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A Newly Emergent Viral Hemorrhagic Fever in Saudi Arabia: Descriptive Epidemiology of Alkhurma Hemorrhagic Fever, 2011-2014

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Rollins School of Public Health of Emory University
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Abstract

Background: The first case of Alkhurma hemorrhagic fever virus (AHFV) was identified in the Kingdom of Saudi Arabia (KSA) in mid-1990s. The first case of AHFV outside Saudi Arabia was detected in south Egypt in 2010. The epidemiology of this virus has yet to be completely determined. We conducted a descriptive epidemiologic assessment of time trends, geographic distribution, and person-related characteristics of cases reported between 2011 and 2014 in the Saudi Arabia.

Methods: A descriptive secondary analysis was conducted of a de-identified dataset collected as a part of the KSA Ministry of Health's (MoH) ongoing AHFV surveillance program from 2011 to 2014. This dataset contained geographic and demographic information (age, gender, nationality and occupation) of laboratory-confirmed cases of AHFV.

Results: A total of 281 laboratory-confirmed cases of AHFV were reported over a period of four years from 2011 – 2014. The number of reported cases decreased from 93 in 2011 to 58 in 2012 and 59 in 2013. The number increased again in 2014 to 71 cases. The monthly distribution of reported cases during the study period revealed two peaks in August and November. Overall, Najran had 75% of all reported cases and it maintained the highest proportion of cases throughout the study period. The ages of cases ranged from one-year-old to 108 years old, with an average age of 37.6 years (standard deviation ± 17.6). The majority of cases were men (63.4%) and Saudi nationals (63.7%). One-third of the cases were housewives and housemaids, 20% held office jobs, and 10.7% worked in jobs that involved direct contact with animals.

Conclusion: Our findings extend existing data on AHFV by providing recent time trends in disease occurrence over a 4-year period. Our results identify Najran as a high-risk area, and housewives and housemaids as a high-risk group. Our study design does not allow for inference on etiology; we had only occupation as an indicator of exposure. Detailed exposure history and analytical studies are needed to characterize modes of transmission and risk factors. Ongoing monitoring of AHFV trends is crucial.

Keywords: Alkhurma Hemorrhagic Fever Virus (AHFV), emerging infections, Saudi Arabia

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Table of Contents

List of Abbreviations

SARS: Severe Acute Respiratory Syndrome

VHFs: Viral hemorrhagic fevers

CCHF: Crimean-Congo Hemorrhagic Fever

RVF: Rift Valley Fever

AHFV: Alkhurma Hemorrhagic Fever Virus

KFDV: Kyasanur Forest Disease Virus

CDC: Centers for Disease Control and Prevention

MoH: Ministry of Health

PCR: Polymerase chain reaction

RT-PCR: Reverse transcription polymerase chain reaction

ELISA: Enzyme-like immunosorbent assay

IFA: Immunofluorescent assay

Chapter 1: Introduction

In recent years, the world has witnessed many changes to ecological systems resulting from natural disasters like floods and from man-made activities such as reforestation and deforestation. Changes in ecological systems can disturb habitats and alter the characteristics of pathogens, provoke microbial adaptation, and alter interactions between different components of the ecosystem, including animals, arthropods, microorganisms and humans (10). In addition, the spread of globalization in recent decades has facilitated the cross-border movement of organisms, animals and humans. These factors have resulted in the emergence of new infectious diseases and the re-emergence of old ones, contributing to increased diversity of pathogens that can impact human health (10,23).

Recent emerging infectious diseases include viral diseases of zoonotic nature, for example, severe acute respiratory disease (SARS) and viral hemorrhagic diseases such as West Nile virus and Ebola virus (10).

Overview of Viral Hemorrhagic Fever

Viral hemorrhagic fevers (VHFs) are caused by enveloped, single-strand RNA viruses that belong to four different virus families: *Arenaviridae* (Lassa virus, Junin virus, and Machupo virus), *Bunyaviridae* (Rift Valley fever virus, Crimean-Congo hemorrhagic fever virus and Hantavirus), *Filoviridae* (Ebola virus and Marburg virus) and *Flaviviridae* (Yellow Fever virus, Dengue virus and Alkhurma virus) (3,8,17).

Most viruses associated with VHFs are zoonotic in that they reside, replicate, and survive in an animal reservoir or arthropod vector. However, the natural reservoirs of some viruses causing VHFs (such as Ebola and Marburg) remain unknown. Ticks might serve as a vector for some VHFs (like tick-borne encephalitis) and mosquitos for others (like dengue fever). Humans are not a natural reservoir of hemorrhagic fever viruses but in the case of certain viruses such as Ebola, Marburg, Lassa and Crimean-Congo hemorrhagic fever, humans who are infected may transmit their infection to others (4). It would seem that the presence and survival of hemorrhagic fever viruses would be restricted to those geographical areas where the reservoir species lives; however, because of current globalization trends, it is possible for the virus to spread beyond the natural habitat of its reservoir. The exportation of animals from their native habitats and the movement of people around the world have increased the permutation of susceptible conditions of viral hemorrhagic fever occurrence worldwide (4,23).

The first outbreaks of Marburg hemorrhagic fever occurred in Marburg and Frankfurt, Germany, and in Yugoslavia after a laboratory worker handled African green monkeys (*Cercopithecus aethiops*) imported from Uganda that were infected with the virus, an example of the risk of transmission outside of the original reservoir, which was African areas where Rousettus bat colonies lived. In another instance, the Ebola virus was transmitted to a nurse in South Africa in 1996 by a medical professional from Gabon, an example of the possibility of transmission of certain viral hemorrhagic fever illnesses via person-to-person contact in places outside the virus's original location (4).

VHFs are multisystem diseases that affect different organs. The severity of illness may vary by type of VHFs, however, most share common initial symptoms and signs including fever, malaise, fatigue, and muscle aches. Some cases show hemorrhagic manifestations such as bleeding under the skin, internal bleeding, and bleeding from bodily orifices (due to increased vascular permeability, which leads to a drop in plasma volume and the development of a coagulation defect) (3- 4). In very severe cases patients may develop serious or fatal complications including coma, seizure, delirium, shock, or renal failure (4).

VHFs like any other viral illness usually has no specific curative treatment. Supportive therapy is the main method of management; antiviral medications have been effective in treating some viral hemorrhagic illnesses such as Lassa fever and hemorrhagic fever with renal symptoms (4). Since no proven vaccines have been developed for most viral hemorrhagic fever illnesses (with the exception of yellow fever and Argentine hemorrhagic fever), prevention efforts must be concentrated on avoiding contact with reservoirs, vectors, and hosts, which requires a full understanding of viral epidemiology, transmission, and pathogenesis (4,9).

Viral Hemorrhagic Fever in Saudi Arabia: Emergence of Alkhurma Hemorrhagic Fever Virus

Saudi Arabia is at high risk for the emergence or reemergence of infectious diseases, particularly of zoonotic origin, that may be transmissible to humans. This is due to its being the site for major mass gatherings of Muslims worldwide. Millions of Muslims travel to the holy city of Makkah and Madinah in the Western Province of Saudi Arabia every year to perform the religious pilgrimages of Hajj and Umrah. As part of the religious rituals, thousands of livestock

animals are imported from neighborhood countries and slaughtered during the Hajj season. In addition, people in Saudi Arabia commonly raise livestock, including sheep and camels, particularly residents of the desert and rural regions (8).

Four types of viral hemorrhagic fever have been identified in Saudi Arabia: Crimean-Congo Hemorrhagic Fever (CCHF), Rift Valley Fever (RVF), dengue fever, and Alkhurma Hemorrhagic Fever Virus (AHFV), which indicates that Saudi Arabia is among the countries at high risk for the emergence of infectious diseases (10,17).

In 1990, there was an outbreak of CCHF in Makkah; since then, the illness has not reappeared in Saudi Arabia. In 1994, dengue fever flared up in Jeddah, and following that, only a few sporadic cases have been reported from Jeddah. From 2000 – 2001, a major RVF epidemic occurred in three areas: Jizan, Asir and Alqunfuda, all located in southwest Saudi Arabia, far away from Makkah city (8,10). Three of the four viral hemorrhagic fever illnesses occurring in Saudi Arabia, namely AHFV, CCHF, and dengue, were confined to the cities of Makkah and Jeddah, both in the Western Province, 80 km apart. The appearance of AHFV and CCHF in these two urban cities is likely associated with the importation of substantial quantities of domesticated animals into Makkah through the Jeddah seaport for the Hajj season (8,10,17).

Alkhurma (also referred to as Alkhumra) is a newly emerging virus first detected in Saudi Arabia in the city of Jeddah in the mid-1990s. The first series of cases involved six patients who were butchers or had contact with sheep before they presented with the illness. Currently, the names Alkhurma and Alkhumra can both be found in published articles, and the Saudi Ministry

of Health uses both names interchangeably. For the purposes of this thesis, the term Alkhurma will be used.

Until 2003, Alkhurma virus was confined to the cities Makkah and Jeddah, in western Saudi Arabia. That year, two laboratory-confirmed cases occurred in Najran, a southern region bordering Yemen. In 2010 and 2011, cases were also reported in Jizan and Taif respectively (22). AHF cases were limited to Saudi Arabia until 2010, when two Italians contracted it after visiting southern Egypt and touring a camel market on the border with Sudan (5,7,12).

Based on published articles, direct contact with animals or animal products is defined as the main mode of transmission. However, vector-borne transmission is also possible. Alkhurma virus belongs to the tick borne *flavivirus* group and possibility of tick and mosquito bites have been reported in the histories of some identified cases (2,17-18,21-22).

Knowledge Gaps in Alkhurma Hemorrhagic Fever Virus

Because Alkhurma virus is a recently emergent virus, its epidemiology and etiological factors remain unsubstantiated. The implementation of prevention and control measures and public awareness campaigns is a formidable challenge when the disease is a relatively new infection, with no clearly established pattern of occurrence, geographical distribution, modes of transmission, and related risk factors. Further investigations are needed to elucidate the epidemiology of Alkhurma virus and characterize its occurrence and distribution by time, place and person, and to address its risk factors and the modes of transmission (1). The Saudi Ministry

of Health should have additional epidemiological information about Alkhurma virus in order to be able to enhance detection and prevention efforts (23).

In this thesis, we will address the descriptive epidemiology of AHFV in terms of disease occurrence and distribution by time, place, and person. We will examine reported cases of AHFV in Saudi Arabia over a period of four years, 2011 – 2014. This will provide a better understanding of the epidemiology of AHFV and help address intervention needs of the Saudi Ministry of Health and enhance expertise to prevent further occurrences of AHFV illness.

Research Questions

1) What are the annual and seasonal time trends in AHFV occurrence in Saudi Arabia during the period of 2011 – 2014?
2) What is the geographic distribution of AHFV in Saudi Arabia during the period of 2011 – 2014?
3) What are the descriptive characteristics of AHFV cases?

Chapter 2: Literature Review

Emergence of Alkhurma Hemorrhagic Fever Virus in Saudi Arabia, 1995 – 2011

Initial Occurrence

The AHFV was discovered in Saudi Arabia in the mid-1990s, since which cases have appeared intermittently. The virus is a genetic and serologic variant of Kyasanur Forest Disease Virus (KFDV) (1-2,5,7-8,10,12-15,21-23).

The AHFV was first isolated in Jeddah in 1995, when a 32-year-old Egyptian butcher presented with acute hemorrhagic fever. The patient lost consciousness and died 2 days after admission from shock and renal and respiratory failure (7,9-10,14,20-21). Initially, the case was confirmed as a *flavivirus* on the basis of an immunofluorescence assay performed with a broadly reactive *flavivirus* monoclonal antibody, 4G2, which can cross-react with other viruses such as dengue, yellow fever, and West Nile. A blood sample was sent to the U.S. Centers for Disease Control and Prevention (CDC) that confirmed that the organism was a *flavivirus* related to KFDV, a member of the mammalian tick-borne virus subgroup genus *flavivirus*, family *flaviviridae,* that had caused hemorrhagic fever in Karnatake state in India (10, 12,14,20-21). In the same year, the same *flavivirus* was detected in five additional cases that presented with dengue-like hemorrhagic fever (8-9,14). The newly detected *flavivirus* was named Alkhurma virus, referring to a small city from which the sheep associated with six original cases originated (1,8, 21,23).

Subsequent Occurrences

After AHFV was first identified in Jeddah, it was not reported again until 2001, when it appeared in Makkah from February 8 – February 23, the Hajj period of that year (8). Since RVF had appeared in Saudi Arabia in September 2000, the Saudi Ministry of Health (MoH) had created and executed strict plans to prevent the occurrence of this disease during the Hajj period. Therefore, during the Hajj period in Makkah in 2001, as part of the active surveillance of VHFs, any patient hospitalized with acute febrile illness was reviewed and clinically assessed. During that time, four patients presented with acute VHFs and were admitted with the inaccurate diagnoses of acute hepatitis or encephalitis. Laboratory tests for dengue, CCHF and RVF were obtained that showed negative results for all three viruses. Blood specimens were then sent to the CDC in Atlanta to test for other types of hemorrhagic fever viruses, and the same *flavivirus* that had been isolated from the 1995 samples – Alkhurma virus – was detected (8). The doctor responsible for overseeing these cases, Tariq Madani, changed the name of the new virus from Alkurma to Alkumra; he stated that Alkurma, the prior name, was not accurate and could have resulted from a typo, as the original six cases were from the Alkhumra district in the south of Jeddah, which is different from Alkurma, a small city 200km east of Makkah (8,17). After this point, a total of 37 cases were identified in Makkah, 20 of which were confirmed by laboratory test over a period of two years, from 2001 through 2003 (1, 8,10,17). Among those 20 confirmed cases, 11 developed the hemorrhagic manifestation and 5 died (1).

Before 2003, AHFV cases were mainly confined to Jeddah and Makkah. However, since then, AHFV cases have been reported in Najran in southern Saudi Arabia, on the border with

Yemen (10). Najran is an agricultural area where people usually raise livestock in the backyards of their homes (10).

Over the period from August 1, 2003 to December 31, 2009, a total of 148 suspected cases that met the clinical case definition of AHFV were reported in Najran; all were found to be negative for RVF and dengue IgM, IgG and Polymerase Chain Reaction (PCR). Seventy-eight out of 148 (52.7%) were laboratory confirmed throughout the 23 districts of Najran; two cases in 2003, one case in 2004, four cases in 2005, one in 2007, twelve in 2008, and 58 cases in 2009 (10,17). Among the 78 confirmed cases, 69 (88.5%) were hospitalized and diagnosed with acute febrile illness and 9 (11.5%) patients were identified by contact tracing.

In a study conducted in Najran from January to April of 2009, active disease surveillance and serologic testing of household contacts identified 28 persons with AHFV. Among these 28 cases, 11 cases (39%) sought medical advice and were hospitalized because their clinical presentation met the case definition of AHFV used for the surveillance system; 17 cases had a subclinical infection and were identified as household contacts (1). Blood samples from those who sought medical advice were tested by using both enzyme-like immunosorbent assay (ELISA) for Alkhurma virus specific immunoglobulin IgM and IgG by using Alkhurma virus antigen, as well as reverse transcription polymerase chain reaction (RT-PCR) for testing the specific viral sequence. ELISA for Alkhurma virus specific IgG was used for samples from follow up testing of household contacts (1).

The were a total of 233 confirmed AHFV cases over a period of three years, from January 1, 2009 through December 31, 2011: 59 cases in 2009, 81 cases in 2010, and 93 cases in 2011. Najran reported the largest number of cases (82%), followed by Jeddah (12%) and Makkah (5.6%). One case was reported in Jizan in 2010 and another in Taif in 2011, marking the first reports of disease in these two cities. Whether this was indeed a first-time occurrence of AHFV in Jizan and Taif, or whether cases in those regions have been undetected or under-reported is unknown (22).

Appearance Outside Saudi Arabia

Although AHFV has been detected only in Saudi Arabia since it was introduced in 1995, two cases of AHFV were detected in two Italian travellers who returned to Italy after visiting Egypt in 2010 (5,7,10,12,18). The first case was a 64-year-old man who had visited a village in southern Egypt, on the Sudanese border, for one week from April 25 - May 1, 2010. There he visited a camel market on April 29 and was bitten on the foot by an undefined arthropod. He returned home about 48 hours after the bite, and during his flight to Italy he experienced a high-grade fever, chills, shaking, anorexia, malaise, nausea, vomiting and blurred vision, which worsened over 5 days. He was admitted to the hospital and found to have AHFV after laboratory confirmation. A second case was also detected in a man who visited the same camel market in Egypt one month after the first case and had a history of undefined arthropod bites (5).

In November 2010, a third case of AHFV was identified in an Italian traveller who had visited the same camel market that the previous two infected travellers had visited. The patient presented with severe muscle weakness and mild confusion that resolved spontaneously after

three (7,12). The patient reported a history of being bitten by undefined arthropods during his tour of the camel market and did not report any direct contact with camels or ingestion of any raw dairy products (12).

A fourth case of AHFV was identified in a 58 year-old Italian female who travelled to south Egypt near the Read Sea and had contact with camels. The patient developed malaise and anesthesia on her return flight and a fever and rash later on. AHFV was confirmed by RT-PCR, followed by a positive Alkhurma virus IgM and IgG (7).

The existence of these four cases indicates the lack of identification of Alkhurma virus in countries other than Saudi Arabia (5,7,12-22).

Seasonal Patterns

Limited information exists on seasonal patterns in Saudi Arabia (14). In the 2006 – 2009 Najran study, the peak numbers of cases occurred at different times each year: December in 2006, January in 2007, May to July in 2008, and March to April in 2009. (1).

Cumulative cases that were reported in Najran over the period from 2003 – 2009 had a peak of occurrences on May and June, decreasing in July, and then increasing again on August (17). In the 2008 – 2009 Najran study, AHFV cases peaked in the summer period (May – August). This could be linked to the breeding season of the flying arthropods, which occurs during the summer months (17).

Another study conducted by Memish and et al. from 2009 –– 2011 shows that the peak numbers of AHFV cases occurred in July and December (22).

Modes of Transmission

Direct contact with animals and animal products has been suggested as the mode of transmission for the disease. The initial clustering of cases in Makkah, the Hajj site, and the neighboring city of Jeddah suggested that the religious ritual of animal sacrifice during pilgrimage is a possible explanation for disease occurrence. Subsequent identification of cases in the region of Najran, which is an agricultural area, suggested that contact with imported livestock from neighboring countries is a possible mode of transmission (17). Based on published and internal data from Saudi MoH, individuals at high risk of getting AHFV include mainly those who come into contact with animals, which include slaughterhouse workers, butchers, shepherds, and livestock sellers. In addition, housewives who prepare meat for cooking and individuals who consume unpasteurized milk might also be at high risk (1,12,17-18,23).

Camels, goats, and sheep have been linked to Alkhurma virus transmission. However, no study has yet detected Alkhurma virus antibodies or virus RNA in these animal species, and no studies on patients infected with AHFV who had a contact with animals reported any abnormalities in the animals (e.g., abortion, disease, death) that would suggest they had infections (1,17-18,23).

On the basis of limited data, it was reported that Alkhurma viral RNA was detected in soft ticks called *Ornithodoros Savignyi* that were collected from the resting places of camels in

Jeddah and Najran market (2,10,15,21-22). Alkhurma viral RNA was also detected in hard ticks called *Hyalomma dromedarii* (2,21-22). However, evidence of transmission of Alkhurma virus through tick bites has not been substantiated (10,15).

Human to human transmission has not been documented (10,23), however notification of a cluster of AHFV within families in Najran over the period of 2003 – 2009 might call attention the possibility of person to person transmission (23). In addition, the role of mosquitoes in Alkhurma transmission remains unsubstantiated (10,15). Due to the scarcity of studies available, neither mosquito nor human-to-human transmission can be completely ruled out (10).

For a descriptive cohort study conducted by Madani et al. on patients infected with Alkhurma virus identified in Najran from August 1, 2003, to December 31, 2009, the research team worked to identify possible sources of infection among detected cases. They clinically examined all animals kept in the backyards of houses in which cases were detected; they also included animals from five abattoirs and markets located in the Najran area. In addition, they collected mosquitoes and ticks from places around randomly-selected houses and animal pens of patients with confirmed AHFV. The examined animals consisted mainly of sheep, goats and camels (23). Of the patients with confirmed AHFV, 81.7% raised livestock in their backyards. These animals were a source of milk and meat for their owners, and some of them lived for more than five years in pens (17). Epidemiological data from this study suggests that direct contact with animals (camels, goats, and sheep) and mosquito bites were likely primary risk factors of AHFV infection among the study population. Of the total patients, 59% reported both histories of animal contact and mosquito bites, 21.8% reported mosquito bites only. The only reported risk

factor for 17.9% of patients was having had contact with animals. Approximately half of the patients reported histories of drinking raw milk, but this was also included history of contact with animals or mosquito bites (17).

In the above study, tick bites were not considered to be associated with the transmission of Alkhurma virus from animals to humans, as only three cases reported having tick bites, and two of those had been exposed to animals as well, and the other to mosquitoes. However, due to the similarity between Alkhurma virus and KFDV, the role of tick bites in the transmission of Alkhurma virus might still be worth investigating (17).

Risk Factors

The majority of reported cases occurred among individuals aged 20 – 50 years. Occurrence was very low among children aged 15 years and under (1,17,22).

In Madani et al.'s study (2010) on reported cases in Najran over the period from 2003 to 2009, the mean age of AHFV patients was 30 years old, and 59% of them were Saudi. As to reported occupation, 28.2% were students, 24.4% were housewives, 10.3% were self-employed, and 37.3 % were in other occupations (17).

In the same study, comparisons were made between the Najran and Makkah outbreaks and some differences were noted. The proportion of females was higher in Najran (38%) than Makkah (10%). Five cases were reported in children under 10 years in Najran, while there were no reported cases in children in Makkah. The proportion of family clusters was higher in Najran

(25%) than Makkah (5%). The mortality rate in Najran was 1.3%, much which is lower than that in Makkah (25%) (17).

These differences are thought to be due to lifestyle differences between the people of Makkah and Najran. Najran is a rural area where all family members live close together in one large house with a backyard in which livestock animals are raised; and all family members, including females and children, share the responsibility of feeding, milking, and cleaning livestock. The difference in mortality rate could be a result of Alkhurma's endemicity in Najran; people living there might have developed immunity due to repeated exposure to the virus.

In another descriptive study of an AHFV outbreak in Najran between 2006 and 2009 involving 28 cases, 50% of the cases were 20 - 39 years old, 46% were male, 10.7% were Saudi, and 89.3% were non Saudi, 14% had jobs involving direct contact with livestock, 18% were housewives, 14% were students, and 25% had other jobs (1).

In an analytic study involving reported cases over a period of three years from 2009 to 2011, over 45 % of cases were in the 25-44 years age group, and just less than 1.5% were children younger than 5 years. Approximately 40% of cases had jobs that involved direct contact with animals. Approximately 26% of cases were housewives and housemaids, which align with the findings of a previous study in Najran in which 38% of cases were women. As a part of their tasks at home, women may have exposures to animal products for food preparation and may have direct contact with animals raised at home. Employment-wise, those with office jobs comprised 22.7% of all cases, technicians comprised 6%, and those with no defined jobs

comprised 4.3% of all cases. The finding that a third of the cases in his study involved individuals whose jobs were not related directly to animal handling or animals products, suggests other modes of disease transmission apart from direct animal contact (22).

Clinical Manifestations and Outcomes

Clinical manifestations of AHFV range from asymptomatic or mild cases to severe, fatal cases (1,12,21). AHFV might be associated with a wide variety of signs and symptoms similar to those associated with other VHFs (1,7,22,24). In most of the previous studies, a diversity of reported clinical manifestations were reported, the most common of which include fever (in 90% - 100% of cases), headache, malaise, chills, arthralgia, muscle ache (in 47%- 85% of cases), retro-orbital pain (in 16%- 55% of cases), nausea and vomiting (in 50 %-70% of cases), diarrhea (in 31%-51% of cases), hemorrhagic manifestations (in 4%-25% of cases), and neurological manifestations (in 1%-23% of cases). A minority of cases developed complications such as encephalitis, fulminant hepatitis, and acute renal failure and required intensive care unit admission (1,22).

According to Memish and Alzahrani, the first set of confirmed cases of AHFV might not have characterized the full spectrum of AHFV-associated illness, and they suggested a pattern of severe to fatal AHFV outcomes. The case fatality rate of 25%-30% might reflect only the detection of severe cases of AHFV and can be biased because it does not include milder cases. Recent data have revealed that mild or even asymptomatic and subclinical cases of AHFV do occur (1,12,21). Out of the 78 confirmed AHFV cases reported in Najran from 2003 – 2009, one patient developed acute renal failure and one showed a slight elevation in creatinine level (17). In

a study conducted by Alzahrani in Najran, out of 28 identified AHFV cases with positive lab tests, 17 were subclinical, 4 of whom developed symptoms and signs during the study period and 13 of whom had no illness manifestation (1). In addition, among 81 cases that were identified nationwide in 2010, only two fatalities were documented; no deaths were reported in 2009 or 2011 (21-22).

Disease Surveillance System in Saudi Arabia

Saudi Arabia: Geography and Regions

The Kingdom of Saudi Arabia occupies 80% of the Arabian Peninsula. The total size of Saudi Arabia is approximately 2,149,690 square kilometers (19). Seven countries and three bodies of water form boundaries with Saudi Arabia. From the west Saudi Arabia is bounded by the Gulf of Aqaba and the Read Sea extends south to Yemen that bounds Southern side of Saudi Arabia. Jordan, Iraq and Kuwait bound Saudi Arabia from the North. To the East, Saudi Arabia is bounded by the Persian Gulf from Ras al Khafji to the peninsula of Qatar, and to Southeast Saudi Arabia borders with Oman and Abu Dhabi emirate in the United Arab Emirates (11).

Geographically Saudi Arabia is divided into five regions. The central region is the heartland of Saudi Arabia in which Riyadh; the capital of Saudi Arabia is located. The Western region, in which the two holiest cities of Islam Makkah and Madinah are located that are visited by millions of Muslims annually for pilgrimage, also the western region contains a busy seaport of Jeddah that is known as Islamic Port of Jeddah, through which thousands to million people pass yearly, additionally many livestock that are imported from neighborhood countries pass Jeddah seaport. The Southern Region (Asir) is mountains area and it is an agricultural area. The eastern region is the richest oil resource in Saudi Arabia in which headquarter of Saudi oil industry is

located in Dhahran city (6). The fifth region constitutes the desert of Rub al-Khali. For administrative purpose, the kingdom of Saudi Arabia is divided into thirteen regions: Riyadh, Makkah, Maddinah, Qasim, Eastren region, Asir, Tabouk, Hail, Najran , Jizan, Albaha, Al-Jouf and Northern boarder region. Each region has a regional governor with the rank of Minister who reports to the Minister of the Interior; (6).

AHFV Surveillance System Structure

AHFV is an immediately notifiable illness in Saudi Arabia and the Saudi MoH has developed an effective surveillance program for it. The surveillance program for AHFV requires the immediate reporting of every single suspected case (as indicated by the case definition criteria) using three standardized forms: the suspected case/clinical history form, an epidemiological investigation form, and a laboratory requisition form (22).

The AHFV surveillance system flow starts from the health facility (governmental or private) and goes to the surveillance officer in charge of viral hemorrhagic fevers at the directorate of health affairs in each region. The surveillance officer checks for the completeness and precision of AHFV reports and then sends a report to the National Directorate of Infectious Diseases in the Preventive Medicine Division at the MoH either by fax, phone, or email. The AHFV surveillance program officer at the MoH then takes responsibility for providing weekly, monthly and quarterly reports for the higher directors at the MoH and sends those as feedback to the directorate of health affairs and health units in each region (22).

Case Definition

A case definition for AHFV was formulated for the Saudi Arabian surveillance program after the identification of four cases in Makkah in 2001. According to Madani (8), a suspected case is any person presenting with acute febrile illness of at least two days duration with negative laboratory tests for RVF, CCHF, and dengue, and also shows at least two of the following: (1) a minimum threefold increase of alanine transferase (ALT) or aspartate transferase (AST) or the presence of clinical jaundice; (2) the development of encephalitis features such as confusion, disorientation, drowsiness, coma, neck stiffness, hemiparesis, paraparesis, or convulsions; (3) hemorrhagic manifestations such as skin manifestations (ecchymosis, purpura, petechiae), bleeding from puncture sites, bleeding from body orifices such as epistaxis and gastrointestinal bleeding (hematemesis, melena, hematochesia), or menorrhagia; (4) a drop in platelet count to less than $100*10^9$/L, or elevation of lactate dehydrogenase (LDH) or more than twice the normal level of creatine phosphokinase (CK) enzyme (8). The same case definition was used in a case control study conducted by Alzahrani in Najran from 2006 – 2009 (1) as well as in Madani's descriptive cohort study of reported cases in Najran from 2003 – 2009 (17).

The case definition that is used currently in Saudi Arabia is the one that was used in a study conducted for reported AHFV cases from 2009 – 2011 by Memish and et al. A suspected case is defined as any case with acute onset of fever and flu-like illness with the presence or absence of hemorrhagic manifestations, hepatomegaly, or elevated liver enzymes and encephalopathy. A probable case is a suspected case with history of contact with animals or animal products and a laboratory confirmation of leukopenia or thrombocytopenia, and/or elevated liver enzymes. A confirmed case is a suspected or probable case with confirmation of PCR of Alkhurma virus (22).

A modification in the case definition was recommended by specialists in the field of arboviral illnesses who were consulted by the Saudi MoH on January 31, 2010, in Riyadh (16). This modification has not adopted by MoH yet. The recommended revised definition is as follows: A suspected case is any case with acute febrile illness and fever of more than 38 °C with one of the following clinical features: hemorrhagic manifestations that are not related to injury, such as bleeding under the skin, from internal organs, or from body orifices; liver involvement such as hepatomegaly and jaundice and neurological involvement such as headache; altered mental status and/or seizure, with one or more of following exposure criteria: recent direct contact with animals or animal products, recent exposure to tick or history of tick bites, history of contact with blood or body fluids of confirmed infected person with AHFV, and working in a laboratory handling AHFV specimens.A probable case is a suspected case with laboratory findings incorporating thrombocytopenia, leucopenia, elevation of liver enzymes, elevated CPK or LDH, and IgM detected by ELISA . A confirmed case is any probable case with one or more of the following laboratory findings: identification of AHFV RNA by real-time or conventional Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), virus isolation using cell culture or suckling mice, fourfold rise of IgG in paired serum samples using ELISA or immune-fluorescent asaay (IFA), neutralization test—preferably plaque reduction for paired sera (16).

Summary

Limited information exists on the seasonal pattern of AHFV in Saudi Arabia, and no existing study discussed whether there was a relation between peak season and hajj period. Since 2003, cases have been reported in cities beyond Makkah and Jeddah (where the initial cases of AHFV occurred). In 2010, four cases were identified outside Saudi Arabia. Risk factors of

AHFV are not completely understood yet. Person-related characteristics including age, gender, nationality and occupation showed some variation between studies.

Despite the existing literature, the epidemiology and etiology of AHFV remain poorly understood. Much remains to be learned about the pattern of occurrence, geographic distribution, modes of transmission, and related risk factors of AHFV. Further investigations are needed to elucidate the knowledge gap in the epidemiology of Alkhurma virus and address its risk factors and modes of transmission.

Chapter 3: Manuscript

Abstract

Background: The first case of Alkhurma hemorrhagic fever virus (AHFV) was identified in the Kingdom of Saudi Arabia (KSA) in mid-1990s. The first case of AHFV outside Saudi Arabia was detected in south Egypt in 2010. The epidemiology of this virus has yet to be completely determined. We conducted a descriptive epidemiologic assessment of time trends, geographic distribution, and person-related characteristics of cases reported between 2011 and 2014 in the Saudi Arabia.

Methods: A descriptive secondary analysis was conducted of a de-identified dataset collected as a part of the KSA Ministry of Health's (MoH) ongoing AHFV surveillance program from 2011 to 2014. This dataset contained geographic and demographic information (age, gender, nationality and occupation) of laboratory-confirmed cases of AHFV.

Results: A total of 281 laboratory-confirmed cases of AHFV were reported over a period of four years from 2011 – 2014. The number of reported cases decreased from 93 in 2011 to 58 in 2012 and 59 in 2013. The number increased again in 2014 to 71 cases. The monthly distribution of reported cases during the study period revealed two peaks in August and November. Overall, Najran had 75% of all reported cases and it maintained the highest proportion of cases throughout the study period. The ages of cases ranged from one-year-old to 108 years old, with an average age of 37.6 years (standard deviation ± 17.6). The majority of cases were men (63.4%) and Saudi nationals (63.7%). One-third of the cases were housewives and housemaids, 20% held office jobs, and 10.7% worked in jobs that involved direct contact with animals.

Conclusion: Our findings extend existing data on AHFV by providing recent time trends in disease occurrence over a 4-year period. Our results identify Najran as a high-risk area, and housewives and housemaids as a high-risk group. Our study design does not allow for inference on etiology; we had only occupation as an indicator of exposure. Detailed exposure history and analytical studies are needed to characterize modes of transmission and risk factors. Ongoing monitoring of AHFV trends is crucial.

Keywords: Alkhurma Hemorrhagic Fever Virus (AHFV), emerging infections, Saudi Arabia

Introduction

Alkhurma (also referred to as Alkhumra) hemorrhagic fever virus (AHFV) is a newly emerging viral hemorrhagic fever. The first case was detected in 1995 in Jeddah, Kingdom of Saudi Arabia (KSA), when a butcher presented with a hemorrhagic fever that turned out to be fatal. In the same year, five additional patients who were butchers or had contact with sheep were identified (9,20).

AHFV is a genetic and serologic variant of Kyasanur Forest Disease Virus (KFDV), a member of the mammalian tick-borne *flavivirus* subgroup, family *flaviviridae* (8,22-23). Before 2003, reports of intermittent cases of AHFV were confined to Jeddah and Makkah in western Saudi Arabia. In 2003, cases were reported in Najran (southern KSA); in 2010, one case was reported in Jizan (southern KSA); and in 2011, another case was identified in Taif (western KSA) (22). (Figure 1)

Earlier studies linked the transmission of AHFV to having direct contact with animals or animal products. However, the detection of Alkurma virus in a soft tick (*Ornithodoros Savignyi*) and a hard tick (*Hyalomma dromedarii)* has drawn attention to the possible role of these vectors in AHFV transmission (16,22).

Although Egypt is the only area beyond the geographical boundaries of Saudi Arabia in which AHFV has been detected, as seen in the identification of the virus in four Italian travellers returning from Egypt, the risk of virus spread to other areas is possible (5,7,10,12,18,21).

Much remains to be learned about AHFV. Therefore, ongoing monitoring of confirmed cases will further elucidate the epidemiology of Alkhurma virus in terms of incidence; distribution by time, place, and person; etiology; and risk of transmission in Saudi Arabia and neighboring countries (1). It is vital to understand disease epidemiology and accordingly establish preventive strategies and preparedness plans. (22). The objective of this study is to describe the epidemiology of AHFV in terms of time trends, geographic distribution, and person-related characteristics (age, gender, nationality, and occupation) by analyzing all reported cases of confirmed AHFV in Saudi Arabia from 2011 – 2014.

Methods

Surveillance System of Alkhurma Hemorrhagic Fever Virus in Saudi Arabia

This study used data collected as a part of the ongoing AHFV surveillance program in KSA. Every identified suspected case (as indicated by the case definition criteria) is required to be reported immediately, using three standardized forms: the suspected case/clinical history form, an epidemiological investigation form, and a laboratory requisition form. The stream of AHFV surveillance system is started from each health facilities (governmental or private) to the in-charge desk officer for viral hemorrhagic fever at the directorate of health affairs in each region. The desk officer checks for the completeness and precision of AHFV reports and then sends a report to the national directorate of infectious diseases in the preventive medicine division at the Ministry of Health (MoH) either by fax, phone or email. Then, the desk officer for AHFV surveillance program at the MoH takes the responsibility to provide weekly, monthly and quarterly reports for the higher directors at MoH and send them as a feedback to the directorate of health affairs and health units in each region (22).

Data Sources

The dataset used in this study was generated as part of the AHFV notifiable surveillance system of the Saudi MoH.

The dataset was de-identified and included epidemiologic and laboratory data on confirmed cases of AHFV during the period of 2011-2014. Specifically, the dataset consisted of demographic information (age, gender, nationality, region and occupation) of 281 cases of AHFV that met the clinical definition used for surveillance program and were confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in the central laboratory of the MoH in Riyadh from 2011 – 2014 (22).

Case Definition

A suspected case is defined as any case with acute onset of fever and flu-like illness with presence or absence of hemorrhagic manifestations, hepatomegaly or elevated liver enzymes and encephalopathy. A probable case is a suspected case with history of contact with animals or animal products with laboratory-confirmed leukopenia or thrombocytopenia, and/or elevated liver enzymes. A confirmed case is a suspected or probable case with confirmation of Polymerase Chain Reaction (PCR) of Alkhurma virus (22).

Study Variables

The initial dataset contained the following variables: age (in years) and time of diagnosis (in years, international weeks and months), which were entered in English; and gender, nationality, occupation and region, which were listed in Arabic. All data was translated to

English by the researcher.

The total number of deaths each year and the total suspected cases were missing in the dataset and were obtained verbally from the person in charge of the AHFV surveillance program at the MoH in Riyadh.

To allow for direct comparison with existing studies by Memish et.al (2014), the continuous variable age was reclassified into six categories, as follows: 0-14, 15-24, 25-34, 35-44, 45- 54 and 55 or older (22).

Nationality was recoded into a dichotomous variable (Saudi vs. non-Saudi).

Occupation was categorized and coded into a numeric variable using a classification that informs exposure history to animals and animal products. The occupation variables were re-classified into the first four occupational categories that were used in Memish et al. (22). The first category included patients who had jobs entailing direct contact with animals (such as butcher, shepherd, livestock laborer, livestock trader, farmer, veterinarian, chef and kitchen worker). The second category consisted of housewives and housemaids, as they might handle uncooked meat from infected animals. The third category consisted of technicians and included blue collar jobs such as construction worker, soldier, driver, restaurant worker, tanning factory employee, sales person, guard and janitor. The fourth category consisted of office jobs such as administrative, employee, NGO-employee, municipality employee, nurse, teacher and student. The fifth category in the Memish et al. study was "no defined job," and it was unclear what jobs were included in this category. Therefore, we defined this category for our study to include all patients the nature of whose jobs was unknown (such as children, freelancers, and unknown). The sixth category consisted of retired and unemployed patients.

Statistical Analysis

Data cleaning and English translation were conducted in Microsoft Excel 2010 (Microsoft, Seattle, WA), Descriptive and analytic statistics were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

A descriptive exploratory analysis of data was performed and summary statistics were derived for all independent variables. Continuous variables were summarized with descriptive statistics (N, mean, standard deviation). Categorical variables were summarized with frequency counts and percentages within each category of a variable.

Bivariate analyses were conducted using the following tests: Chi Square test for associations between each independent variables (age, gender, nationality, occupation and region) with the dependent variable (year of diagnosis), and associations between all the independent variables with each other. A Chi-Square test for linear trend examined changes in reported cases over four years; and analysis of variance (ANOVA) examined differences in age within categorical independent variables.

Ethics

We used a secondary de-identified dataset from the AHFV surveillance program managed by the Saudi MoH. Data was de-identified before it was made available for analysis and did not include any subject identifiers that might link the information to particular patients. Therefore, this research did not meet the definition of human subject research and it was determined to be exempt from review by the Institutional Review Board of Emory University.

Results

Descriptive Analysis

A summary of the demographic characteristics and geographic distribution of 281 confirmed AHFV cases during the period from 2011 – 2014 are analyzed by year (Table 1) and by nationality (Table 2).

Annual and monthly trend of AHFV cases

The trend for cases was non-linear; the highest frequency of cases occurred in 2011 (n= 93), decreasing to 58 (20.64%) in 2012 and 59 (21%) in 2013, then increasing to 71 cases (25.27%) in 2014.

The monthly distribution of reported cases during the study period revealed two peaks, one in August (12.1% of all cases) and the other in November (12.1% of all cases), with some increases shown in March, June, and July. The lowest numbers of cases occurred in January and May (Figure 2). Examining the pattern of cases by international weeks (IW) revealed that the highest proportion of cases occurred in the fourth quarter (40-52 weeks).

Region

Breaking down the frequency of AHFV cases by region, three-fourths of reported cases over the study period occurred in Najran, and almost 14% of cases were reported in Jeddah. Makkah accounted for 8% of all reported cases, 2.5% of cases were reported in Taif, and no cases were reported in Jizan.

Age

The mean age of patients across the entire study period was 37.6 years (SD=17.6 years). The age distribution revealed that 129 cases (45.9%) were 25-44 years old, 81 cases (28.8%) were 45 years and older, and 22 cases (7.8%) were under 15 years of age (six of which were under 5 years). The youngest reported AHFV patient was one year old and the oldest was 108 years old.

Nationality and Gender

The proportion of reported AHFV cases was higher among Saudi nationals (63.7%) than non-Saudis. Cases were predominantly male (63.3%).

Occupation

The largest occupational group represented among those with AHFV over the study period was housewives and housemaids, which represented around one-third of the total cases. The second largest occupational group was those with office jobs (about 20% of cases), followed by technicians (16.4%) and unemployed and retired (13.5%). Those with jobs involving the direct handling of animals constituted 10.7% of all AHFV cases. The smallest occupational group represented among AHFV cases was “no defined job” (6.4%).

Bivariate Analysis

Examining the association between each independent variable (mentioned above) and the dependent variable (year of diagnosis) showed statistically significant associations for region, age, and occupation group with year of diagnosis (p-value <0.05).

Najran continued to have the highest proportion of AHFV cases by year, while Taif continued to have lowest proportion (except in 2013, when Taif reported one case while Jeddah reported no cases). Jeddah was the second highest risk region for three years (2011, 2012, and 2014).

The mean age of cases decreased each year of the study period: 41.7 years (±17.3) in 2011, 36.7 years (±17.5) in 2012, 35.4 (±17.1) in 2013, and 34.6 years (±17.9) in 2014 (p-value= 0.04).

Examining the association between all independent variables with each other showed statistically significant associations between occupation group and age, gender and nationality (p-value <0.05).

The housewives and housemaids group was entirely female. The average age of patients among this group was 43.4 years (± 15.7), 71.9% were Saudi nationals, and 28% were non-Saudis. The office jobs group was younger on average than other occupational groups, with an average age of 24.7 years (±11.5). In this group, 80.4% were Saudi nationals and 19.6% were non-Saudis. The technicians group was all male and their average age was 35.7 years (± 10.5). The unemployed and retired patients were older, with an average age of 51.3 years (±20.7). Those with jobs involving the direct handling of animals were all male with an average age of 37.1 years (±13.7); 30% of them were Saudi and 70% were non-Saudi. The average age of patients in the smallest occupation group, "no defined job," was 24.8 years (± 22.9) (Table 2, Table 3). Overall, the mean ages by occupation were different, and this was statistically

significant. The differences in age were mainly driven by the younger age of Office Jobs and no defined Job groups, as well as the older age of the Unemployed/Retired group.

Case Fatality Rate

Our dataset lacked individual-level outcome data on the confirmed cases of AHFV; we only had total number of deaths in each year. Two deaths were reported in 2012, three deaths in 2013, and no deaths in 2011 and 2014. Therefore, the case fatality rate based on the available information was 1.8%.

Discussion

The occurrence of AHFV in Saudi Arabia during 2011 to 2014 showed a fluctuating trend. High-risk region for occurrence was Najran. High-risk groups were housewives and housemaids, and office jobs.

Reported cases of AHFV in Saudi Arabia fluctuated from 2011 to 2014, in contrast to the steadily increasing trends from 2009 to 2011 as reported in a previous study (22).

Information about seasonal patterns of AHFV was limited in the literature. However, some studies revealed that peak numbers of cases occurred in the summer season and attributed this to the parallel peak activity and breeding periods of mosquito and ticks, which are thought to have a role of transmission of Alkhurma virus (1,17,22). Based on our observation that the seasonal pattern of AHFV cases over a four-year period revealed peaks in August and November, we think the August peak might be attributable to the summer season as explained in

the literature and that the November peak might be related to Hajj weeks or the weeks following the Hajj period. The Hajj periods were November 4-9 (44-45 IW) in 2011, October 24-29 (43-44 IW) in 2012, October 13-18 (41-42 IW) in 2013, and October 1-6 (40-41 IW) in 2014. This could indicate the possibility of the presence of Alkhurma virus in livestock that was imported from neighboring countries during the Hajj period.

The geographical distribution of AHFV cases in our study corroborated the findings in the literature that AHFV cases have been reported in regions beyond Jeddah and Makkah where the initial cases of AHFV were detected (1,10,17,22). The high rate in Najran could be attributed to its being an agriculture area. The people in Najran often keep animals in their backyards where ticks and mosquitos could be present. In a study conducted on AHFV outbreak in Najran, ticks were collected around houses with confirmed AHFV patients (17). A second possibility is that those reporting the disease in Najran might be doing a more comprehensive and precise job of surveillance and notification compared with other regions in Saudi Arabia (22). Finally, people in Najran might be less aware of control measures around Alkurma virus, such as adopting protective measures in the direct handling of animals, avoiding exposure to ticks or mosquitos, and recognizing the importance of pasteurization (not consuming raw dairy products). They might be at higher risk of exposure to Alkhurma virus.

The report of seven confirmed cases in Taif in our study might be an indication that AHFV is under-reported in some regions in Saudi Arabia, particularly those that have not yet reported a case of AHFV. Subclinical cases of AHFV might exist without being detected and clinical cases might be missed or misdiagnosed. The first case reported in Taif in 2011, before it

was confirmed as AHFV in a health facility in Jeddah, it was thought to be dengue infection case. Therefore, cases of AHFV may be missed or misdiagnosed as other viral hemorrhagic fever or zoonotic diseases in some regions in Saudi Arabia (22).

The average age of all patients in the four-year study period (37.6 years) was slightly younger than the average age of patients reported from 2009 to 2011 (38.9 years). However, the proportion of cases among children younger than 6 years old was higher in our study (2.5%) than in the previous study (1.3%) (22).

Epidemiological data from our study suggests that only 10.7% of cases occurred among those who worked in direct contact with animals. This was discordant with the 2009 – 2011 study, which shows that 41.2% of reported AHFV cases had jobs that involved direct contact with animal (22).

Our finding that AHFV occurrence was highest among occupation groups not involving direct contact with animals suggests that other modes of transmission are likely. Other evidence points to vector-borne transmission, based on reports of clusters of AHFV within five families in Najran (17), the detection of Alkhurma virus in *Ornithodoros Savignyi* and *Hyalomma dromedarii* ticks, the presence of *Ornithodoros Savignyi* in livestock places in Jeddah and Najran, and the reports of four cases of Italian travellers who reported a history of tick bites after visiting a camel market in Southern Egypt. Animals might act as reservoirs for the virus, with ticks being the vector for transmission of the virus to humans. (17,21-22). Consumption of unpasteurized milk has likewise been thought to be a risk factor for transmission of AHFV.

Milk-borne transmission of another mammalian tickborne flavivirus such as tickborne encephalitis has been reported (21). Further investigations should be conducted to identify the role of human-to-human transmission, vector-borne transmission, and raw milk consumption in AHFV occurrence (10,17,21).

Because a comprehensive assessment of the exposure history of each reported case was missing from our dataset, we could not exclude direct contact with animal histories in occupation groups not involving direct contact with animals that had high proportions of AHFV cases. Direct contact with animals could still be the main possible mode of transmission in those occupation groups because it is not unusual for Saudis to raise animals on farms or in backyards at home, particularly in agricultural, rural, and dessert areas. Our finding that there was a high proportion of Saudi nationality among the total cases of AHFV and among the two highest proportion occupation groups (housewives and housemaids, and office jobs) supports this idea. We think that Saudis whose main jobs are not related to direct contact with animals are more likely to come into contact with animals compared to non-Saudis because the homes of non-Saudis are usually not suitable for keeping livestock, thus directly limiting their potential contact with animals (22).

The high proportion of AHFV cases among housewives and housemaids might be explained by the possibility of their exposure to the Alkurma virus through contact with raw meat during food preparation. A second possibility, previously suggested in the literature, is that housewives, especially in rural areas, are potentially exposed to livestock and ticks when taking care of livestock, milking livestock, and cleaning pens (1,22).

We observed that the proportion of AHFV was higher among Saudi females than non-Saudi females, and the proportion of Saudis among the housewives and housemaids group was higher than that of the non-Saudis. This could support the idea above that Saudi housewives are more likely to come into contact with animals that they own and raise compared to non-Saudi housewives (22). This observation of a high proportion of cases among housewives and housemaids group corroborated findings in the two studies in the literature: a study of AHFV from 2009 – 2011 showed that women represented more than 40% of all cases, and the housewives and housemaids group was the second largest group among AHFV cases (22), and a study conducted of AHFV cases reported in Najran from 2003 to 2009 showed that 38% of cases were female and the proportion of houswives was 24.4% (17).

The case fatality rate showed a significant reduction compared to earlier case fatality rates (1.8% vs. 25-30%) of reported AHFV cases in Saudi Arabia. The prior high case fatality rate was explained in the literature as possibly biased, resulting from the detection of severe cases of AHFV only (1,12,21). Another explanation is that the reduction in case fatality rate in recent years is a reflection of improvements in the AHFV surveillance system, rapid diagnostic tests, the ability to detect mild or subclinical cases, aggressive management of symptomatic cases, and increased awareness. In addition, it may be related to a change in the pathogenicity of the Alkhurma virus strain (22).

The present study is based on an analysis of secondary data consisting of a portion of the ongoing AHFV surveillance program's full dataset. Therefore, not all relevant variables were available.

The data contained the epidemiological forms of laboratory-confirmed cases only—suspected cases and clinical forms were not included in the dataset. This limited our ability to calculate trends of cases in terms of incidence rate and prevalence.

Our study included confirmed cases only; the total numbers of suspected cases were not available in our dataset. We were able to find out the total number of all suspected cases in Najran in each year by contacting the person in charge of the AHFV surveillance program at the MoH in Riyadh. The number of suspected cases from Makkah and Jeddah were not reported in the final dataset of the AHFV surveillance program. Over the study period, the number of suspected cases in Najran was as follows: in 2011, 154 cases (75 positive), in 2012, 100 suspected cases (40 positive), in 2013, 380 suspected cases (50 positive), and in 2014, 151 suspected cases (47 positive).

Occupation was the only indicator for exposure. Although occupation was an important indicator for prediction of possible modes of transmission of Alkhurma virus among reported cases, a comprehensive assessment of the exposure history of each reported case is needed in order to obtain better knowledge about etiology and modes of transmission.

Important information such as date of developing symptoms, date of recovery, and the

progression of each case that might have been obtained from clinical history forms was missing. Missing such information limited our ability to calculate survival rate per month and per region, which permits comparison of level of reporting, diagnosis and management system of AHFV between regions, and reveals presented gaps in each region. Knowing the outcome of each case would have been necessary to detect the possible risk factors that affected the disease's progression.

Our study is a descriptive analysis; therefore we were able to identify high-risk groups and areas for generating hypotheses about possible etiology, but this analysis precludes inference of modes of transmission and risk factors.

The strength of present study is that it includes the most current data obtained from the AHFV surveillance system of the MoH, with all of the laboratory-confirmed cases of AHFV in KSA. Therefore, the results of this study are suitable for comparison with the previous results found in the literature, and can inform efforts to modify current measures and policy for prevention, control, diagnosis and management of AHFV in Saudi Arabia accordingly.

Although no cases of AHFV have yet been reported beyond Saudi Arabia and Egypt, AHFV is expected to have a wider geographical distribution (21). This paper will enrich the literature with additional information about the epidemiology of AHFV (regional distribution, age, gender, nationality, occupation and seasonal pattern).

We conducted a descriptive analysis of the most recent data of AHFV cases including time, geographic distribution and demographic information. We discussed our results in detail and compared them with what has been published in the literature in order to generate hypotheses about possible etiology and modes of transmission and address the gaps in knowledge for further studies. To this end, our study will help the Saudi MoH better understand recent trends in AHFV and the epidemiology of the disease, identify gaps in their present surveillance strategy, and redirect efforts for better surveillance, clinical detection, control, prevention and management.

The observation of a high proportion of cases in Najran, which borders Yemen, and the occurrence peak numbers of AHFV cases in the weeks following the Hajj period call attention to the possibility that the virus is present in neighboring countries. That AHFV occurrence was highest among occupation groups that did not involve direct contact with animals suggests that other modes of transmission are likely. However, due to the fact that the occupation variable was only an indicator for exposure, direct contact with animals cannot be excluded.

This implies that efforts are needed to improve the AHFV surveillance program. Additionally, public health policies need to target controlling the livestock trade, especially importation from neighboring countries, and increasing the awareness of protective measures that can be taken while handling animals or animal products. Further investigations should be conducted to identify the role of human-to-human transmission, vector-borne transmission, and raw milk consumption in AHF occurrence (10,17,21).

Chapter 4: Conclusion and Recommendations

Conclusion

The occurrence of AHFV in Saudi Arabia during 2011 to 2014 showed a fluctuating trend. High-risk region for occurrence was Najran. High-risk groups were housewives and housemaids, and office jobs. The observation of a high proportion of cases in Najran, bordering Yemen, and the occurrence of peak numbers of AHFV cases in the weeks following the Hajj period when many foreign visitors are present calls attention to the possibility that the virus is circulating in neighboring countries. This implies that public health policies need to target controlling the livestock trade, especially importation from neighboring countries (10). The occurrence of AHFV cases in Taif for the first time suggests the possibility of AHFV underreporting in other regions in Saudi Arabia that have not reported cases yet.

Because AHFV occurrence was highest among occupation groups not involving direct contact with animals, other modes of transmission may be likely. However, due to the fact that the occupation variable was the only indicator for exposure, direct contact with animals cannot be excluded. This implies that efforts are needed to improve the AHFV surveillance program. Additionally, more efforts need to target increasing the awareness of taking protective measures while handling animals or animal products. Further investigations should be conducted to identify the role of human-to-human transmission, vector-borne transmission, and raw milk consumption in AHF occurrence (10,17,21).

Recommendations

1) Continuous monitoring and evaluation of AHFV surveillance program by conducting further studies in areas of uncertainty.

2) To overcome the limitations that we faced in our dataset, we recommend including the following data in the final dataset of the MoH's AHFV surveillance system based in Riyadh. These data are important for understanding clinical epidemiology of illness and establishing a clinical care assessment program:

- A record of every single suspected case in the final dataset. This is required to provide trends of an illness over a period of time. Trend analysis is important for providing information for needs assessment, surveillance program evaluation, and improvement of established policies for control, prevention, and management of AHFV. Additionally, it gives a more precise comparison of levels of AHFV across different geographical regions. Trends permit prediction of future frequencies and rates of occurrence that will help in policy planning.
- An exposure history is needed in addition to occupational information to determine the mode of transmission of the virus and risk group to exposure to the virus. An AHFV patient's history of contact with blood or body fluid has to be considered in the reporting of exposure history for further investigation of human-to-human transmission of AHFV.
- A clinical history including presenting symptoms and physical signs, date of onset of symptoms, date of presenting to health facilities, date of lab test, date of diagnosis and progress of cases. This information is important for providing potential clues for the diagnosis. Additionally, it is required to detect the fatality of the disease and to address gaps in diagnostic and management capabilities.

3) Improvement of AHFV detection system by enhancement of detection of subclinical and asymptomatic cases through widening investigations to involve any close contacts with cases and not only the cases that present to health facilities.

4) Train heath care providers on the disease reporting, detecting suspected cases, and diagnosis of viral hemorrhagic fever diseases to enhance timely reporting and avoid underreporting or misdiagnosis of illness.

5) Conduct strong active surveillance program for livestock to investigate presence of the virus, especially livestock that cases have come into contact with or those that show abnormal manifestations of morbidity such as spontaneous abortion (21). Control livestock trade and importation with neighboring countries (10).

6) Conduct further large-scale studies on more tick species found in livestock areas in Saudi Arabia and neighboring countries (10).

7) Conduct a study to examine animal products (e.g., milk and meat) of experimentally infected animals in order to study the association between consumption of raw animal product and transmission of Alkurma virus (10).

8) Strengthen prevention and control measures by increasing health education and awareness of the disease.

- Promote use of protective tools in direct contact with animals or animal product; encourage wearing protective gloves during animal handling.
- Enhance awareness of people to avoid tick bites and exposure to mosquitos, and avoid drinking unpasteurized milk.

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Appendix: Figures and Tables

Figure 1: Map of Kingdome of Saudi Arabia, with regions where Alkhurma Hemorrhagic Fever Virus cases were reported (represent by red spot) (23)

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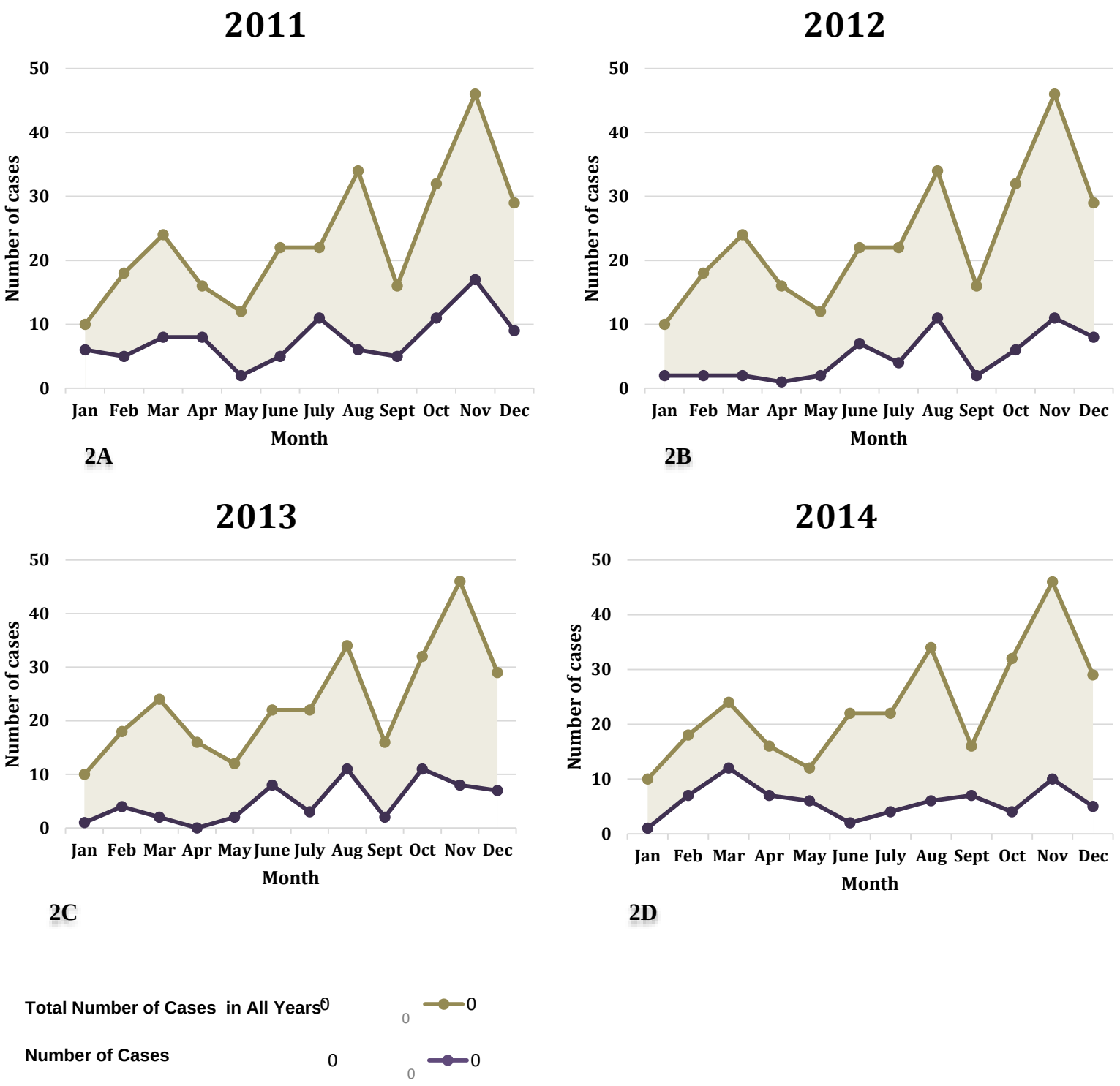

Figure 2: The monthly distribution of Alkhurma Hemorrhagic Fever Virus over four years period in Saudi Arabia (2011-2014); 2A) Year 2011, 2B) Year 2012, 2C) Year 2013, 2D) Year 2014

Table 1: Demographic characteristic of patients with Alkhurma Hemorrhagic Fever Virus in Saudi Arabia by year (2011-2014)

	Year				
Demographic Characteristics	**2011**	**2012**	**2013**	**2014**	**Total**
Age group (year) *					
0-14	4 (18.2)	6 (27.3)	3 (13.6)	9 (40.9)	22
15-24	11 (22.5)	7 (14.3)	17 (34.7)	14 (28.6)	49
25-34	21 (29.6)	17 (23.9)	17 (23.9)	16 (22.5)	71
35-44	24 (41.4)	12 (20.7)	5 (8.6)	17 (29.3)	58
45-54	14 (36.8)	9 (23.7)	10 (26.3)	5 (13.1)	38
55+	19 (44.2)	7 (16.3)	7 (16.3)	10 (23.3)	43
Gender					
Male	51 (28.7)	39 (21.9)	43 (24.2)	45 (25.3)	178
Female	42 (40.8)	19 (18.5)	16 (15.5)	26 (25.2)	103
Nationality					
Saudi	60 (33.5)	30 (16.8)	42 (23.5)	47 (26.3)	179
Non-Saudi	33 (32.4)	28 (27.5)	17 (16.7)	24 (23.5)	102
Occupation group *					
Direct contact with animals jobs	2 (6.7)	9 (30)	6 (20)	13 (43.3)	30
Housewives and housemaids	38 (42.7)	16 (18)	15 (16.9)	20 (22.5)	89
Office jobs	13 (23.2)	12 (21.4)	12 (21.4)	19 (33.9)	56
Technicians	18 (39.1)	8 (17.4)	10 (21.7)	10 (21.7)	46
No defined jobs	3 (16.7)	3 (16.7)	8 (44.4)	4 (22.2)	18
Un-employee/Retired	19 (50)	9 (23.7)	8 (21.1)	2 (5.3)	38
Missing	0	1 (25)	0	3 (75)	4
Region * ¶					
Najran	75 (35.4)	40 (18.9)	50 (23.6)	47 (22.2)	212
Makkah	7 (30.4)	1 (4.4)	8 (34.8)	7 (30.4)	23
Jeddah	10 (25.6)	17 (43.6)	0	12 (30.8)	39
Taif	1 (14.3)	0	1 (14.3)	5 (71.4)	7
Total	93	58	59	71	281 (100%)

* p-value <0.05 (Chi-square test for association)

¶ p-value might be false positive because there were regions that had zero case in some year

Table 2: Demographic characteristics of patient with Alkhurma Hemorrhagic Fever Virus in Saudi Arabia by nationality (2011-2014)

	Nationality		
Demographic Characteristics	**Saudi**	**Non-Saudi**	**Total**
Age group (year)			
0-14	14 (7.8)	8 (7.8)	22 (7.8)
15-24	31 (17.3)	18 (17.7)	49 (17.4)
25-34	38 (21.2)	33 (32.4)	71 (25.3)
35-44	36 (20.1)	22 (21.6)	58 (20.6)
45-54	31 (17.3)	7 (6.9)	38 (13.5)
55+	29 (16.2)	14 (13.7)	43 (15.3)
Gender			
Male	107 (59. 8)	71 (69.6)	178 (63.4)
Female	72 (40.2)	31 (30.4)	103 (36.7)
Occupation group *			
Direct contact with animals jobs	9 (5.0)	21 (20.6)	30 (10.7)
Housewives and housemaids	64 (35.8)	25 (24.5)	89 (31. 7)
Office jobs	45 (25.1)	11 (10. 8)	56 (19.9)
Technicians	18 (10.1)	28 (27. 5)	46 (16.4)
No defined jobs	13 (7.3)	5 (24.5)	18 (6.4)
Un-employee/Retired	27 (15.1)	11 (10. 8)	38 (13.5)
Missing	3 (1.7)	1 (1)	4 (1.4)
Region			
Najran	134 (74.9)	78 (76.5)	212 (75.4)
Makkah	15 (8.4)	8 (7.8)	23 (8.2)
Jeddah	23 (12.9)	16 (15.7)	39 (13.9)
Taif	7 (3.9)	0	7 (2.5)
Total	179 (100%)	102 (100%)	281 (100%)

* p-value <0.05 (Chi-square test for association)

Table 3: Mean Age and Gender Distribution among Occupation Groups with Alkhurma Hemorrhagic Fever Virus in Saudi Arabia (2011-2014)

Occupation Groups	Gender	N	Mean Age (year)	Standard Deviation
Direct contact with animals jobs	Male	30	37.1	13.7
Housewives and housemaids	Female	89	43.4	15.7
Office jobs	Female	10	16.9	9.4
	Male	46	26.4	11. 3
Technicians	Male	46	35.7	10.5
No defined jobs	Female	3	3.3	2.5
	Male	15	29.1	22.8
Un-employee/Retired	Female	1	22	
	Male	37	52.1	20.4
Missing	Male	4	38.5	6.5