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between 2001 and 2011**

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## Surveillance of Rift Valley Fever in Iran between 2001 and 2011

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### Abstract

Rift Valley fever virus (RVFV) is an acute zoonotic viral disease that mostly affects ruminants with an occasional spill over as human infection. Following the outbreak of RVF in Saudi Arabia in 2000, surveillance of both animal and human population in Iran increased until 2011. During this period 1206 ovine, 405 caprine, 325 bovine and 28 camel samples were tested for RVFV in nine provinces in Iran. None of these samples tested IgG positive. Moreover, amongst 37 clinically suspected human cases of patients with RVF symptoms, none of these samples tested positive for RVFV. Despite the fact that no positive cases in human or animal populations were identified in Iran, surveillance and monitoring of viral haemorrhagic fevers including RVFV will continue.

**Keywords:** RVF, caprine, ovine, bovine, camel, human

### 1) INTRODUCTION

Rift Valley Fever (RVF) is a viral disease affecting livestock. RVF virus (RVFV) is a member of the Bunyaviridae family, genus *Phlebovirus*<sup>1</sup>. Rainy seasons and flooding prepare the ground for the hatching of the primary vectors, multiple species of mosquitoes known as floodwater *Aedes*, which feed on nearby mammals. High levels of viremia in these animals lead to infection of secondary arthropod vector species and to subsequent infection of other mammals and livestock, in which it causes abortions and death in susceptible animals.<sup>2</sup>

The first human infection with RVFV was reported after the isolation of the virus in 1930. There were no extensive human disease outbreaks until 1951, when an estimated 20,000 persons were infected during an epizootic in cattle and sheep in South Africa.<sup>3</sup>

The route of transmission from animals to humans is from RVFV carrying arthropod vectors, aerosols of blood or amniotic fluid, or other direct contact with infected animals.<sup>4</sup>

RVF in humans has a wide spectrum of clinical manifestations, from asymptomatic infection or a benign febrile illness, to severe illness in approximately 1%-3% of cases, which can include retinitis, encephalitis, hepatitis and haemorrhagic fever.<sup>5</sup>

RVF is mainly reported in Africa. In 2000, the first confirmed RVF outbreak outside Africa was reported in two 'RVF hotspot' countries bordering Iran; namely the Kingdoms of Saudi Arabia and Yemen. Because of livestock and meat imports from Saudi Arabia to Iran, enhanced monitoring of RVFV in susceptible species was undertaken.<sup>4,6</sup>

### 2) MATERIALS AND METHODS

#### 2.1. Study area

A surveillance and control programme of viral hemorrhagic fevers in Iran, established by three collaborating organisations in 1999, including the Centre for Disease Control (CDC) at the Ministry of Health (MOH), Pasteur Institute of Iran (PII) (with the establishment of Arboviruses

and viral Haemorrhagic- Fevers Laboratory known as a National Reference Lab) and the Veterinary Organisation, all organised at the National Level. These three organisations instituted a National Expert Committee on Viral Haemorrhagic Fevers (NEC). In the framework of the NEC, an active collaboration on RVF monitoring has been undertaken over a span of 10 years between 2001 and 2011 in the nine provinces of Iran which have led to this report. Geographical distribution of these provinces were as follows: Khorasan province (Northeast Iran), Tehran province (Northern Iran), Isfahan province (Central Iran), Fars province (Southern Iran), Kerman province (Southeast Iran), Sistan va Baluchistan (Southeast Iran), Kurdistan (Western Iran), Hormozgan province (Southern Iran) and Bushehr province (Southwest Iran). These provinces were selected randomly from among the 30 provinces of the country.

## 2.2. Sampling

Animal and human sampling was conducted at various intervals between 2001 and 2011. During this period, a total number of 405 caprine samples were gathered from suspected goats in four provinces (155 from Hormozgan, 157 from Fars, 80 from Bushehr and 13 from Tehran). To monitor seroprevalence of RVF among suspected sheep, 1206 ovine samples were collected in five provinces (283 from Bushehr, 102 from Fars, 296 from Isfahan, 118 Kurdistan and 407 from Hormozgan). Three provinces were selected to collect a total number of 325 bovine samples from suspected cattle (51 from Sistan va Baluchistan, 147 from Hormozgan and 127 from Bushehr). In 2001, 28 suspected camel sera were collected from Sistan va Baluchistan province. All animal samples were collected by the Iranian veterinary organisation. Animal samples were collected mostly from regions with casual reports of abortion.

At the same time, 37 suspected human cases with RVF symptoms from Kerman (17), Bushehr (16) and Khorasan (4) provinces were sent to the Arboviruses and Viral Haemorrhagic Fever laboratory (National. Ref. Lab) at the Pasteur Institute of Iran, to be examined for RVF (Figure 1).

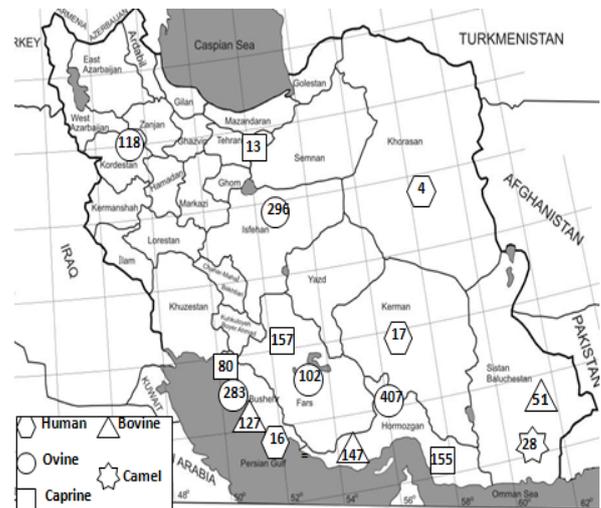


Figure 1. Geographical distribution of human and animal sampling in Iran

## 2.3. IgG detection for human and animal sera

For ELISA, the wells were coated overnight at 4°C with Ag RVF, and the Ag negative control was diluted at 1:800 in 1X Phosphate Buffered Saline (PBS). The plates were then washed 3 times with Phosphate Buffered Saline Tween-20 (PBST). Diluted positive control and negative control and human or animal samples (caprine, ovine, bovine and camel) in Phosphate Buffered Saline Tween-20 Milk (PBSTM) at 1:100 were added to the plate and incubated for 1 h at 37°C. The plates were then washed 3 times with PBST. Anti IgG human or animal conjugated with Horseradish Peroxidase (HRP), diluted in PBSTM at 1/350, was added to the plate and incubated for 1 hour at 37°C. After washing, 3,3',5,5'-Tetramethylbenzidine (TMB) was added and after a while the reaction was stopped with sulphuric acid 4N. Ultimately, the OD was read at 450 nm by ELISA reader.<sup>7</sup> Positive and negative RVFV antigens and also positive and negative controls were obtained from Pasteur Institute of Dakar as WHO collaborating center.

## 2.4. RT\_PCR for human sera

Viral RNA was extracted from 140 µl of serum using QIAamp Viral RNA Kit according to the instructions of the manufacturer (Qiagen GmbH, Hilden, Germany). The samples were subsequently analysed by RT-PCR using specific primers NSCa (5'-CCTTAACCTCTAATCAAC-3') and NS2g (5'-TGATTGTCAGAGTGGTCGTC-3'), which amplified an 800 bp fragment. Amplification cycles were as follows: denaturation at 95°C for 30 S annealing at 55°C for 30 sec and extension at 72°C for 1 min. The final extension step was at 72°C for 5 min. Amplified DNA fragments were visualised after electrophoresis in 1.5% agarose gel and visualised under ultraviolet light.<sup>8</sup> Positive (band 780 bp)

and negative controls were obtained from Pasteur Institute of Dakar.

To ensure the accuracy of the assays and eradicate potential false results, both serological and molecular assays were performed three times per sample.

### 3) RESULTS AND DISCUSSION

The study took place between 2001 and 2011. No RVFV IgG positive cases were detected in 405 caprine, 1206 ovine, 325 bovine and 28 camel samples. In addition, none of the 37 suspected human cases with RVF symptoms tested positive for RVFV using serological and molecular tests.

RVF is a viral disease affecting livestock, especially sheep and goats, causing abortion in females and a high mortality rate in newborn animals.<sup>9</sup> Humans can be infected directly by contact with blood or abortion products of infected animals, or indirectly by mosquito bites.<sup>5</sup>

Animal serum samples were nevertheless collected from regions with some casual reports of abortion; none were IgG positive for RVFV. An explanation for this is that other diseases could have caused the animal abortions.

### 4) CONCLUSION

Although disease is usually introduced by natural means<sup>10</sup>, but the role of efficient surveillance and regional monitoring should not be omitted as protective strategies. For instance, by monitoring the situation of disease in hot spot countries and having put the import ban on live cattle in some years of outbreak, the potential risk of disease introduction would be declined.<sup>11</sup> Thus, despite the fact that no positive cases in human or animal population in studied areas were detected, successive surveillance and monitoring of viral haemorrhagic fevers, including RVF would seem logical, and can be effective for the prevention of the potential introduction of such uninvited zoonosis, as Iran lies in close proximity to the 'hot spot countries' Saudi Arabia and Yemen, which have notable outbreaks.<sup>3, 4</sup> In this regard, because of the aerosol infectivity and risk of dissemination of the virus, a need exists for on-going surveillance and monitoring of outbreaks in the country and region.

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