through this surveillance activity, a seasonal Influenza A H1N1 case was confirmed by the National Influenza Center at NMIMR after it tested negative for VHF. The Influenza screening was attempted because of the clinical information on the patient which included respiratory symptoms. Most febrile illnesses are treated as malaria partly because they do not require complex diagnostic equipment and facilities [9]. Previously, we detected high exposure levels of Dengue virus antibodies in suspected and confirmed malaria patients [10]. This has added to our suspicion and finding of the possible circulation

of Dengue virus that have been undetected or misdiagnosed. In Africa, Dengue is endemic in over 33 countries with the serotype-2 being noted to predominantly cause outbreaks in most parts of the continent [9]. The confirmed Dengue-2 virus in our study validates the data in the sub-region and supports other studies in the country that detected Dengue -2 virus from a traveler [1] and suspected malaria children [12]. The findings made in our VHF surveillance activity within a period of 2 yr. supports the need for an improved routine surveillance activity for these VHF and other emerging viruses of zoonotic and outbreak potential in order to minimize spread and/or possibly forestall outbreaks.

Conclusion

The findings made in our VHF surveillance activity within a period of 2 yr. supports the need for an improved routine surveillance activity for these VHF and other emerging viruses of zoonotic and outbreak potential in order to minimize spread and/or possibly forestall outbreaks.

DECLARATIONS

Ethical considerations

The study was permitted by the Public Health and the Disease Surveillance Divisions of the Ghana Health Service. Requirement of ethical approval was waived because the study constituted routine surveillance for disease outbreak.

Funding

None

Competing Interests

No potential conflict of interest was reported by the authors.

Author contributions

JHB, and WKA conceptualized and designed the study. FAB and BS permitted and assisted with participants recruitment and revision of manuscript. JHKB, TWA, EK, KA, GM, CA, DP and SN processed the clinical specimens, analysed the data and edited the manuscript. JHKB, WKA, EYB and IAA EFA, TKA interpreted the data, read and edited the manuscript. JHKB and TWA wrote the initial draft. WKA, EYB and IAA revised the draft. All authors read and approved the final manuscript.

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Availability of data

Data is available upon request to the corresponding author.

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