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The first serological detection and risk factors analysis of Rift Valley fever virus in sheep and goats in Fars province, southern Iran

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Abstract
Rift Valley fever is a vector-borne zoonosis that can affect various species, including ruminants and camels. The present study reports the first serological detection of the Rift Valley fever virus in sheep and goats, along with an analysis of risk factors in Fars province, located in the south of Iran**.** The province of Fars was distributed into three climate zones, and three cities were accidentally chosen for each climatic zone. Two epidemiologic units were selected in each city, and samples of all the sheep and goats were collected from each unit. In total, 540 serum samples (391 from sheep and 149 from goats) were gathered and tested by a commercial ELISA kit. Out of 540 samples tested, 12 (2.2%) were found to be seropositive for the Rift Valley fever virus, with 10 from sheep and 2 from goats, indicating the presence of specific antibodies. The correlation between seropositivity and risk factors such as age, sex, climate, animal type, and, history of abortion was not significant. In conclusion, the Rift Valley fever virus is not endemic in Fars province. Further studies are recommended to investigate the distribution of mosquito vectors and their genotype in Fars province, isolate the virus, and develop vaccines.

Introduction

Rift Valley fever virus (RVFV) belongs to the Phlebovirus genus within the Bunyaviridae family and is responsible for causing an arboviral disease (1). The RVFV is transmitted to humans mainly through the bites of infected mosquitoes or through direct contact with infected animals and their products (2). Some people became infected after touching and doing autopsies on animals later identified as RVFV-infected. There are several

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mosquito vectors, mainly Culex and Aedes genera, that transmit RVFV (3). Ruminants are primarily infected by the bite of an infected vector, but other types of transmission encompass animal-to-animal infection via direct contact with infected tissues or fluids, as well as the potential spread through needle reuse during vaccination procedures (4). The clinical manifestations of RVFV can vary widely, ranging from abrupt deaths and abortions to mild or non-specific symptoms. The severity of the disease is influenced by factors such as the virulence of the infecting virus strain as well as the species, breed, and age of the affected animals. Abortion rates may reach 85–100 % in herds that have been damaged. Younger animals exhibit a heightened vulnerability to the disease and are more likely to die, with mortality rates as high as 70-100% in lambs. (5). Young animals affected by RVFV display a range of clinical signs, including fever, lethargy, apathy, abdominal pain, nasal discharge, anorexia, bloody diarrhea, abortion, and increased mortality rates. Clinical pathology revealed severe leukopenia as a common finding in Rift Valley fever disease (RVFD) (5). Infection in humans can clinically cause encephalitis or hemorrhagic fever in severe cases. While RVFV is endemic to various regions of the African continent, its recent emergence in the Middle East, northern Egypt, and the Comoros Archipelago has underscored its expanding geographical range. This development has heightened concerns regarding the possibility of disease outbreaks in Europe (6). From 2001 to 2011, a sero-epidemiological investigation was undertaken in Iran's nine provinces to analyze samples obtained from different ruminant species and individuals with suspected clinical symptoms for the presence of RVFV. Findings revealed that none of the samples, whether from animals or humans, tested positive for the virus (7). Due to the simple transmission of disease-carrying mosquitoes from neighboring countries such as Iraq, the risk of contracting this virus increases in Iran. Livestock movement and illicit trading activities between the two countries, along with the trade of animal products, are significant risk factors for RVFD in the studied area. Findings from a 2010 study conducted on sheep in Iraq revealed that 8.88% of the sampled animals had tested positive for the virus in their serum (8). Numerous species from the Culicidae family are known as disease-carrying mosquitoes in various studies conducted in Iran, principally in the western provinces such as Kurdistan and Kermanshah (9). As a result of the widening borders between Iran and Iraq, the risk of contracting this disease increases in Iran (10). Epidemiological research provides valuable insight into zoonotic diseases among animals and humans in a specific area. Such studies aid public and animal health authorities and researchers develop effective control and prevention programs for the disease. In one notable study conducted by Fakour et al., the outbreak of RVFV was examined, yielding seropositive results (11) among 288 ruminants in the Kurdistan province of Iran, the prevalence rate was seen at 1.74 % (11). No documented data exists regarding the prevalence of RVFV in Fars province, Iran. Consequently, the objective of the present study was to determine the seroprevalence of RVFV and identify potential risk factors such as animal sex, farming systems, age of the animal, and history of abortion in sheep and goats in Fars province by using enzyme-linked immunosorbent assay.

Materials and methods

Study Design

A cross-sectional investigation was conducted by collecting blood samples from sheep and goats in the province of Fars. This province, which covers an area of over 133000 km² and has an average annual rainfall of about 230 mm in the south of Iran, is situated between latitudes 2703 to 31040°N and longitudes 50036 to 55035°E. Based on the weather, Fars province was classified into three regions: warm, cold, and moderate. Three cities were randomly chosen from each region using the Iran Veterinary Organization's Geographical Information System (GIS). Next, a sample of every sheep and goat was taken from each of the two epidemiological units in the chosen city. During the sampling process, relevant information regarding animal sex and farming systems was documented. Additionally, the age of the animals and their abortion history were gained by interviews with the individuals responsible for managing the herds. These collected data played a crucial role in the subsequent analysis of potential risk factors.

Blood sampling and analyses

A total of 391 sheep and 149 goats were included in the study, and blood samples were collected from them through the jugular vein using a 10 mL sterile syringe. To ensure the integrity of the collected blood samples, they were transported on ice to the Razi Vaccine and Serum Research Institute, Shiraz branch. At the institute, the samples underwent centrifugation at 3000 rpm for 10 minutes to separate the sera, which were then stored at -20°C until further examination.

Enzyme-Linked Immunosorbent Assay Method

To identify antibodies against RVFV, a commercially available competitive ELISA antibodies kit (ID VET, France) was employed following the provided guidelines. The optical density (OD) of the samples was measured at 450 nm, with the OD value of each sample divided by the OD value of the negative control. The results were expressed as sample/negative percentage (S/N%) using the readings obtained from the ELISA reader. S/N% was calculated by dividing the OD sample by the OD negative control and multiplying it by 100. Samples exhibiting S/N% values below 40% were considered positive, those falling between 40% and 50% were considered doubtful, and samples with S/N% values exceeding 50% were categorized as negative.

Statistical analysis

All statistical procedures were performed with SPSS version 21 software (SPSS Inc., 2012). Descriptive analysis was utilized to determine the frequencies of serological data. The relationship between the risk factors and RVFV seroprevalence was assessed through the Chi-square test ($\chi 2$), and variables with a univariate P value below 0.05 were subsequently included in the logistic regression test to calculate odds ratios. The significance level was set at $P \leq 0.05$.

Results

Out of 540 animals, 12 (2.22%, 95% CI: 1.27%-3.84%) were positive serologically for RVFV. The majority of animals, 508 (94.07%, 95% CI: 91.75%-95.77%), were seronegative for RVFV antibodies, while 20 (3.70%, 95% CI: 2.41%-5.65%) animals were categorized as suspect. The distribution of seronegative, seropositive, and suspect animals for RVFV antibodies across different cities can be found in Table 1. No animal has been detected positive in Eglid and Khorambid city in a cold climate and Jahrom and Darab in a warm environment for RVFV. Table 2 displays the data of chi-square tests at the animal level. The suspect animals were considered seronegative to perform the chi-square test. After initial screening, multivariable models were constructed for significant factors ($p \leq 0.5$), and climate, age, type of animal, farming system, and history of abortion were used in logistic regression analysis. None of the factors had a significant effect on the rate of RVFV in the final model (Table 3).

Discussion

This research was the first hearing on the prevalence of RVFV infection in domestic ruminants in Fars province. The results showed 2.6% and 1.3% seroprevalence of the virus in sheep and goats, respectively. While earlier reports from Kurdistan province, western Iran, showed zero seroprevalence of RVFV (7), recently, it was reported to be 1.74% and 1.65% in ruminants and aborted sheep, respectively (1, 11).

Table 1. The frequency of seronegative, seropositive, and suspect animals for RVFV antibodies in different cities

Climate/Cities	Tested	Negative		Positive		Suspect	
	No	No	%	No	%	No	%
Cold							
Sepidan	60	48	80.00	6	10.00	6	10.0
Eglid	60	60	100.00	0	0.00	0	0.00
Khorambid	60	60	100.00	0	0.00	0	0.00
Temperate							
Shiraz	60	58	96.67	2	3.33	0	0.00
Marvdasht	60	52	86.67	1	1.67	7	11.67
Pasargad	60	59	98.33	0	0.00	1	1.67
Warm							
Jahrom	60	60	100.00	0	0.00	0	0.00
Fasa	60	51	85.00	3	5.00	6	10.00
Darab	60	60	100.00	0	0.00	0	0.00
Total	540	508	94.07	12	2.22	20	3.70

Table 2. Relationship between potential risk factors and RVFV seroprevalence

Risk factors	Number of tested	Number of positive	Seroprevalence (%)	$\chi 2$	*p- value*
Climate				1.53	0.464
Cold	180	6	3.33		
Temperate	180	3	1.67		
Warm	180	3	1.67		
Age				1.76	0.185
Up to 2 years	133	1	0.75		
Above 2 years	407	11	2.70		
Gender				0.15	0.703
Female	476	11	2.31		
Male	64	1	1.56		
Animals type				0.733	0.392
Sheep	391	10	2.56		
Goats	149	2	1.34		
Farming system				1.53	0.215
Village	480	12	2.50		
Nomadic	60	0	0.00		
History of abortion				1.11	0.292
No	388	7	1.80		
Yes	152	5	3.29		

The prevalence of infection has been increasing in recent years (4). One of the reasons for the increase in the infection rate in Iran can be the possibility of transmission of the virus from neighboring countries such as Iraq and Saudi Arabia through mosquitoes. Climate change, drought conditions, and the presence of fine pollen can aid in transmitting arthropod vectors of this disease from neighboring countries, in particular Iraq, to Iran. Livestock transportation and grazing in border lands between two neighboring countries, Iraq and Saudi Arabia, can be another factor in the spread of infection (12). Also, the prevalence of virus-carrying mosquitoes (five genera and eleven species of the family Culicidae) has been reported in different regions of Iran, such as Kurdistan and Kermanshah (9). Sheep is the most sensitive animal species to RVFV (13), and it is considered an indicator to identify an area as endemic (14).

Table 3. Odds ratios (OR) of risk factors for RVFV seroprevalence

Risk factors	OR	95.0% C. I	*p-value*
Climate			
Cold	1		
Temperate	0.389	0.090-1.676	0.205
Warm	0.391	0.093-1.648	0.201
Age			
Up to 2 years	0.365	0.043-3.090	0.355
Above 2 years	1		
Animals type			
Sheep	1		
Goats	0.485	0.095-2.466	0.383
Farming system			
Village	1		
Nomadic	0.000	0.000-.	0.997
History of abortion			
No	1		
Yes	1.585	0.470-5.345	0.458

CI: Confidence interval

In the current study, the risk factors associated with the prevalence of infection through serological testing were investigated. In the study of the relationship between the age of animals and the seroprevalence of RVFV. In the current study, although there was no significant difference between the two age groups, the older animals had a higher seroprevalence. With increasing age, from the point of view of time, the chances of livestock contact with carriers increase. On the other hand, with increasing age, the size of livestock also increases, which increases the level of contact between livestock and transporters. In other studies, the relationship between age and seroprevalence of RVFV was insignificant (12). No significant correlation was observed between the age of animals and the seroprevalence of RVFV, in conducted research in Kurdistan, Iran (1). In agreement with our results, their results showed that the prevalence was higher in ages older than 1 to 3 years, which was attributed to the start of feeding livestock from grasslands. The possibility of contact between the animals and the vector mosquito has increased in the animal’s graze in pastures close to water and areas where mosquitoes live. In most studies, RVFV seroprevalence is higher non-significantly, in adult animals (12). Since the higher prevalence in young people is a sign of the recent outbreak of the disease, a higher seroprevalence in adult animals in the current study can indicate that there has been no recent outbreak in the region. This issue is also consistent with the reported records because in the event of an epidemic of abortions, it is impossible to ignore it, and considering that no reports of casualties and complications of the RVFR have been observed, it can be stated, although the virus is circulating, it has not yet become endemic.

In agreement with previous studies conducted in Iran, no significant relationship between gender and seroprevalence of RVFV in our study was observed (11). In some studies, the seroprevalence of RVFV has been reported to be higher in females than in males (15, 16). However, the reason for this is unclear. It may be explained by differences in the management applied in various farms (17) or the population composition of sheep and goat breeding herds, which indicates a very high population of female animals compared to males.

In the regional survey of Fars province for the prevalence of RVFV infection, the relationship between the cities and the three climatic regions was insignificant under study and the prevalence of infection; however, in some cities, such as Sepidan, with high rainfall, a higher infection serum prevalence was observed, which means creating a suitable environment for the virus carrier. There was no significant relationship between rural or nomadic management systems and the prevalence of RVFV. The evidence shows the effect of factors such as the regional ecology, agriculture, and the type of surface water on the mosquito population that carries RVFV. the spread of mosquitoes can affect the spread of the RVFV in ruminants followed by disease outbreaks (17).

The RVF causes serious economic damage due to abortion in sheep, goats, and cattle, and in many cases, it causes the death of newborn animals (18). Studies have shown that even though livestock farmers did not report the occurrence of abortion storms, the prevalence of RVFV seroprevalence among animals with a history of abortion is several times higher (19). According to the results of this research and the low prevalence of RVF, it can be said that RVFV was not the leading cause of abortion in animals with a history of abortion, and other pathogenic factors were also involved in this.

Conclusion

The results gained from the current study showed that 2.2% of the samples tested positive for specific antibodies against the Rift Valley fever virus. However, the analysis revealed that factors such as age, sex, climate, and abortion history did not have a significant impact on the seroprevalence of Rift Valley fever in sheep and goats in Fars province. According to the results obtained from this research, the Rift Valley fever virus is not endemic in Fars province. However, due to the high volume of livestock transportation and traffic in Fars province, control strategies are highly recommended to prevent the spread of infection and the area becoming endemic. In addition, practical and regular periodic monitoring and treatment, the use of vaccines and control of vector mosquitoes should be put on the agenda.

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Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of Interest

The authors declare that they have no conflict of interest.

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