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A retrospective study of Rift Valley fever in Saudi Arabia

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Summary
A retrospective study was undertaken to examine domestic ruminant sera for Rift Valley fever (RVF) virus antibodies. The sera were collected between 1992 and 1995 from cattle, sheep and goats from various locations in Saudi Arabia. The standard capture enzyme-linked immunosorbent assay system was employed to detect specific RVF antibodies in the animals and the results indicated an absence of RVF antibodies. This finding confirms the assumption that Saudi Arabia was free from RVF up until at least 1995 and most probably before the 2000 epidemic. The finding also confirms that RVF was not endemic in Saudi Arabia.

Keywords
Arbovirus – Retrospective study – Rift Valley fever – Saudi Arabia – Viral disease.

Introduction

Rift Valley fever (RVF) is an arboviral disease of ruminants and humans and is caused by a phlebovirus of the Bunyaviridae family. Although the virus has a segmented genome, no antigenic variants have been reported to date. The disease causes abortions in pregnant animals and high mortality in young lambs and kids (3, 4). The disease in humans is usually mild in the endemic areas of Africa, and can be manifested by influenza-like symptoms. However, it can be fatal in areas when introduced for the first time. This was the case in Saudi Arabia during the outbreak that occurred in 2000 (T. Madani, personal communication, 2000).

Since RVF was first recorded in the literature in 1931 (2), the disease has been confined to Africa and Madagascar. There were no confirmed cases of the disease outside Africa until outbreaks occurred simultaneously in the south-western region of Saudi Arabia (Jazzan) and the Republic of Yemen during the late summer and autumn of 2000.

Before the recent outbreak in Jazzan, Saudi Arabia had remained clinically free from the disease, and no RVF virus had been isolated from ruminants or insect vectors.

This study was undertaken in order to determine whether or not ruminants in Saudi Arabia had RVF antibodies prior to the Jazzan outbreak.

Materials and methods

Test sera

Test sera were collected at various locations across Saudi Arabia from indigenous adult cattle, sheep and goats (Fig. 1). All the donor animals had been present in their respective localities for at least one year. The collections were made between November 1992 and January 1995. The sera were inactivated at 56°C for 30 min. and stored at –20°C until used.

Procedure for the enzyme-linked immunosorbent assay test for the detection of Rift Valley fever virus antibodies

In principle, the double sandwich enzyme-linked immunosorbent assay (ELISA) (capture) was employed using an ELISA kit which had been modified in order to detect RVF virus antibodies in the test sera (1).

The procedure was as follows:

All the volumes of the reagents were added as 100 µl per well unless stated otherwise.

– **Step 1:** coating of the plates

Mouse anti-RVF virus serum was diluted 1:4,000 in phosphate buffered saline (PBS) pH 7.4, and 100 µl was added to each

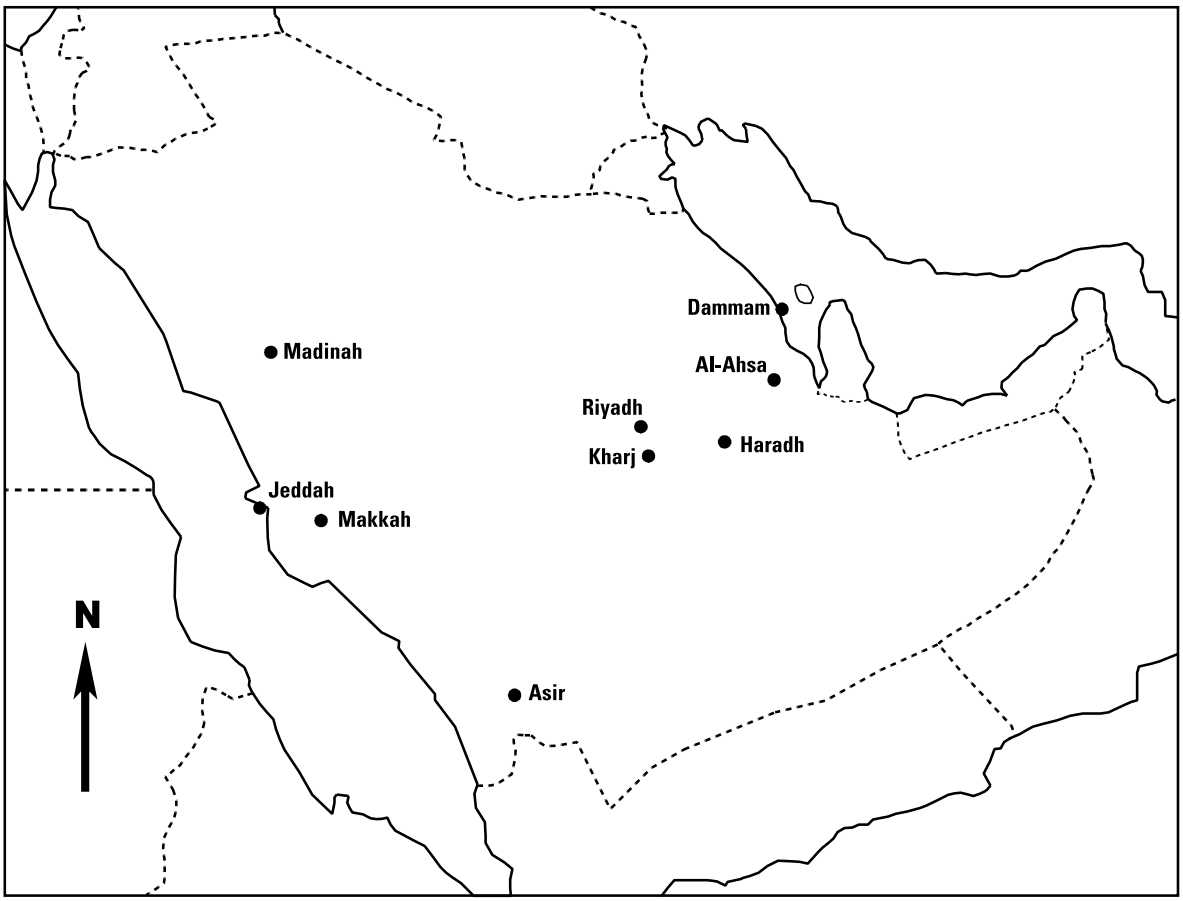

Fig. 1
Map of Saudi Arabia showing the sampling locations

well. The plates were covered and incubated in a humid chamber either for 1 h at 37°C or overnight at room temperature (~26°C). After the incubation period, the plates were washed by flooding and emptying, three times, using PBS pH 7.4 containing 0.1% Tween 20.

– **Step 2:** addition of the blocking buffer

The blocking buffer was PBS pH 7.4 containing 10% skimmed milk (SM). Of this, 200 µl was added to each well and the plates were incubated for 1 h at 37°C.

– **Step 3:** addition of the RVF virus antigen

Rift Valley fever virus inactivated by irradiation was used. It was diluted 1:400, in PBS pH 7.4 containing 5% SM, and added to each well. The plates were incubated in a humid chamber for 1 h at 37°C and washed as above.

– **Step 4:** addition of the test and positive and negative control sera

Each of the test or control sera was diluted 1:20, in PBS pH 7.4 containing 5% SM, and then serial two-fold dilutions were made. The plates were then incubated in a humid chamber for 1 h, at 37°C and washed as above.

– **Step 5:** addition of the conjugate

The respective antispecies antibody (IgG) conjugated to horse raddish peroxidase enzyme (IgG HRPO) was used for each animal species. Each well of the plates received 100 µl of the respective conjugate at a dilution of 1 in 2,000 in PBS pH 7.4 containing 5% SM. The plates were incubated in a humid chamber for 1 h at 37°C and washed as above.

– **Step 6:** addition of the substrate

The substrate used was azinobis-ethylbenzothiazoline sulphuric acid, used at a concentration of 40 mg/100 ml of 0.1M phosphate citrate buffer, pH 4.0. Each well received 100 µl of the substrate solution.

The reaction was left in the dark to develop for 30 min at room temperature (~26°C); then, as recommended by the manufacturers, 100 µl of the stop solution was added and the reaction was read at 405 nm in an ELISA reader.

Table I
Enzyme-linked immunosorbent assay antibodies against Rift Valley fever virus in the sera of cattle, sheep and goats in various locations in Saudi Arabia

Region	Location	Sheep and goats			Cattle		
		Number tested	Number positive	Percentage positive	Number tested	Number positive	Percentage positive
Western and North-western	Jeddah	30	0	0	0	0	0
	Makkah	20	0	0	0	0	0
	Madinah	20	0	0	0	0	0
Eastern	Al-Ahsa	43	0	0	54	0	0
	Dammam	22	0	0	19	0	0
Central	Riyadh	23	0	0	13	0	0
	Haradh	17	0	0	23	0	0
	Kharj	13	0	0	21	0	0
South-western	Asir	35	0	0	0	0	0
Total		223	0	0	130	0	0

Controls were included in the test. Both positive and negative sera were used, as was a true negative antigen. A negative antigen is prepared and used in the same way as the positive antigen but it is diluted and does not contain the virus.

Results

The results of the serological survey are shown in Table I. Neither the test sera examined, nor the negative control, showed a reaction in the ELISA test. However, the positive serum showed a typical titration curve.

Discussion

The sera examined were negative for RVF virus antibodies.

The sera were collected from animals which had been in their respective localities for at least one year. Some of them were from herds, which were being kept as sentinels, between the years 1992 and 1995, for other purposes. The fact that these animals were free from RVF antibodies indicated that the virus was not active in Saudi Arabia between 1992 and 1995. This finding is well supported by the absence of any record of overt clinical RVF virus infection, in either domestic animals or humans in Saudi Arabia, until the recent RVF outbreak in the south-western region of the country in 2000.

It would have been useful to have examined sera from animals in Jazzan before the recent outbreak, but unfortunately this was not possible. However, sera from Asir, in the southwest of Saudi Arabia, were included in the study. These sera also gave negative results. Asir recorded pockets of RVF virus infection during the recent 2000 outbreak.

Une étude rétrospective de la fièvre de la Vallée du Rift en Arabie saoudite

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Résumé
Une étude rétrospective a été réalisée pour mettre en évidence la présence éventuelle d'anticorps de la fièvre de la Vallée du Rift dans le sérum de ruminants domestiques. Les prélèvements de sérum ont été effectués de 1992 à 1995 sur des bovins, des ovins et des caprins provenant de diverses localités d'Arabie saoudite. Le dépistage des anticorps spécifiques de la fièvre de la Vallée du Rift a été réalisé par la méthode normalisée de dosage immuno-enzymatique par capture antigénique. Les résultats ont révélé l'absence d'anticorps. Ils valident ainsi l'hypothèse selon laquelle l'Arabie saoudite était indemne de fièvre de la Vallée du Rift au moins jusqu'en 1995 et, plus que vraisemblablement, jusqu'à l'épizootie de l'an 2000. En outre, ils confirment que la maladie ne présentait pas de caractère enzootique en Arabie saoudite.

Mots-clés
Arabie saoudite – Arbovirus – Étude rétrospective – Fièvre de la Vallée du Rift – Maladie virale.

Estudio retrospectivo de la fiebre del Valle del Rift en Arabia Saudí

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Resumen
Los autores describen un estudio retrospectivo destinado a detectar, en el suero de rumiantes domésticos, anticuerpos contra el virus de la fiebre del Valle del Rift (FVR). Las muestras de suero, procedentes de bovinos, ovinos y caprinos de varias localidades de Arabia Saudí, fueron extraídas entre 1992 y 1995. Para detectar anticuerpos específicos contra la enfermedad se utilizó la técnica de referencia, un ensayo inmunoenzimático de captura. Las pruebas revelaron la ausencia de anticuerpos en los animales, lo que viene a confirmar la hipótesis de que la enfermedad estuvo ausente de Arabia Saudí hasta por lo menos 1995, y muy probablemente hasta poco antes de la epidemia de 2000. Esos resultados confirman también que la FVR no era endémica en el país.

Palabras clave
Arabia Saudí – Arbovirus – Enfermedad vírica – Estudio retrospectivo – Fiebre del Valle del Rift.

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