Original Article

Seroprevalence and Risk Factors Associated with Akabane Virus Infection in Sheep and Goats in Fars Province, Iran

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ABSTRACT

Akabane disease is an arthropod-borne viral disease that affects ruminants. This teratogenic pathogen causes severe economic losses in ruminants worldwide and in Iran; however, it has not received enough attention in Fars province, Iran. Therefore, this study aimed to determine the influence of age, gender, climate, farming system, and history of abortions on the seroprevalence of the Akabane disease in sheep and goats in Fars province. In the present study, Fars province was divided into three climates, and three cities were randomly selected from each climatic region. In each city, two epidemiologic units were selected, and all sheep and goats in each unit were sampled. Overall, 540 serum samples (391 sheep and 149 goats) were collected and examined with the commercial ELISA kit. The results showed that 83 out of 540 (15.4%) samples were seropositive and had antibodies against the Akabane virus (AKAV). The effect of gender and age on the rate of the AKAV was not significant. Animals in warm climates were 4.218 times more likely to have antibodies against the AKAV than animals in cold climates. Females were 1.32 times more likely to exhibit seropositivity. The odds of AKAV infection were higher in animals with an abortion history than in healthy animals. The findings of the present study indicated that the prevalence of the AKAV was high in small ruminants in Fars province. Therefore, it is necessary to conduct more studies to control the risk factors involved in the spread of this virus.

Keywords: Abortion, Akabane disease, Fars province, Serological survey, Sheep and goats

1. Introduction

The Simbu serogroup of the *Bunyavirus* genus members includes the Akabane virus (AKAV), which causes abortions, fetal damage, stillbirth, and various congenital abnormalities in ruminant species (1, 2). AKAV is an arthropod-borne virus and a member of the genus *Orthobunyavirus* in the family Bunyaviridae (3, 4). This virus causes the epizootic and sporadic prevalence of abortions, premature births, stillbirths, and congenital defects described by arthrogryposis, hydranencephaly, or microanencephaly in cattle, sheep, and goats (5, 6). The virus is transmitted mainly through biting midges of *Culicoides* species (5). Viraemia happens in every case, and the virus can reach the fetus through transplacental distribution (1). AKAV is widely prevalent in tropical and temperate areas in Australia (7, 8), Southeast Asia (9), East Asia (10, 11), the Middle East (12), and Africa (13).

To date, virus isolation, histopathology, immunohistochemistry, serology, and genetic analysis (14) have been used for the laboratory verification of AKAV infections. Serology is the most applied and frequently used method. Special antibodies have been detected against the AKAV using the virus neutralization test and enzyme-linked immunosorbent assay (ELISA) (15, 16). Although AKAV is identified as a significant pathogen that causes abortions and congenital defects in ruminants, it has not received enough attention in Fars province, Iran. The population of goats and sheep in Fars province is approximately 2.47 and 3.18 million heads, which ranks first and second in the animal population in Iran, respectively. Therefore, they play an important role in the economies of farmers in this province by producing milk and red meat (Agriculture Statistics of Iran, 2020).

Moreover, animal husbandry, especially sheep breeding, is critical to southern Iran and affects people's lives. The main benefit of sheep breeding is related to reproduction, and any disturbance in lambing causes economic loss (17). Considering the ecological conditions of Fars province and the suitable climate for the growth and reproduction of mosquitoes, AKAV might exist among the sheep and goat populations of the province. It is obvious that knowing the epidemiological situation is important in terms of taking measures to prevent and control the Akabane disease (18). There is no documented report investigating the prevalence of AKAV in Fars province, Iran; therefore, the current study aimed to estimate its seroprevalence and identify the potential risk factors in sheep and goats in Fars province using the ELISA.

2. Materials and Methods

2.1. Study Design

A cross-sectional study was conducted on blood samples collected from sheep and goats in Fars province, Iran. This province is located between latitudes 27°3' to 31°40'N and longitudes 50°36' to 55°35'E in the south of Iran in an area of about 133,000 km^2 with a mean annual rainfall of about 230 mm. In this study, Fars province was divided into three warm, cold, and temperate regions based on weather conditions. Based on the Geographical Information System of the Iran Veterinary Organization, three cities were randomly selected in each region, and then all sheep and goats in two epidemiological units in each city were sampled. The data on the animal's gender and the farming system were recorded during sampling. Their age and history of abortion were identified by interviewing the person in charge of the herd for use in the analysis of risk factors.

2.2. Blood Sampling and Analyses

In total, blood samples were collected from 391 sheep and 149 goats through the jugular vein using a 10 mL sterile syringe. Animals (sheep and goats) of both genders were bled in two age groups: up to two years and equal to or more than two years. The blood samples were stored on ice while being transferred to the Razi Vaccine and Serum Research Institute (Shiraz branch), where sera were

separated. The samples were centrifuged at 3,000 rpm for 10 min, and sera were separated and stored at −20°C until they were examined by the commercial ELISA kit for the presence of antibodies against the AKAV.

2.3. Enzyme-Linked Immunosorbent Assay Method

The commercial competitive ELISA antibody kit (ID VET, France) was used to detect antibodies against the AKAV, according to the manufacturer's instructions. The optical density (OD) of the samples was read at 450 nm by dividing the OD value of the samples by the OD value of the negative control. For each sample, the results were presented as sample/negative percentages (S/N%) using the OD from the ELISA reader: S/N%=OD sample/OD negative control×100. Samples that showed S/N% of lower than 30%, between 30% and 40%, and >40% were regarded as positive, doubtful, and negative, respectively.

2.4. Statistical Analysis

All statistical analyses were conducted in the SPSS software for Windows (version 16.0.0, SPSS Inc., 2007). The frequencies of serological data were identified by descriptive analysis. The relationship between the risk factors and the AKAV seroprevalence was evaluated by the Chi-squared test ($\chi 2$). The associated variables with a univariate *P*-value of <0.05 were tested by the logistic regression test to calculate the odds ratios. The level of significance was set at $P \leq 0.05$.

3. Results

Out of 540 animals, 83 (15.37%, 95%CI: 12.57%-18.66%) were serologically positive for the AKAV. The frequency of seronegative, seropositive, and suspect animals for AKAV antibodies in different cities is shown in Table 1. While no animal was detected as positive in Shiraz, 65% of the tested animals in Darab had a positive serological test for the AKAV. Table 2 summarizes the results of Chi-squared tests at the animal level. Climate, gender, age, and history of abortion significantly affected the AKAV serostatus ($P<0.05$). These factors were used in the logistic regression analysis, revealing that gender and age did not have a significant effect on the rate of the AKAV in the final model (Table 3). Animals in warm climates were 4.218 times more likely to have antibodies against the AKAV than animals in cold climates. Females were 1.32 times more likely to exhibit seropositivity. The odds of the AKAV infection were higher in animals with an abortion history than in healthy animals (OR=1.724, $P=0.05$).

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

Climate/Cities	Tested	Negative		Positive		Suspect	
	No	No	%	No	%	No	%
Cold							
Sepidan	60	49	81.7	5	8.3	6	10.0
Eglid	60	46	76.7	11	18.3	3	5.0
Khorambid	60	57	95.0	1	1.7	2	3.3
Temperate							
Shiraz	60	55	91.7	0	0.0	5	8.3
Marvdasht	60	54	90.0	5	8.3	1	1.7
Pasargad	60	59	98.3	1	1.7	0	0.0
Warm							
Jahrom	60	41	68.3	16	26.7	3	5.0
Fasa	60	54	90.0	5	8.3	1	1.7
Darab	60	20	33.3	39	65.0	1	1.7
Total	540	435	80.6	83	15.4	22	4.1

Table 2. Relationship between potential risk factors and Akabane seroprevalence

Risk factors	Number of tested	Number of positive	Seroprevalence (%)	$\chi 2$	P- value
Climate				69.56	0.001
Cold	180	17	9.4		
Temperate	180	6	3.3		
Warm	180	60	33.3		
Age				7.01	0.008
Up to 2 years	133	30	22.6		
Above 2 years	407	53	13.0		
Gender				4.64	0.031
Female	476	79	16.6		
Male	64	4	6.3		
Animals type				0.258	0.612
Sheep	391	62	15.9		
Goat	149	21	14.1		
Farming system				0.465	0.495
Village	481	72	15.0		
Nomadic	60	11	18.3		
History of abortion				9.53	0.002
No	388	48	12.4		
Yes	152	35	23.0		

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

Risk factors	OR	95.0% C.I	P-value
Climate			
Cold	1		
Temperate	0.408	0.118-0.804	0.016
Warm	4.218	2.303-7.726	0.001
Age			
Up to 2 years	1		
Above 2 years	1.322	0.933-8.317	0.066
Sex			
Female	2.785	0.755-2.316	0.329
Male	1		
History of abortion			
No	1		
Yes	1.724	0.999-2.975	0.050

CI: Confidence interval

4. Discussion

In this study, the seroprevalence of the AKAV, which is one of the infectious factors of abortion in ruminants, was investigated in Fars province, Iran, using the competitive ELISA method. This is the first report on the spread of the AKAV infection in Fars province that investigated the spread of this virus and the role of various factors in its spread. The temperature and humidity of Fars province are optimum for the reproduction, growth, and activity of the *Culicoides* vectors (mosquitoes) (19), which might lead to the transmission of the AKAV.

The AKAV has spread all over the world. The first outbreak of this virus was reported in Iran in 1992 (20). In recent studies, the infection rate of the AKAV in a population of 57 animals, including cattle, sheep, and goats, was estimated to be 1.75% in Shahrekord, Iran (10). In Semnan province, Iran, the serum examination of cows showed that the prevalence of the AKAV infection ranged from 0-75%, and compared to the bluetongue virus with no infection, it was classified as having higher importance (21). In Khuzestan province, which is located near Fars province, the results showed that 72.39% and 85.87% of the investigated sheep and cattle, respectively, were seropositive for the AKAV (18, 22). In addition, the prevalence of AKAV in ruminants in Saudi Arabia, which is located in the neighborhood of Iran, was reported to be about 70% (23). In Turkey, close to Iran, the prevalence of serum antibodies against the AKAV in cattle, sheep, and goats was 44.74%, 22.90%, and 14.52%, respectively (24). In China, the prevalence of AKAV infection in sheep and goats was 12% and 18.15%, respectively (25). In Nigeria, 4.3% of the studied sheep were evaluated as positive for the presence of antibodies against the AKAV (4). These cases show the global spread of the virus and that the prevalence rate varies across geographical areas (18).

In addition to ecosystem-related factors, such as global warming, changes in seasons, and humidity, other factors, such as animal migration, changes in demographic structure, and increased human travel, also play an important role in the occurrence of mosquito-related diseases, such as the AKVA. The seasonal distribution of *Colicoides* is strongly related to climatic changes, such as temperature and rainfall (2). In some tropical regions, these carriers are present all year round (2), while in regions with a moderate climate, the number of adult vector mosquitoes decreases significantly during the cold seasons of the year (2). This change in the prevalence of the vector mosquito can have a significant effect on the epidemiology of the AKVA (2). In tropical and subtropical regions, there is a trend in the seasonal transmission of the virus vector in such a way that the highest prevalence of infection is seen in wet summer months and the lowest transmission rate is seen in cold winter months (25). As seen in the results of this study, the prevalence rate was highest in hot areas, followed by cold and temperate areas. A connection between AKVA and other related viruses has also been reported with the prevalence of fetal and congenital defects in temperate regions. Although the life cycle of the vector is disrupted due to cold weather and there are few and limited vectors (2), this issue can be partly related to the wind-dependent spread or spread by an infected host from an endemic region to a temperate region (2). In the present study, the prevalence of infection in temperate and cold regions was lower than in warm regions, which indicates the existence of several factors, such as the connection between cities, the type of nomadic migration, and also the importance of the transfer of different races between this province and other neighboring provinces. Fars province is located in the south of Iran, with cold, moderate, and hot climatic regions where animal husbandry in the form of raising ruminants is very common. In addition to the climatic conditions of the province and the development of the livestock industry, human traffic also plays a major role in the spread of carrier-related pollution in this province. Considering the proximity of the cities studied in this research, it is crucial to increase the knowledge of livestock farmers about this disease to prevent the transmission of infection between cities and the increase of seropositive animals, develop new techniques to manage the transfer of livestock, as well as the entry and cultivation of different breeds with each other, and control livestock farms.

The percentage of seropositive animals in different cities of Fars province was in the same range. Studies have shown that the prevalence of AKAV infection can differ across various regions of the same province. In a similar study conducted in Khuzestan, the prevalence of infection in different cities of the province differed from each other (22). These results are in agreement with other studies on the AKAV (2, 18). This virus is transmitted by mosquitoes, which do not have the same prevalence in all regions, which justifies differences in the prevalence of AKAV in various regions. However, the prevalence of mosquito vectors was not investigated in this study.

The spread of the virus is considered high in many countries with warm and temperate climates, especially in areas where seropositive animals for bluetongue disease have been reported (11). A previous report showed that the serum prevalence of bluetongue virus in Fars province for sheep, goats, and cattle is 70.7%, 55.3%, and 20%, respectively (11). It is interesting to note that the agents of both the bluetongue and Akabane viruses are transmitted through *Colicoides*. However, in the study conducted by Mohajer et al. (21), there was no correlation between the prevalence of these two viruses. In countries with a moderate climate, the prevalence of infection is usually high in the hot and humid months of summer and autumn due to the high presence of virus-carrying mosquitoes (6, 22).

Several studies have shown that AKAV causes reproductive disorders such as stillbirth and abortion (5, 6). Moreover, this virus has been found to be the most related to abortion among viruses in its family

(5, 6). In warm weather, the prevalence of AKAV infection can be largely responsible for abortion in livestock. The results obtained from this study indicate that weather, age, gender, and history of abortion have a significant relationship with the prevalence of this infection, while the type of livestock and farming system (nomadic or rural) did not have a significant relationship with the prevalence of the disease. A similar study revealed that the history of abortion and the breed of animals are significantly related to the risk of infection, but their age and gender did not show a significant relationship with virus infection (18). It was also found that the seroprevalence of the AKAV infection is significantly related to lactation conditions (lactating or heifer) (21). In another study, age, race, and geographical location showed a significant positive relationship with the prevalence of infection, while history of abortion did not show a relationship with the prevalence of infection (22). Furthermore, gender showed a strong relationship with the prevalence of infection, which is in accordance with the present results (5). Usually, the prevalence of AKAV is higher in animals with a history of abortion (6). A study conducted on aborted fetuses using a PCR molecular test showed 44.9% and 78.2% seroprevalence of the AKAV at animal and herd levels, respectively (5). According to the results obtained in this study, the incidence of abortion in the livestock of Fars province could be a risk factor for the AKAV seroprevalence in farms.

The obtained results from the present study indicate that the prevalence of AKAV is high in small ruminants in Fars province, Iran. Considering the regional conditions of Fars province, which has warm, moderate, and cold climates, as well as the presence of different ecosystems in this province, the conditions are suitable for completing the life cycle of mosquitoes that carry the virus. In addition, due to the geographical and cultural location of Fars province and its touristic nature, transportation and human traffic are also very high, which increases the risk of virus transfer not only in this province but also in neighboring provinces. Lack of vaccination and effective control programs in Fars province can increase spread of the infection. Considering the possibility of the arrival of other livestock from other provinces of the country, such as Khuzestan, which is located near Fars province, as well as buying and selling livestock between the cities of Fars province, preparing a suitable control program and raising the knowledge of livestock farmers is essential. Moreover, since the AKAV had a higher prevalence in animals with a history of abortion, it is necessary to carry out more studies to trace the virus, identify its genotype, and determine which mosquitoes carry this virus in Fars province. However, the effect of gender and age on the spread of the AKAV was not significant in this study.

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Authors' Contribution

Study concept and design: M.M; M.H; M.B; F.T; M.S; F.S

Acquisition of data: M.M;M.H;F.T;M.S

Analysis and interpretation of data:

Drafting of the manuscript: M.M; M.H; M.B; F.T; M.S; F.S

Critical revision of the manuscript for important intellectual content: M.M;M.H;M.B;F.T;M.S;F.S

Ethics

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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