

## Vertical Sero-Epidemiological Surveillance of Rift Valley Fever in Sentinel Sheep in Saudi Arabia

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**Abstract:** A 3 years longitudinal survey was conducted using sentinel sheep to monitor antibodies against Rift Valley Fever Virus (RVFV) in six different localities in Saudi Arabia where no previous evidence of RVF exists and no vaccination against RVF is practiced. Two hundred and forty, 6 months old Noaimi lambs of comparable body weight were used. They were divided into six sentinel flocks of 40 lambs each. The animals were stationed in local farms at Al-Hasa, Riyadh, Al-Kharj, Tabouk, Al-Madinah al-Monawarah and Jeddah regions. All lambs were ELISA-negative for antibodies against RVFV and none of them was vaccinated against RVF. The lambs were regularly examined for clinical abnormalities while serum samples were collected from each lamb upon arrival and at 2 months intervals thereafter. The sera were tested for the presence of IgG antibodies against RVFV using sheep IgG-sandwich ELISA Method. Out of 3740 serum samples collected from the six flocks over the 3 years period, none was found to be positive for anti-RVFV antibodies. Furthermore, none of the lambs exhibited clinical signs suggestive of RVF. These results indicate absence of viral activity in the studied areas during the survey period. Such results are of significant importance to the prevention and control measures in the Saudi Arabia as well as to the adjacent countries.

**Key words:** Rift Valley fever, epidemiology, virus, surveillance, sentinel animals, zoonotic diseases, arboviral disease, Saudi Arabia

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### INTRODUCTION

Rift Valley Fever (RVF) is a major zoonotic disease of ruminants and man caused by a mosquito-borne virus of the genus *Phlebovirus*, family Bunyviridae (Flick and Bouloy, 2005). Until the year 2000, RVF Virus (RVFV) was confined to continental Africa and Madagascar where it caused extensive losses in sheep, goats, cattle and camels, beside significant human morbidity and mortality (Al-Afaleq *et al.*, 2003). In August 2000, RVFV emerged for the first time outside Africa, causing a devastating outbreak in South-Western Saudi Arabia and adjoining parts of Yemen. Approximately 40,000 ruminants died and tens of thousands aborted during the outbreak in Saudi Arabia, in addition to 883 clinically severe human cases with 124 deaths (Al-Afaleq and Hussein, 2011). No data were published on the number of animals that died or

aborted in Yemen; however, 1325 human cases with 166 deaths were concurrently recorded in the NorthWestern part of that country (Al-Afaleq and Hussein, 2011). The outbreak has been brought under control within 4 months through rigorous measures including mass ruminant vaccination, vector control, restriction of animal movement and continued monitoring. However, concerns about the virus escaping into other regions outside the outbreak area remained high. This prompted us to undertake sero-epidemiological surveys to ascertain whether or not the virus has reached those regions. These studies are of paramount importance due to the fact that potential mosquito vectors capable of transmitting RVFV occur widely throughout Saudi Arabia (Buttiker, 1981; Jupp *et al.*, 2002; Alahmed and Kheir, 2005; Ahmed *et al.*, 2011).

Two sero-epidemiological surveys were undertaken: the first was a cross-sectional study on different species of domestic ruminants covering all regions of Saudi Arabia (Al-Qabati and Al-Afaleq, 2010; Al-Afaleq *et al.*, 2012) while the second which is described in this communication is a 3 years longitudinal survey using sentinel sheep flocks stationed at various parts of Saudi Arabia other than the South Western region where the 2000 outbreak of RVF occurred and where annual vaccination of ruminants is practiced.

## MATERIALS AND METHODS

**Animals:** Two hundred and forty, 6 months old Noaimi lambs of comparable body weight were used. The animals were born and raised on a farm in Northern Saudi Arabia where RVF is unknown to occur. None of the lambs was vaccinated against RVFV. However, they were vaccinated against sheep pox, clostridial diseases, pasteurellosis and Peste des Petits Ruminant (PPR). All of the lambs were clinically normal.

The lambs were divided into six “sentinel flocks” of forty lambs each, transported by truck and stationed at designated regions representing different parts of the kingdom presumed to be free from RVF, namely: Al-Hasa, Riyadh, Al-Kharj, Tabouk, Al-Madenah Al-Monawarah and Jeddah regions.

**Samples collection:** On arrival, the lambs were examined clinically, ear-tagged and bled by jugular venipuncture using a separate needle and plain vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) for each sample. Collected blood samples were centrifuged at 1000 rpm for 10 min and sera were separated and stored at -20°C until tested.

**Detection of anti-RVfV antibodies:** Enzyme-Linked Immunosorbent Assay (ELISA) kits (National Institute for Communicable Diseases, Johannesburg, South Africa) were used to test serum samples for the presence of anti-RVfV antibodies. The assay format used was IgG-sandwich ELISA and the tests were carried out and their results interpreted using cut-off points according to the manufacturer’s instructions (Paweska *et al.*, 2003a, b; 2005).

**Study design:** Animals of all six sentinel herds were subjected to regular clinical observation and were bled upon arrival and at 2 months intervals thereafter for serum collection. The sera were tested for the presence of anti-RVfV IgG antibodies using ELISA test. Sheep were kept under standard husbandry conditions in adequate housing pens at the designated areas (Fig. 1), fed on

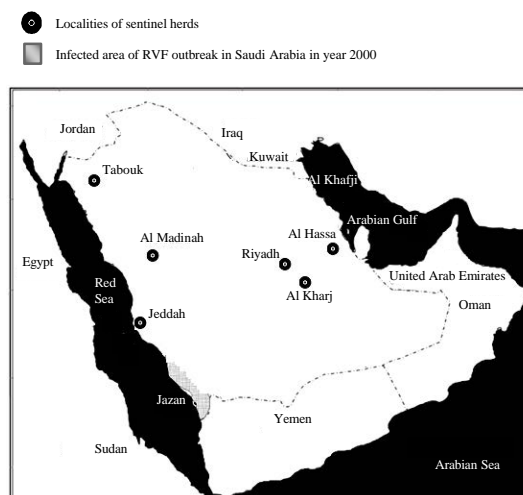


Fig. 1: Map of Saudi Arabia showing the localities of sentinel herds

commercial pellets (16% crude protein) and watered *ad lib*. The duration of the study was 36 months starting July 2005.

## RESULTS

Upon arrival, all lambs were found to be clinically normal and serologically negative for RVF. Close clinical monitoring of all six herds revealed no clinical signs indicative of RVF or other infectious diseases throughout the duration of the study. During this period, however, a few lambs died from causes unrelated to RVF.

A total of 3740 serum samples collected from the six herds at 2 months intervals during the study period were tested for the presence of anti-RVfV IgG antibodies. All tested sera during the entire 36 months study period were serologically negative for anti-RVfV antibodies. For confirmatory purpose, all samples were retested and all of them were confirmed negative.

## DISCUSSION

Establishment of a surveillance system such as the use of sentinel animals to monitor the activity of RVfV in endemic areas and determine its spread to new localities is a standard procedure for evaluating the status of RVfV in many countries (Davies, 1975; Elfadil *et al.*, 2006). We have recently reported the first cross-sectional survey to investigate the presence of the virus in parts of Saudi Arabia other than those where the first RVfV epizootic occurred in 2000. A total of 3480 sheep, goats, cattle and camels from 22 different localities in the kingdom were

tested for antibodies against RVF virus. All of them were negative except 4 (0.26%) out of 1508 sheep and 3 (0.33%) out of 913 goats which tested positive for anti-RVFFV antibodies. This extremely low rate and the fact that all of the tested animals were clinically normal, coupled with the absence of any known virus activity in the surveyed localities suggested that those rare cases might have been false positives or vaccinates smuggled from the original outbreak area in Southwestern Saudi Arabia (Al-Afaleq *et al.*, 2012).

The present study which constitutes the first comprehensive longitudinal sero-epidemiological surveillance of RVFFV in areas outside the outbreak region, confirms these results. The selected study areas represented different ecosystems with varying temperatures, rainfall and plant cover intensities. In this study, sentinel sheep were closely monitored for three consecutive years during which no evidence was found of seroconversion or clinical signs suggestive of RVF. These results indicate absence of viral activity in the studied areas during the entire survey period. On the other hand, a survey carried out in 2006 in some parts of South-Western Saudi Arabia that were previously stricken by RVF revealed a few IgM-positive cases indicating persistence of the virus in a cryptic form in that region (Elfadil *et al.*, 2006; Al-Afaleq and Hussein, 2011). The results of the present study indicate that the control measures implemented by Saudi Arabian authorities have so far been successful in containing RVF and preventing it from spreading into new localities in the kingdom. A similar conclusion was reached in our earlier sero-epidemiological studies on this disease in the kingdom (Al-Afaleq *et al.*, 2012). The obtained results are vital for the country's prevention and control programmes of RVF. Such data is equally important to the adjacent countries and beyond in drawing up their prevention measures.

It should be pointed out, however that Saudi Arabia is subject to several risk factors rendering it highly vulnerable to the re-emergence of this disease at any time. These risk factors include major environmental and ecological changes associated with agricultural development and water conservation projects, importation of millions of sheep, cattle and camels from RVF-enzootic areas for religious rituals, influx of 2-3 million visitors each year for Hajj (pilgrimage) and Umrah (lesser pilgrimage) purposes as well as the impact of global climatic changes (Fagbo, 2002; Davies and Nunn, 1998; Al-Afaleq and Hussein, 2011).

### CONCLUSION

It is therefore essential that RVFFV monitoring be maintained in different parts of the country using sentinel

animals, early warning techniques and other surveillance procedures, coupled with knowledge of prevailing risk factors. This is important in order to ensure early detection of RVF and implementation of appropriate control measures, particularly since no animal vaccination against RVF is practiced outside the enzootic area in South-Western Saudi Arabia rendering local animals in non-enzootic areas fully susceptible to infection while potential arthropod vectors exist throughout the country (Jupp *et al.*, 2002; Davies and Martin, 2003).

### ACKNOWLEDGEMENT

Researchers would like to thank King Abdul-Aziz City for science and technology (Project No. 3-23-WH) for their kind financial support.

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