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Yellow Fever outbreak in Darfur, Sudan in October 2012; the initial outbreak investigation report

Mohammed A. Soghaier[a,*], Ahmed Hagar[a], Mohammed A. Abbas[b], Mutasim M. Elmangory[c], Khalid M. Eltahir[d], Amadou A. Sall[e]

[a] *Directorate of Epidemiology & Zoonotic Diseases, Sudan Federal Ministry of Health, Sudan*
[b] *Integrated Vector Management Unit, Sudan Federal Ministry of Health, Sudan*
[c] *National Public Health Laboratory, Sudan Federal Ministry of Health, Sudan*
[d] *WHO Country Office, Khartoum, Sudan*
[e] *Pasture Institute, Dakar, Senegal*

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Summary
Introduction: Sudan is subject to repeated outbreaks, including Viral Hemorrhagic Fever (VHF), which is considered to be a very serious illness. Yellow Fever (YF) outbreaks in Sudan have been reported from the 1940s through 2005. In 2012, a new outbreak of YF occurred in the Darfur region.
Objective: To identify the potential for an outbreak, to diagnose the disease and to be able to recognize its cause among the initial reported cases.
Methodology: >This is a descriptive and investigative field study that applies standard communicable disease outbreak investigation steps. The study involved clinical, serological, entomological and environmental surveys.
Results: The field investigation confirmed the outbreak and identified its cause to be YF.
Conclusion: National surveillance systems should be strong enough to detect VHFs in a timely manner. Local health facilities should be prepared to promptly treat the initial cases because the case fatality ratios (CFRs) are usually very high among the index cases.

Introduction

Yellow Fever (YF) is a mosquito-borne viral hemorrhagic fever that affects an estimated 200,000 people and causes 30,000 deaths annually [1]. YF is a member of the flavivirus genus (group B arbovirus)

* Corresponding author. Tel.: +249 912387175.
E-mail addresses: soghaier@gmail.com, soghaier@hotmail.com (M.A. Soghaier).

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[2]. It has a high case fatality ratio (CFR), reaching 50% in some severe manifestations [3].

The disease is believed to have three identified transmission cycles in Africa: (1) sylvatic (jungle); (2) intermediate (savannah); and (3) urban [4]. The sylvatic (jungle) transmission cycle involves transmission of the virus between non-human primates and sylvatic mosquito species found in forest areas (*Aedes* (*Stegomyia*) *africanus*) [5]. Humans generally contract the infection when they are in the jungle during occupational or recreational activities [3—5]. The YF intermediate (savannah) transmission cycle is mediated both by domestic and sylvatic mosquitoes that infect humans living or working in areas bordering forest [6]. Occasionally, large human outbreaks are mediated by the mosquito species involved in the savannah cycle [7]. Urban transmission cycle involves transmission of the virus between humans and domestic mosquitoes, *Ae*. (*Stegomyia*) *aegypti* [1,4]. Urban transmission can lead to large epidemics of the disease due to the ease of communicability. The transmission cycle is very important with regard to planning and implementing appropriate control strategies, which usually involve mass vaccination, vector/surveillance control and case management [8].

Sudan is one of the African YF belt countries. YF was first documented in 1940 in the Nuba Mountains, South Kordofan State [9]. In 1959, the Sudan experienced the largest outbreak ever reported in Africa to date, with 15,000 cases and 1500 deaths reported from Blue Nile state [1]. The most recent YF outbreak occurred during 2005 in South Kordofan [10]. That outbreak included 605 reported suspect cases and 163 deaths, which resulted in a CFR of 27%. From the beginning of October 2012, the country experienced a new YF outbreak in its most vulnerable region (Darfur). The present work is the report of the initial outbreak investigation that was performed.

Methodology

Design

This was a descriptive field investigation study that applied the standard communicable disease outbreak investigation steps [11]. In this study, epidemiological description, ecological, entomological and serological surveys were conducted.

Study area

The study area consisted of the two Darfur states, Central and South Darfur, in the western part of Sudan. The total populations for the two states are 1,022,740 and 3,485,815, respectively [12]. The area is currently suffering from the complications of civil war and longstanding conflicts.

Study population

The study population consisted of patients who were diagnosed with hemorrhagic fever and had been reported to the national directorate of epidemiology and zoonotic disease within the Federal Ministry of Health of the Republic of the Sudan through the national communicable disease surveillance system. Other people who were epidemiologically linked to these reported cases, in addition to domestic animals and insects that were present at the areas from which cases were reported, were also included in the study population.

Study timing

The study was conducted from October 2—20, 2012. During this time period, all standard operational steps of communicable disease outbreak investigation were performed, including preparation, field investigations and laboratory confirmation of the organism responsible for the outbreak.

Context background

On October 1, 2012, the national surveillance system of the Directorate of Epidemiology and Zoonotic disease with the Federal Ministry of Health of the Sudan reported 7 cases with suspected Viral Hemorrhagic Fever (VHF). These cases were from the Khour Ramla village of the Nertiti locality, which belongs to the newly formed Central Darfur state. These cases presented with sudden onset fever, body aches, vomiting and bleeding manifestations. Some cases also presented with jaundice. Five of these cases died, with a CFR of 71.4%. Some of these patients were referred from Nertiti rural hospital to Nyala hospital in the capital city of South Darfur state (the detailed case description will be provided below).

The National Directorate of Epidemiology and Zoonotic disease conducted its systematic discussions and reviews. An investigation team was immediately sent to assess the situation in the field, to explore the potential for an outbreak and to find the possible causes and sources of the event.

The team consisted of an epidemiologist, an entomologist, public health surveillance officers and a laboratory technician from FMOH. The team arrived in the field by October 10 through the

United Nations Humanitarian Air Service (UNHAS), which was facilitated by the World Health Organization (WHO) & WFP country offices. The federal outbreak investigation team met and merged with the Central Darfur State Epidemiology Department team. The investigation began after the official briefing meetings with the stakeholders and the community leaders.

Steps of the outbreak investigation

Preparation to investigate

Immediately after the construction of the investigation team, the team conducted a brief meeting and distributed the responsibilities and the field tasks between them. The head of the investigation team opened the contact channels with the Central and South Darfur states and shared the main objectives of the mission. The head of the team also secured the required field tools and commitments. The team members conducted a thorough literature review of the basic concepts of hemorrhagic fevers, their epidemiology, transmission and ways to investigate the outbreak. The team revised all records of similar problems reported to the FMOH during the last several years, particularly from areas with similar geography and demographics.

Verification of the diagnosis and confirmation of the outbreak

Because the serum samples that the National Public Health Laboratory (NPHL) received were not tested until the departure of the team, the team revised the symptomatology and clinical presentations of the reported cases from the clinic registers (Nertiti rural hospital and Nyala). We have interviewed the health care providers who attended the reported patients (medical assistants, nurses and medical doctors) in each hospital. According to the documented descriptions, the investigation team suggested the following deferential diagnoses:

- Crimean Congo Hemorrhagic Fever CCHF;
- Rift Valley Fever RVF;
- Yellow Fever YF;
- Complicated Malaria;
- Dengue, Zika virus infection and other VHFs;
- Viral Hepatitis disease, particularly Hepatitis A (HAV), Hepatitis B (HBV), Hepatitis C (HCV) and Hepatitis E (HEV).

We ranked these differential diagnoses because the majority of cases were reporting fever and bleeding. In addition, the area had just experienced a very heavy rainy season. Most of the community members are farmers and animal pastoralists and have close, direct contact with their animals. Due to the fact that few patients were reporting jaundice, we ranked CCHF and RVF first. We ranked YF third because few patients had reported jaundice. However, we considered the sylvatic cycle of YF transmission because the area that had reported the index cases is very close to the jungle. Wild monkeys inhabit the jungle, and people usually go there with their animals for rearing. The team conducted field entomological studies, collected additional blood samples and shipped them to the NPHL in Khartoum for confirmation and demarcating out between the possible diagnoses.

Case definition

When the team reached the field, we made a very broad case definition as a method to include as many diseased cases as possible for blood sample collection for testing and confirmation. Our field case definition was any patient who developed a sudden high-grade fever $\geq 38\,^{\circ}C$ with or without bleeding, jaundice, vomiting or prostration after October 1. We used this case definition to guide our hospital visits, to facilitate active household case searching and to assist with clinical records revision.

Active case finding

The team conducted a systematic active case-finding survey based on the above field case definition. The team visited the two main hospitals of the affected area (Nertiti rural hospital and Nyala) and actively assessed the present medical records, admission sheets, laboratory registers and pharmacy logbooks. Additionally, ''shoe-leather epidemiology'' was used for active case finding at the village and household levels within the residential areas from which the index cases were reported. The investigation team visited the following villages during the field outbreak investigation missions:

- Khour Ramla,
- Fukodoko,
- Rijl Aldalaba,
- Toor,
- Tibbi,
- Jurfambari,
- Norma.

Based on the above-mentioned case definition, the investigation team generated the initial

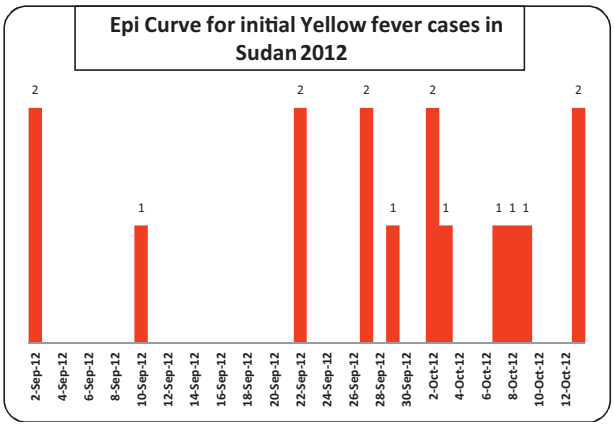

Figure 1

outbreak line list. Nine additional cases were added as a result of active case finding, bringing the total to 16 cases for the initial line list. Fresh blood samples were collected from the acutely febrile patients during the household visits (the samples were used for serology and blood films for malaria).

Descriptive epidemiology

The team analyzed the data descriptively, applying the classical epidemiological traits of person, place and time to gain insights on the reported cases. One of the most interesting findings was that the majority (81.2%) of the cases were young adult males, were related and were in close contact with animals (38% pastoral). Another interesting finding was that most of the cases were initially treated with anti-malarial medications, including the recommended treatment lines that are based on a positive immuno chromatographic (ICT) test for Falciparum Malaria. Family history of the disease was confirmed because two families had reported the deaths of immediate family members during the investigation period and the illness had manifested identically in both, with fever and bleeding (hematemesis). Bleeding presented in 80% of cases, while jaundice presented in 19% of cases. Further descriptions of the overall outbreak and the other reported cases will be published separately (Figs. 1 and 2).

Hypothesis generation

Before laboratory confirmation of the collected blood samples, the investigators generated three hypotheses based on the available descriptions for the reported cases and the surrounding environment, in addition to information from previous outbreaks.

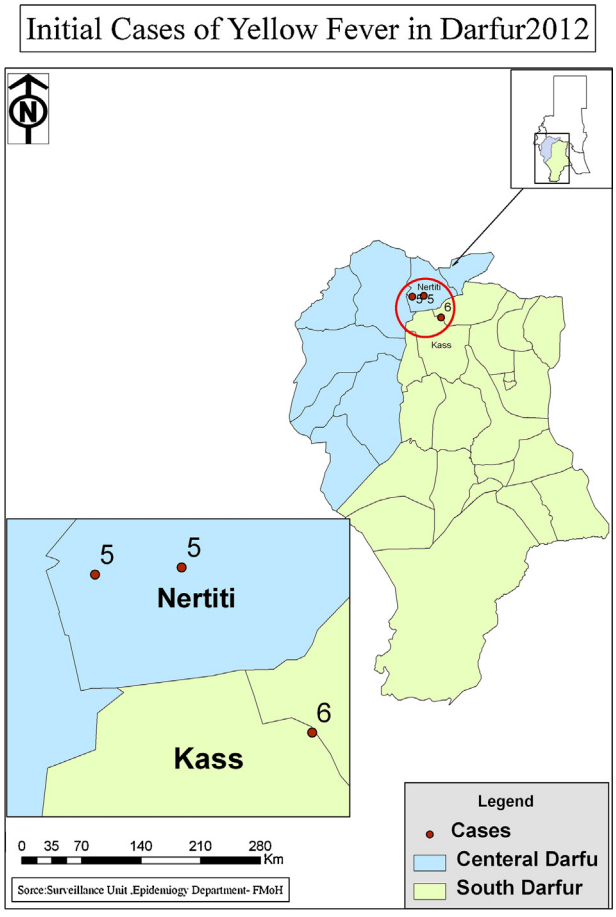

Figure 2

(a) Because the majority of the cases were young adults, most cases presented with fever and massive bleeding, and most patients were involved with animal rearing activities. When combined with the high case fatality rate, we assumed that these events could be explained by an outbreak of CCHF or RVF.
(b) The majority of the cases were young adults and were involved in animal rearing. Additionally, the index cases reported were from the Khour Ramla and Fukodoko areas, which are very close to the forest, which is inhabited by wild monkeys. Young men will often take their animals into the forest. We assume that this behavior could explain an outbreak of sylvatic cycle transmission Yellow Fever.
(c) The area is endemic to malaria. Additionally, the preliminary results indicated that a vast majority of the cases were Malaria ICT-positive and had a history of taking anti-malarial medications. Considering the natural history of the disease, we thought that this case might be an outbreak of complicated malaria.

Hypothesis evaluation

To evaluate the above three hypotheses, we conducted serological surveys from the febrile individuals (7 samples were collected from the different villages). We conducted field entomological surveys at the affected villages and found an elevated house index (>20%) for *Ae. Aegypti* and *Anopheline* mosquitoes in Nertiti. Additionally, we performed a hospital-based cross-sectional study to roughly estimate the proportion of positive Malaria cases from all of the febrile cases that attended the outpatient clinic at the Nertiti rural hospital from October 10—11. We identified 14/37 who were Malaria ICT-positive.

Additional environmental studies

The investigation team met with the animal resource and veterinary authority in the Nertiti locality to receive a brief report regarding animal heath in the affected villages one month prior to the outbreak. In particular, we asked about the presence of abortion storms and deaths among the animals. The locality veterinary authority provided us with updates regarding animal health. Additionally, the veterinary authority helped our investigators collect the required samples of hard ticks from some animals for the purpose of monocular biology. The team captured one wild monkey near the Khour Ramla village but failed to sample it due to security concerns, as the area is currently suffering from the complications of longstanding conflicts.

Implementation of control/prevention measures

The team immediately began talking to the Central Darfur and South Darfur state stakeholders, with the goal of advocating for the improvement of case management capacities. This situation was especially true for the Nertiti rural hospital, which was in need of field blood bank facilities to decrease case fatalities. The team recommended strengthening the hemorrhagic fever surveillance system to monitor the situation and to prioritize the interventional plan. The team recommended strengthening malaria case diagnosis and treatment activities to facilitate sorting between malaria and other febrile illnesses.

Communication of findings

When the field investigation mission was completed, the team conducted several debriefing meetings with the stakeholders and community leaders in the affected states. The goal of the meetings was to explain the possibilities of the outbreak, the steps that should take place and all expected interventions. The team disseminated the findings of the investigation field trip among the seniors and colleagues within the directorate of epidemiology and zoonotic disease and the general directorate of public health and the EHA.

Laboratory results of the outbreak investigation

Serological studies

Out of 7 tested samples, 4 were found to be positive for YF IgM using the captured ELISA technique [13], and quantitative RT-PCR [14], which was conducted at the Institute of Veterinary Research — UK (University of Khartoum).

The 4 positive samples and other negatives were processed and sent to the WHO reference laboratory for YF (Institute of Pasture Dakar — Senegal) for further confirmation of the presence of IgM antibodies against YF, Dengue, Chikungunya, West Nile, CCHF, RVF, and Zika viruses. Two samples were positive for anti-YF IgM at relatively high titers (1/3200 and 1/12,800). They were positive for anti-YF IgM antibodies and were confirmed by plaque reduction neutralization tests (PRNTs) [15] and real time RT-PCR for the YF virus (detecting the viral genome). In addition to these 2 patients, 1 more sample was positive for YF virus by real time RT-PCR. One sample was negative for anti-YF IgM, likely because the sample has been taken at a very early stage of the infection (1 day immediately after the onset of the disease), and the antibody response was not detectable at that time [16]. Further genetic sequencing revealed a link between the current virus and the Uganda 2010 virus, but not to the Sudan 2005 outbreak. Additional samples that were confirmed later during the outbreak were tested by the American Naval Medical research Unit (NAMRU-3) in Cairo. The overall outbreak description will be reported and published separately. The results of the initial blood testing are shown in Table 1.

Malaria testing

Of the 7 blood films that were prepared from the febrile patients during the active case search, we identified 2 that were positive with the ring stage of the Falciparum Malaria parasite; both samples showed hyper-parasitemia (+++). During

Table 1 Summary of cases and initial laboratory testing.

ID	Age in years	Sex	Locality	Date of onset	YF PCR	YF ELISA IgM	CCHF PCR	RVF PCR	Dengue PCR	Zika PCR	HAV PCR	HEV PCR	HBV PCR	HCV PCT	Malaria blood film
1	17	Male	Nertiti	2-Sep-12	NA*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	20	Male	Nertiti	2-Sep-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	30	Male	Nertiti	10-Sep-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	50	Male	Nertiti	27-Sep-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	20	Male	Nertiti	27-Sep-12	+ve	+ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	+ve
6	23	Male	Nertiti	29-Sep-12	+ve	+ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	−ve
7	30	Male	Nertiti	3-Oct-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	15	Male	Kass	2-Oct-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	9	Female	Kass	2-Oct-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	20	Male	Kass	22-Sep-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	30	Female	Kass	8-Oct-12	−ve	−ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	+ve
12	18	Male	Kass	9-Oct-12	+ve	+ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	−ve
13	48	Male	Nertiti	7-Oct-12	−ve	−ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	−ve
14	20	Male	Nertiti	13-Oct-12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	27	Male	Kass	22-Sep-12	+ve	+ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	−ve
16	8	Male	Kass	13-Oct-12	−ve	−ve	−ve	−ve	−ve	−ve	NA	−ve	−ve	−ve	−ve

NB: * NA means not applicable that because blood samples were not collected from these patients due to mortality or inaccessibility or test was not performed.

the Nertiti hospital-based study, we observed that 37.8% (14/37) of samples were positive for ICT for *M. falciparum*.

Entomological studies

The team examined 6 locations (villages) and found hard ticks and *Anopheline* mosquitoes in high densities in both the adult and aquatic stages (house index > 20% for *Ae. Aegypti* and *Anopheline* mosquitoes in Nertiti). However, we found no evidence of the presence of *Ae. Aegypti* at the households that reported the cases.

Further discussion

Sudan is known to have repeated outbreaks of YF. During 2005, a major outbreak occurred in the South Kordofan state [10]. According to the natural history of the disease, the excessive movement between parts of the country may play a role in its re-emergence and overall dynamics [17]. It has been observed that immediately after the implementation of peace agreements between conflicting parts of the country, these YF outbreaks took place. This may have facilitated excessive movement. Some of the community members believe the recent YF outbreak in Darfur region is somehow related to the deployed African union soldiers (United Nations Advance Mission In Darfur (UNAMID)), as the majority of the soldiers belong to the West Africa and African YF belt [18]. This phenomenon is also supported by the absence of evidence of the circulation of the 2005 virus. Furthermore, many parts of the Sudan are currently active fields for oil production and gold mining. The latter is particularly suspect because so many people are involved in the mining activities and come from many parts of the country, in addition to other neighboring countries [19—21]. However, the Sudan government, through the directorate of epidemiology and zoonotic disease in collaboration with WHO, is planning to conduct a national YF risk assessment study to prioritize the high-risk states. These results are intended to guide and advocate for a protective YF mass vaccination campaign during the next year and then to include YF vaccination in the routine national EPI schedule. Sudan currently adheres to the IHR 2005 by administering YF vaccinations to all travelers abroad and issuing those travelers standard immunization certificates [22].

Conclusion

VHF outbreaks can occur at any time and can rapidly spread everywhere. Consequently, routine national surveillance systems should be strong enough to detect these outbreaks in a timely manner. Local health facilities should be prepared to manage and promptly treat the initial reported cases because the CFRs are usually very high among the index cases.

Conflicts of interest

All authors declare that no competing interests exist that may have played a role in the results of the outbreak investigation mission.

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