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 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.
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 radish 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.
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 Jazan 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.

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### Table I

<table>
<thead>
<tr>
<th>Region</th>
<th>Location</th>
<th>Number tested</th>
<th>Sheep and goats</th>
<th>Cattle</th>
<th>Percentage positive</th>
<th>Percentage positive</th>
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<tr>
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</tr>
<tr>
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</tr>
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<td>0</td>
</tr>
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<td>South-western</td>
<td>Asir</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>223</td>
<td>0</td>
<td>0</td>
<td>130</td>
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Une étude rétrospective de la fièvre de la Vallée du Rift en Arabie saoudite

A.I. Al-Afaleq, E.M.E. Abu Elzein, S.M. Mousa & A.M. Abbas

Résumé

Mots-clés

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

A.I. Al-Afaleq, E.M.E. Abu Elzein, S.M. Mousa & A.M. Abbas

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
References


