INTRODUCTION

Rift Valley fever virus (RVFV) is a mosquito-borne virus, and is the causative agent of Rift Valley fever (RVF), a zoonotic disease characterized by an increased incidence of abortion or fetal malformation in ruminants occurring in epizootic periods associated with heavy rainfall. Infected humans can also lead to clinical manifestations that in severe cases cause encephalitis or hemorrhagic fever. The virus is endemic throughout much of the African continent. However, the emergence of RVF in the Middle East, northern Egypt and the Comoros Archipelago has highlighted its geographical range expansion, increasing the concerns over the potential for further incursion into Europe. It extends from Syria in the Middle East, right down to Mozambique in southeastern Africa.

RVF virus belongs to the Bunyaviridae family within the genus Phlebovirus. It is an enveloped virus containing a capsid and single-stranded RNA genome. Young animals are significantly more susceptible and a lot more likely to die. Severity of the disease varies by species, with lambs being “extremely susceptible”, calves “highly susceptible” and humans “moderately susceptible”.

Animals and human become infected through a bite from an infected mosquito, and there are a number of mosquito vectors that have been shown to transmit RVFV, predominantly of the Culex and Aedes genera. Other modes of transmission include animal-to-animal infection through direct contact with infected tissues or fluids, through the re-use of needles during vaccination; particularly in regions with limited resources. The lactating animals may also potentially infect their young ones via milk feeding.

Clinical signs of RVF tend to be nonspecific, which make individual cases difficult to diagnose, but high levels of mortality in young animals, high abortion rates and flu-like symptoms in humans are major indications of the infection. Clinical signs in young animals include fever, lethargy and listlessness, abdominal pain, nasal discharge, anorexia, bloody/fetid diarrhoea, abortion and mortality. In adult sheep and cattle, abortion is the outstanding sign, but the mortality rate in adult sheep is higher (20–30%) than in cattle (10%). In less severe cases in cattle there is febrile disease and disagalactia and some animals develop emaciation with jaundice. In fatal cases, sudden death is preceded by a high fever for 1–2 days. Several veterinarians and veterinary staff were infected after han-
duling and performing necropsies on animals that were only later identified as infected with RVF virus. Under the surveillance plan from 2001 to 2011, samples from different animals like sheep, goats, cattle, and camels including clinically suspected humans were examined for RVFV in the nine provinces of Iran through a seroepidemiological study. None of the animal or human samples were found positive for the virus. However, successive surveillance and monitoring of diseases like RVF is necessary for the prevention of the potential introduction of such uninvited zoonosis [by simple transfer of disease-vector mosquitoes through wind or air currents and travel of many pilgrims (Hajj yatra), as Iran lies in close proximity to the ‘hot spot countries’ Saudi Arabia, Iraq and Yemen. This trend become more pronounced by purchase of remnants, including sheep and cattle, from Iraq as well as formal and informal exchanges of these animals in the border areas of Kurdistan, Kermanshah, Ilam, East Azerbaijan provinces in the northwest and west provinces of Iran. In the year 2000, the disease outbreak was reported for animals and humans in Saudi Arabia. The result of the study carried out by Muhsen in the year 2010 on 1215 sheep sera in Iraq revealed a seropositivity of 8.88%. This can be considered as warning sign for RVFV IgG antibodies by IIFA using a modified competitive ELISA (ID Screen Rift Valley Fever Competitor Multi-Species ID-Vet, Grables, France), which detects both IgG and IgM antibodies directed against the nucleoprotein of RVFV. This test has been shown to have a high sensitivity (91–100%) and high specificity (100%) in both tests done by the manufacturer and an independent ring trial. The competitive ELISA was performed according to the instructions of the manufacturer and all samples were run once. The absorbance was read at 450 nm. To control the validity of each plate, the mean value of the two negative controls (ODNC) were calculated and the plate was considered valid when OD NC controls divided by ODNC should be <0.3. For each sample the competition percentage was calculated by dividing (OD sample/ODNC) × 100. If the value was ≤40%, the sample was considered positive. A value ≥50% was a negative result and the values in between 40 and 50% indicated a doubtful result.

Additional confirmation of positive results in previous test was performed with indirect immunofluorescence assay (IIFA). All collected sera were screened for anti-RVFV IgG antibodies by IIFA using a modified commercial RVFV IIFA slide test kit (Euroimmun, Lübeck, Germany). Slides containing a mixture of infected and non-infected Vero E6 cells on one field (positive field) and non-infected Vero E6 cells on a negative control field were used. Sheep, goat and cattle sera were diluted 1:100 with sampling buffer, and 25 μl of the diluted samples were used. Sheep, goat and cattle sera were diluted 1:100 with sampling buffer, and 25 μl of the diluted samples were used.

The aim of the present study was to investigate the presence of antibodies against RVFV in three species of ruminants (cattle, sheep and goats) in Iran’s Kurdistan Province, while recording the clinical symptoms and history of animal abortion. This is the first study to determine antibodies against RVFV and report positive serology in animals of Iran.

MATERIAL & METHODS

Study area

The major prevailing ruminants (cattle, sheep and goats) of both sexes, i.e. male and female in the age groups of ≤1 yr, 1–3, 3–5 and ≥5 yr-old, were selected randomly during winter, spring, summer and autumn of the year (from January 2016 to December 2016) in Kurdistan Province located in western Iran. This cross-sectional study was focused on the border area of Kurdistan Province, Iran.

Sample collection

At first, clinical examination was performed including mucosa, lymph nodes, body temperature, heart rate, respiratory rate and the history of abortion in herd based on a previously prepared questionnaire. Next, 10 ml blood was taken from the animals’ jugular veins using sterile syringes and serum tubes without additives.

Ethical statement

All animals used in the experiments were handled in compliance with the Guide of National Institute of Health, USA for the care and use of laboratory animals, and the study was approved by the local ethical committee.

Tests

Serum samples were separated and were kept frozen at -20ºC. Separated sera were tested serologically using competitive ELISA (ID Screen Rift Valley Fever Competition Multi-Species ID-Vet, Grables, France), which detects both IgG and IgM antibodies directed against the nucleoprotein of RVFV. This test has been shown to have a high sensitivity (91–100%) and a high specificity (100%) in both tests done by the manufacturer and an independent ring trial. The competitive ELISA was performed according to the instructions of the manufacturer and all samples were run once. The absorbance was read at 450 nm. To control the validity of each plate, the mean value of the two negative controls (ODNC) were calculated and the plate was considered valid when OD NC controls divided by ODNC should be <0.3. For each sample the competition percentage was calculated by dividing (OD sample/ODNC) × 100. If the value was ≤40%, the sample was considered positive. A value ≥50% was a negative result and the values in between 40 and 50% indicated a doubtful result.
were applied to a biochip and incubated for 30 min at room temperature. After a first washing step for 10 min with phosphate-buffered saline (PBS), pH 7.2; 0.2% Tween 20, 25 μl of donkey anti-sheep IgG Cy3 (Indocarboxyanin)-labelled antibodies (Dianova, Hamburg, Germany) was used for small ruminants and goat anti-bovine IgG Cy3-labelled antibodies (Dianova) for cattle samples, respectively. After the second incubation step at room temperature in the dark for 30 min, the slides were washed again for 10 min, dried and covered with sampling buffer and cover plates. Slides were then examined on a fluorescence microscope.

Statistical analysis

Statistical analysis was performed using SPSS Version 21 software in order to determine the prevalence of the RVF in the study sera. The association between the RVF variable and the variables of clinical findings including age, sex, season, and species were screened using Chi-square ($\chi^2$) statistical test. The $p$-values ≤ 0.05 were considered statistically significant.

RESULTS

A total of 288 ruminants (118 cattle, 142 sheep and 28 goats) were examined for RVFV seropositivity. The results of competitive ELISA and IIFA tests were positive for five (1.74%) of the 288 animals which included two cattle of 118 (1.7%), and three sheep of 142 (2.11%). None of the animals, seropositive to RVF showed clinical symptoms related to the disease. The results of this study did not show any statistically significant relationship between serologic test and season, age, sex, and the animal’s species variables ($p$ ≥ 0.05). The results are shown in Table 1.

DISCUSSION

In recent years, the distribution and nature of RVF has changed significantly. It has become a great veterinary concern to dairy producers, wildlife managers and veterinary diagnosticians because of the frequent occurrence of sporadic cases and outbreaks among domestic and wild ruminants. Very little information is available about the epidemiology and disease potential of RVF in Iran’s domestic livestock.

In this study, five of the total 288 animals studied were found positive in serology tests done using competitive ELISA and IIFA. The IIFA, which has been designed for the human diagnostic market, was adapted for serological testing on animals, and the results obtained clearly correlated with that of the ELISA. In other words, 1.74% of animals had a report of RVF infection. The present study is the first research on seroprevalence of RVFV in Iran reporting positive serological results, indicating the presence of virus in the region under study.

Increasing formal and informal exchanges of animals in border areas of the Kurdistan Province of Iran, with Iraq, and increasing annual pilgrimages to the neighboring countries such as Iraq and Saudi Arabia and the focusing on border areas of the Kurdistan Province for sampling might be the reasons for the positive results in this study. There are a number of mosquito species, predominantly in Culex and Aedes genera that have been identified as vector of RVFV in Kurdistan and Khuzestan provinces in Iran, which can be a serious threat for animals and humans.

In this study, no significant relationship was observed between season and age with serologic test of RVFV. Four seropositive animals were in the age group 1–3 and all five seropositive cases were observed in samples of the spring season. In this age and season, the animals are usually released into the pasture for grazing, where they are likely to be exposed to infected mosquitoes and subsequent RVFV infection.

The lack of significant relationship between animal species and the results of the serologic test is consistent with another seroprevalence study on RVF carried out by Aghaa and Rhaymah. Although no significant relationship was obtained between sex and serologic test, four cases of seropositive animals were female. Abortion is the most important clinical symptom of RVF in animals. At the time of sampling, the animals with an abortion history were sampled more often.

The outbreak in Saudi Arabia and Yemen in 2000 resulted in the death of an estimated 40,000 animals from a range of species, with 8000–10,000 abortions in ruminants. Uncontrolled movement of livestock during an outbreak is responsible for introducing RVF to new areas. For example, the virus that caused the Saudi Arabia’s outbreak in 2000 was found to be the same strain that caused the outbreak in 2008.
that caused the 1997–98 outbreaks in East Africa\textsuperscript{20–21}. Therefore, an incursion of this zoonotic virus into Europe and the Middle East could potentially have a devastating impact on the livestock industry in multiple countries, along with a significant impact on animal and human health\textsuperscript{1}.

In a serological study conducted on some ruminants in north Turkey, no positive case was reported\textsuperscript{22}. But in two serological studies carried out in Iraq (the western neighborhood of Iran and Kurdistan Province of Iran), the tests revealed positive results for 1215 sheep samples (8.88%) in Ninevah Province\textsuperscript{11–13}. Another study carried out in Saudi Arabia in the Basrah and 368 goat samples (2.99%) in Ninevah revealed positive results for 1215 sheep samples (8.88%) in Basrah and 368 goat samples (2.99%) in Ninevah Province\textsuperscript{12–13}. 8. Radostits OM, Gay CC, Hinchcliff KW, Constable RD.\textit{Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats}. X edn. London, UK: Saunders Elsevier Company 2014; p. 1205–7.

Conflict of interest
All of the authors declare that they have no conflict of interest.

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