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Original Article

A Serological Survey on Antibodies against Akabane Virus in Sheep in Southwest of Iran

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Abstract

Background and Aims: Akabane disease causes epidemics of abortions, stillbirths and congenital malformations manifested as arthrogryposis and hydranencephaly or microanencephaly in sheep, goats and cattle. Akabane virus replicates in arthropods and is transmitted by either mosquitoes or midges. Outbreaks of the Akabane virus have been reported from many countries in Southeast Asia, Middle East, Africa and Australia. The aim of this study was to evaluate the prevalence of Akabane virus infection and correlation of this infection with host and environmental determinants in sheep in Khouzestan province, the Southwest of Iran.

Materials and Methods: In this study, serum samples of 360 sheep were randomly collected from 6 cities of Khouzestan province and were examined by ELISA assay.

Results: Seroprevalence of Akabane virus infection was 39.72% (95% CI: 34.67-44.78%). Statistical analysis showed history of recently abortion and breed of sheep are significantly associated with infection ($p<0.05$) but sex and age of sheep are not significantly associated with infection ($p>0.05$).

Conclusion: The results of the present study confirm that the Akabane virus infection exists in Khouzestan province, Iran. Considering the local weather conditions and the facility of vector-borne transmission, the health authorities should take measures to prevent and control the infection.

Keywords: Akabane virus, Epidemiology, Prevalence, Serology, Iran

Introduction

Akabane disease is a viral disease in cattle, sheep and goats. The virus is classified into the genus orthobunyavirus in the family Bunyaviridae (1). Akabane disease causes epidemics of abortions, stillbirths, premature births and congenital malformations manifested as arthrogryposis and hydranencephaly or microanencephaly (2). Akabane virus replicates in arthropods and is transmitted by either mosquitoes or midges (3).

Akabane is named after the Japanese village where the virus was first isolated from mosquitoes *Aedesvexans*, *Culextritaeniorhynchus* in 1959 (4). In 1969-70, a major epizootic of Akabane occurred in Israel affecting 3,000 dairy calves, 700 lambs, and 600 kids (5). In Australia, the Akabane

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virus was first identified in 1972; however, sporadic undiagnosed outbreaks had been observed as early as the mid-1940's. Antibodies of the virus have been found in ruminants from many countries in Southeast Asia, Middle East, Africa and Australia (6-17). In recent years, with the importation of ruminants from abroad, the incidence of many diseases, especially abortion diseases, has increased. Surveys on abortion diseases in domestic ruminants have been carried out, but most were restricted to brucellosis, campylobacteriosis, neosporosis, and other diseases. Being one of important pathogenic microorganisms for abortion diseases,Akabane virus has not received enough attention. So far, little has been known about Akabane virus infection in ruminants (18, 19). In order to develop an effective prevention and control program to fight against abortion diseases in ruminants, it is important to obtain epidemiological information on the prevalence of Akabane disease.

While sheep are a major livestock species in Iran, studies of Akabane disease have not been given the same priority as some other diseases. Thus, the objective of this study was to determine the seroprevalence and risk factors of Akabane virus infection in sheep in Khouzestan province in the Southwest of Iran, in 2016.

Methods

Sampling. A total number of 360 serum samples were randomly collected from apparently healthy sheep of various ages, breeds and either sexes during January and February 2016, from 19 flocks in 6 different city in Khouzestan province. This tropical province is 64057 Km^2 and is located between latitude 29˚58′ to 32˚58′ N and longitude 47˚42′ to 50˚39˚ E in the southwest of Iran.

In a total number of 360 samples, there were 324(90.00%) female and 36 (10.00%) male sheep. Age was determined by tooth replacement in sheep. The animals were divided into five age groups: suckling (≤ 1 year old), juvenile (1 to 2 years old), young (2 to 3 years old), sub-adult (3 to 4 years old) and adult (>4 years old). The blood samples were collected from the jugular vein with sterile tubes of venoject without anticoagulant and the sample shipped from cities of sampling to laboratory in ice pack and then centrifuged at 3000 rpm for 10 min. Then, serum was separated and stored at −20 ˚C until enzyme like immunosorbent assay (ELISA) examination.

Serological test. The ELISA test was performed according to the producer instructions (ID Vet innovative diagnostics, France). The optical density (OD) of the samples was measured at 450 nm. S/N percentage was calculatedfor each sample:

$$S/N = \frac{OD\ sample}{OD\ negative\ control} \times 100$$

Samples with the S/N:

-Less than or equal to 30% were considered positive.

-Less than or equal to 40% and greater than 30% were considered doubtful.

-More than 40% were considered negative.

Statistical analysis. Statistical analyses were performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). The association between age, sex, breed, history of abortion and geographic location were analyzed by Chi-square test and logistic regression. Differences were considered statistically significant when $p < 0.05$.

Results

Among the 360 sera, 143 samples (39.72%, 95% CI: 34.67 – 44.78%) were positive and had antibodies to Akabane virus. All of 19 sampled flocks were seropositive against Akabane virus. The lowest prevalence rate was for a flock in Hendijan with 5.56% and the highest prevalence rate was for a flock in Dezful with 93.33%.

The prevalence of Akabane virus infection in female and male sheep were 38.89% and 47.22%, respectively. There was no significant difference between these sex groups ($\chi 2 = 0.62$, df = 1, $p > 0.05$). The odds of infection in ram in comparison with ewe was 1.41 (95% CI: 0.70 – 2.81) (Table 1).

Table 1: Prevalence against Akabane virus antibodies in sheep from Khouzestan province, Iran.

Category	Groups	Prevalence	Odds Ratio	95% CI for OD	P-Value
Sex	Female	38.89%(126/324)	-	-	-
	Male	47.22%(17/36)	1.41	0.70-2.81	0.33
Abortion	Delivered normally	33.58%(87/263)	-	-	-
	History of recently aborted	63.93%(39/61)	3.59	2.00-6.42	<0.001
Age	Suckling	23.53%(4/17)	-	-	-
	Juvenile	30.43%(14/46)	1.42	0.39-5.14	0.59
	Young	39.29%(22/56)	2.10	0.61-7.28	0.24
	Sub-adult	45.98%(40/87)	2.77	0.84-9.16	0.10
	Adult	40.91%(63/154)	2.25	0.70-7.22	0.17
Location	Hendijan	20.00%(14/70)	-	-	-
	Baq-Malek	28.00%(14/50)	1.56	0.66-3.64	0.31
	Ahvaz	37.14%(26/70)	2.36	1.11-5.06	0.03
	Behbahan	40.00%(20/50)	2.67	1.18-6.00	0.02
	Shushtar	44.33%(29/60)	3.74	1.73-8.12	0.001
	Dezful	66.67%(40/60)	8.00	3.62-17.71	<0.001
Breed	Arabi	10.81%(4/37)	-	-	-
	Mix	23.53%(16/68)	2.54	0.78-8.26	0.12
	Torki	38.78%(19/49)	5.23	1.60-17.11	0.006
	Afshari	40.00%(18/45)	5.50	1.66-18.20	0.005
	Bakhtiari	43.66%(31/71)	6.39	2.05-19.97	0.001
	Lori	61.11%(55/90)	12.96	4.23-39.77	<0.001

Statistically significant differences were evident between infection and history of recent abortion, so that 39(63.93%) of ewes with history of recently abortion and 87(33.58%) of normally delivered ewes were seropositive to Akabane virus ($\chi2 = 12.41$, df = 1, $p < 0.001$). The odds of infection in ewes with history of recently aborted in comparison with normally delivered ewes was 3.59 (95% CI: 2.00-6.42) (Table 1).

There was no statistically significant difference between infection and age, so that 4 from 17 of suckling lambs, 14 from 46 of juveniles, 22 from 56 of young animals, 40 from 87 of sub-adult animals and 63 from 154 of adult animals were seropositive to Akabane virus ($\chi2 = 5.04$, df = 4, $p > 0.05$) (Table 1).

As the Table 1 shows 26(37.14%) sheep from Ahvaz, 20(40.00%) sheep from Behbahan, 14(28.00%) sheep from Baq-Malek, 29(48.33%) sheep from Shushtar, 40(66.67%) sheep from Dezful and 14(20.00%) sheep from Hendijan had antibodies against Akabane virus. Statistically significant differences were evident between infection and location ($\chi2 = 34.49$, df = 5, $p<0.001$).

Differences between breeds were also observed ($\chi2 = 30.70$, df = 5, $p<0.001$). 61.11% of Lori sheep, 43.66% of Bakhtiari sheep, 38.78% of Torki sheep, 10.81% of Arabi sheep, 40% of Afshari sheep and 23.53% of Mix sheep had antibodies against Akabane virus (Table 1).

Multivariate logistic regression showed that breed and history of abortion were risk factors for infection (Table 2).

Discussion

Akabane virus has been shown to be an important pathogen causing abortions and congenital malformations in ruminant (20). Forman and et al. in 2008 reported that 14 outbreak of Akabane virus was occur in Asia between 2002 and 2006 (21). However, prevalence of this virus infection have not reported in Iran since 1992 (22). This study has shown that 39.72% of sheep were seropositive to Akabane virus in Khouzestan province, Iran.

Table 2: Risk factors based on multivariate logistic regression for Akabane virus infection in sheep from Khouzestan province, Iran.

Category	Groups	Prevalence	Odds Ratio	95% CI for OD	P-Value
Abortion	Delivered normally	33.58%(87/263)	-	-	-
	History of recently aborted	63.93%(39/61)	5.08	2.56-10.10	<0.001
Breed	Arabi	10.81%(4/37)	-	-	-
	Mix	23.53%(16/68)	2.57	0.75-8.81	0.13
	Torki	38.78%(19/49)	2.52	0.69-9.19	0.16
	Afshari	40.00%(18/45)	6.21	1.74-22.17	0.005
	Bakhtiari	43.66%(31/71)	5.79	1.77-19.02	0.004
	Lori	61.11%(55/90)	14.63	4.56-46.91	0.001

Seroprevalence of Akabane virus infection in ruminants were reported in several locations. In 1980, 38% of sheep were seropositive to Akabane virus infection in Japan (12). Saudi Arabia is southern neighbor of Iran and two seroprevalence of Akabane virus infections in sheep were reported; 11% in 1988 and 17% in 1998 (6, 7). Other reports were 18.15% in China, 17.37% in Cyprus, 17% in Kenya, 13% in Sudan, 4.4% in Nigeria and 0% in Indonesia (10, 13-16, 23).

Detect of risk factor of vector borne disease was difficult. The present study also revealed that prevalence rates were highly associated with locality, breed, and recent abortion but were not associated with sex and age of animals.

The prevalence rate was significantly higher in Dezful city than in other cities. It means that Akabane virus infection was associated with locality. This result confirmed records of Mohamed et al. in 1996 (13). The difference between reported seroprevalences in different countries show clearly that Akabane virus infection was associated with locality.

The prevalence rates were also significantly lower in Arabi sheep and in Mix sheepthan in other breed. Arabi sheep and Mix sheep are known as indigenous breed in Khouzestan. Generally, exotic breeds are more susceptible than indigenous breeds. Elhassan et al. showed that Akabane virus antibodies prevalence was highly associated with breeds (1).

The prevalence rates were also higher in ewes which had history of recently abortion than in ewes which had delivered normally. This result confirmed records of Jun et al., Elhassan et al. and Oluwayelu et al. that discussed the associations between this virus and abortion suggesting that this virus may significantly increase abortion risk among sheep (1, 14, 23).

The present study revealed that prevalence rates were not associated with sex. This result confirmed records of Oluwayelu et al. but didn't confirm with reports of Elhassan et al. in 2014. In addition, no statistical significant differences were found in seroprevalence of Akabane virus infection between age groups in this study but Oluwayelu et al. and Elhassan et al. were reported a significant difference (1, 14).

Temperature, international trade, geographic status of areas,lifestyle of people and wildlife characteristic are of important factors that all of them can influence prevalence of Akabane virus in the area. Thus, Akabane virus prevalence should be investigated from several perspectives in an area, however, these views are sometimes interrelated and should be taken into consideration altogether and not in isolation.

The main Akabane virus vector species in Khouzestan is unknown. The presence of Culex species and other important mosquito vectors for Akabane virus in Iran could also contribute to the transmission of the virus among different vertebrate hosts. Species of mosquito implicated in Akabane virus infection circulation can be targeted with insecticides either at their larval or adult stages. Drier summers and wetted winters may prevent mosquito summer population growth

through a shortage of larval habitats at the critical times of the year; however, drought elsewhere causes a concentration of both mosquitoes and vertebrates around persistent water bodies which could promote transmission even under average, dry conditions.

The present study clearly indicated the widespread prevalence of Akabane virus infection in sheep in Khouzestan, Iran. Since a vaccination program for Akabane is not established in Iran, a seropositive result indicates Akabane infection in the domestic populations.

Absence of clinically recognized symptoms of Akabane virus infections with noticeable outbreaks of abortion and fetal malformations may lead to underestimation of the importance of the disease in the area. This situation may, however, change and the disease can pose considerable threat with the increasing trend toward raising of exotic breed to satisfy the rising local demand for lamb meat, milk and other dairy products. Therefore, according to local weather conditions and facility of vector-borne transmission, prevention and control measures should be considered by health authorities.

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