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

Joseph HK Bonney1*, Theodore W Asigbee1, Erasmus Kote1, Keren Attiku1, Franklin Asiedu-Bekoe2, Gifty Mawuli1, Evelyn Y Bonney1, Ivy A Asante1, Christopher Abana1, Deborah Pratt1, Stephen Nyarko1, Badu Sarkodie1, William K Ampofo1.

1Department of Virology, Noguchi Memorial Institute of Medical Research, College of Health Sciences, University of Ghana, Accra, Ghana; 2Disease Surveillance Department, Ghana Health Service, Accra, Ghana; 3Public Health Division, Ghana Health Service, Accra, Ghana.

Received December 2019; Revised January 2020; Accepted February 2020

Abstract

Background: Viral hemorrhagic fevers (VHFs) are infectious illnesses that can cause severe 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 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.

Conclusion: Our results buttress the need for a routine surveillance activity for VHFs to minimize spread and possibly forestall outbreaks.

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 (≥ 38°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,
Viral pathogens from suspected viral hemorrhagic fever patients
Bonney et al., 2020. https://doi.org/10.46829/hsijournal.2020.6.1.1.31-35

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's 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 VHF's 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 were 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 VHF's were collected from health facilities across the country by trained health staff (Figure 1). The standardize 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: 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 (rT-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 VHF's 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 QIAamp 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 rT-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

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    | Superscript One Step RT-PCR | S-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 |

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.
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).

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

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 VHF as described on the case investigation forms (Table 2). A total of 57 (38%) had their temperatures measured or reported of history of

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 hemorrhagic 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 swelling 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 VHFs 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 VHFs.

DISCUSSION

The detection of active Dengue fever cases through our routine hospital-based febrile surveillance for VHFs 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 VHFs 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 VHFs. 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 H1N1case was confirmed by the National Influenza Center at NMIMR after it tested negative for VHFs. 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[11] 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 VHFs 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 VHFs and other emerging viruses of zoonotic and outbreak potential in order to minimize spread and/or possibly forestall outbreaks.

DECLARATIONS

Ethical considerations

The study and 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.

Acknowledgements

We are grateful to the Ghana Health Service for the approval and permission, the staff of the Virology Department of Noguchi Memorial Institute for Medical Research for their support and technical assistance and the staff of all the health directorates across the country where clinical specimens were collected for their co-operation.

Availability of data

Data is available upon request to the corresponding author.

REFERENCES

1. Centers for Disease Control and Prevention (CDC); National Center for Emerging and Zoonotic Infectious Diseases (NCEZID); Division of High-Consequence Pathogens and Pathology (DHCPP) (2011) Bats: Learning about bats and rabies - Rabies. In: CDC.gov. http://www.cdc.gov/rabies/bats/education/index.html
2. Roca A, Afolabi MO, Saidu Y, Kampmann B (2015) Ebola: A holistic approach is required to achieve effective management and control. J
Viral pathogens from suspected viral hemorrhagic fever patients
Bonney et al., 2020. https://doi.org/10.46829/hsijournal.2020.6.1.1.31-35