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Original Article

Seroprevalence and Risk factors of Akabane Virus Infection in cattle from Khouzestan Province of Iran

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Abstract

Background and Aims: Akabane virus is an arbovirus in the genus Orthobunyavirus of the family Bunyaviridae that can affect ruminants such as cattle, sheep and goats. This arthropod-borne virus is transmitted by either mosquitoes or midges and has been identified as a cause of outbreaks of reproductive disorders (abortions, premature births, and stillbirths) and congenital malformations (arthrogryposis, hydranencephaly and microencephaly) in cattle, sheep and goats. The aim of the present study was to determine the prevalence and risk factors of Akabane virus infection in cattle from Khouzestan province, the Southwest of Iran.

Materials and Methods: In this study serum samples of 361 cows were randomly collected from 9 cities of Khouzestan province and were examined by commercial ELISA kite.

Results: Seroprevalence of Akabane virus infection was 85.87% (95% CI: 82.27-89.47%). Univariate statistical analysis showed that breed and age of cows ($p<0.05$) plus location and sex ($p<0.001$) were significantly associated with infection but history of recently abortion and type of management are not significantly associated with infection ($p>0.05$). Multivariate logistic regression showed that age, sex, breed, history of recently abortion, type of management and location justify 30.7% of infection fluctuations.

Conclusions: The results of the present study confirmed that cattle in Khouzestan province are highly infected with Akabane virus. These findings call for continuous monitoring of the disease among ruminants in order to ascertain the actual burden and increase awareness of the disease. This will facilitate early detection and aid the development of appropriate control measures against the disease in this area.

Keywords: Akabane virus, Epidemiology, Serology, Cattle, Khouzestan

Introduction

Akabane disease is a viral disease in cattle, sheep and goats caused by Akabane virus which classified into the genus orthobunyavirus in the family Bunyaviridae (1). "Akabane" is the name of the village where the virus was first isolated. Subsequent serological studies have classified Akabane virus in the Simbu group (one of the serological group in the family Bunyaviridae (2). Akabane virus has been known to cause outbreaks of abnormal deliveries in cattle, such as abortions, premature births, stillbirths and calf deformities noted as congenital arthrogryposis-hydranencephaly or microanencephaly syndrome (1, 3, 4).

Akabane virus was originally isolated from mosquitoes, Ades vexans and Culex tritaeniorhychus, in Japan in summer of 1959 (2). Akabane virus replicates in arthropods and is transmitted by either mosquitoes or midges(5).

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Akabane virus has been reported in a number of countries on the African continent, the Middle East, Southeast Asia and Australia (6). In 1969-70, a major epizootic of Akabane occurred in Israel affecting 3,000 dairy calves, 700 lambs, and 600 kids (7). In Australia, the Akabane 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.(۲۰-۸)

There are some evidence on the virulence differences in the Akabane virus isolated, as Akabane virus has been isolated from normal bull and sheep in Australia. Nevertheless, the typical gross lesions of aborted fetuses with arthrogryposis and hydranencephaly caused by Akabane virus infected are less frequent in Japan in recent year. In addition, the virus was isolated not only from those affected calves but also from those calves without clinical symptom in Taiwan. These might indicate that there were differences in the virulence of isolates .(۲)

Khouzestan is one of the provinces that located in the southwest of Iran. This tropical province is 64057 Km2 and is located between latitude 29˚58′ to 32˚58′ N and longitude 47˚42′ to 50˚39˚ E (Fig. 1). The weather of this province is hot and more than the others and is very suitable for the vectors of Akabane virus. Because of the interaction between vector and climatic factors, outbreaks of Akabane disease usually follow a well-defined distribution, occurring in areas adjoining regions where vectors are endemic (6). To date, there is not any report of Akabane virus infection in cattle from Khouzestan, therefore, this study was carried out to determine the extent of this viral infection in this region.

Methods

Sampling. A total of 361 serum samples were collected from apparently healthy cattle of various ages, breeds and either sexes during January and February 2016, in 9 different city (Shadegan, Dezful, Hendijan, Shushtar, Susangerd, Ahvaz, Behbahan, Ramhormoz and Baq-Malek) in Khouzestan province.

From the 361 samples, there were 322 (89.20%) females and 39 (10.80%) males. The animals were divided into three age groups: young (≤ 2 year old), sub-adult (2 to 4 years old) and adult (>4 years old) according to dental formula (21). The variables of breed (native, holstein and crossbreed) history of abortion (yes or no) and management (industrial and nonindustrial) were collected

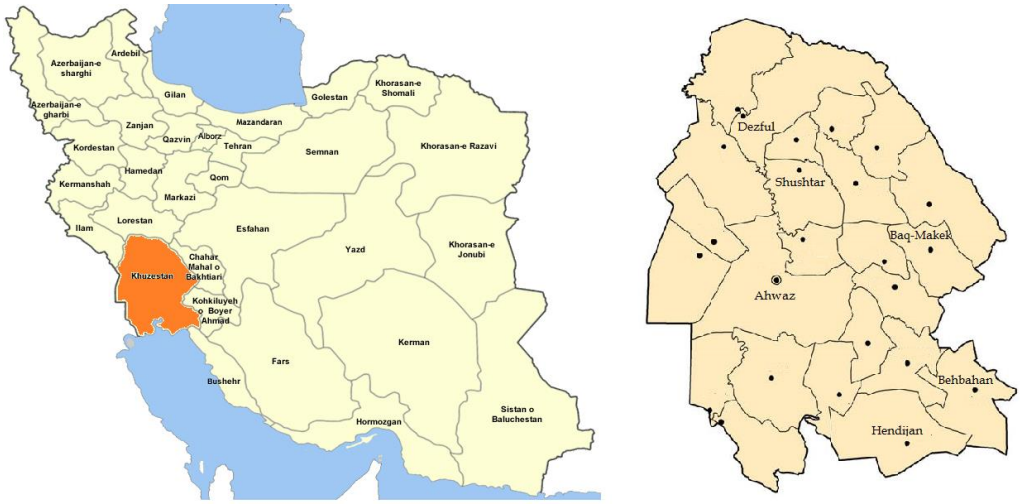

Fig. 1. Khuzestan province of Iran

Table 1: Prevalence of Akabane virus antibodies in cattle in Khouzestan, Iran.

Category	Groups	Prevalence	Odds Ratio	95% CI for OR	P-Value
Sex	Male	66.67%(26/39)	-	-	-
	Female	88.2%(284/322)	3.74	1.77-7.89	<0.001
Abortion	Delivered normally	88.20%(269/305)	-	-	-
	History of recently aborted	88.24%(15/17)	1.004	0.22-4.57	0.99
Age	Young	76.47%(52/68)			
	Sub-adult	85.43%(129/151)			
	Adult	90.85%(129/142)	1.27	1.095-1.47	0.002
Location	Shadegan	60.00%(24/40)	-	-	-
	Dezful	66.67%(28/42)	1.33	0.54-3.28	0.53
	Hendijan	87.50%(35/40)	4.67	1.51-14.46	0.008
	Shushtar	89.74%(35/39)	5.83	1.74-19.61	0.004
	Susangerd	90.00%(36/40)	6.00	1.79-20.15	0.004
	Ahvaz	92.50%(37/40)	8.22	2.16-31.27	0.002
	Behbahan	95.00%(38/40)	12.67	2.67-60.05	0.001
	Ramhormoz	95.00%(38/40)	12.67	2.67-60.05	0.001
	Baq-Malek	97.50%(39/40)	26.00	3.24-208.80	0.002
Breed	Native	74.60%(47/63)	-	-	-
	Holstein	87.84%(65/74)	2.46	1.001-6.04	0.05
	Crossbreed	88.39%(198/224)	2.59	1.29-5.22	0.008
Management	Nonindustrial	85.02%(227/267)	-	-	-
	Industrial	88.30%(83/94)	1.33	0.65-2.71	0.43

Table 2. Odds ratio in multivariate logistic regression

Category	Groups	Odds Ratio	95% CI for OR	P-Value
Age	-	1.27	1.07-1.52	0.003
Geographical location	Shadegan	-	-	-
	Dezful	2.15	0.8-5.76	0.13
	Hendijan	5.39	1.67-17.43	0.005
	Shushtar	5.41	1.58-18.5	0.007
	Susangerd	6.29	1.83-21.61	0.004
	Ahvaz	5.8	1.27-20.22	0.02
	Behbahan	17.38	3.55-84.91	<0.001
	Ramhormoz	15.97	3.28-77.68	0.001
	Baq-Malek	36.29	4.42-298.29	0.001

from all cattle according to observation and interview.

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 linked immunosorbent assay (ELISA) examination.

Serological test. The ELISA test (Competitive ELISA for the detection of anti-

G1 antibodies in ruminant serum and plasma) 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 calculated for each sample and Samples with the S/N Less than or equal to 30% , Less than or equal to 40% and greater than 30% and More than 40% were considered positive, doubtful and negative respectively (22).

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

Results

Among the 361 sera, 310 samples (85.87%, 95% CI: 82.27–89.47%) were positive and had antibodies to Akabane virus. Statistical analysis showed that infection depends on age and, so that 76.47% of young (52 from 68), 85.43% of sub-adult (129 from 151) and 90.85% of adult (129 from 142) animals were seropositive to Akabane virus which shows a rise in infection rate with aging ($\chi2= 7.87$, df = 2,$p<0.05$). Logistic regression showed that the odds of infection between the age based on year and disease is 1.27 (95% CI: 1.095-1.47)($P<0.05$) and with increased 1 year odds of infection increased 27%. Also 6.2% of fluctuation of infection was justified by age(Table 1).

The prevalence of Akabane virus infection in female and male cattle were 88.2% and 66.67%, respectively. There was significant difference between these sex groups ($\chi2 =11.58$, df=1, $p<0.001$). The odds of infection in female in comparison with males was 3.47 (95% CI: 1.77–7.89) and 5.2% of fluctuation of infection was justified by sexuality (Table 1).

There was no statistically significant differences between infection and history of abortion, so that 15(88.24%)of cows with history of recently abortion and 269(88.2%) of normally delivered cows were seropositive to Akabane virus ($\chi2 = 0.00002$, df =1,$p>0.05$). The odds of infection in cows with history of abortion in comparison with normally delivered cows was 1.004 (95% CI: 0.22-4.57) and 0.01% of fluctuation of infection was justified by history abortion (Table 1). Differences between breeds were also observed ($\chi2 = 8$, df = 2, $p<0.05$).

Native cows with 74.60% prevalence rate have lower chance to be infected with Akabane virus in comparison with Holstein breed (87.84%) and crossbreed animals (88.39%). Also 3.5% of fluctuation of infection was justified by breed (Table 1).

Although relative frequency of seropositive cases were higher in intensively managed animals, according to statistical analysis, management type had no significant role on prevalence rate ($p>0.05$). Univariate logistic regression showed that the odds of infection in industrial cattle in comparison with nonindustrial was 1.33 (95% CI: 0.65-2.71) and 0.3 % of fluctuation of infection was justified by management type (Table 1).

As the Table 1 shows, infection rates varied among different cities. Shadegan with 60% and Baq-Malek with 97.5% prevalence rates were the lower and higher infected places. Significant differences were evident between infection and location ($\chi2 = 47.37$, df = 8, $p < 0.001$) and 19.92% of fluctuation of infection was justified by geographical location(Table 1).

Multivariate logistic regression showed that age, sex, breed, history of recently abortion, type of management and location justified 30.7% of fluctuations of infection but age and geographic location were risk factors for infection (Table 2).

Discussion

Akabane disease is widespread throughout the world. Fourteen outbreak of Akabane disease was occurred in Asia from 2002 to 2006 (23). Outbreak of Akabane disease was first occurred in Iran in 1992 (24). Since that time

so far, there isn't any report of the occurrence of this disease from Iran.

The prevalence rates of Akabane virus infection in cattle and sheep have been reported in surveys conducted in many countries or areas and vary among different geographical areas (3, 9, 22). Recently Oluwayelu et al. (2016) reported 70.1% of examined cattle in Nigeria were seropositive to Akabane virus. Seroprevalence survey of Akabane virus infection in two sentinel herds of calves in Eastern and central regions of Saudi Arabia showed that in Al-Ahsa oasis (Eastern region and closer to Iran) the rate of Akaban virus infection was 70%, while in other herd none of the examined cattle had antibodies to this virus (25). Other reports were 20.32% in China, 87% in Israel, 0.14% in Turkey, 29.4% in Sudan and 7% in Indonesia (1, 19, 26-28). There are different between these reports. Because this disease is vector borne and its transmission related to some factors and exactly detecting risk factors are difficult. As the result of the present study, the prevalence rates were highly associated with locality, breed, sex and age of animals but were not associated with history of recently abortion and type of management.

The present study showed that the odds of infection was increased with increase of age. This result confirmed reports of Oluwayelu et al. (2016) and Elhassan et al. (2014), who reported a significant difference but didn't confirm recently records of Ahi et al. (2015). Whereas infection in adult cattle is common in endemic areas, reports of clinical disease are rare but neurological disease associated with infection in cattle 2 to 7 years of age has been observed (29).

The present study revealed that prevalence rates were associated with sex. This result confirmed reports of Elhassan et al. in 2014 but didn't confirm recently records of Ahi et al. (2015) and Oluwayelu et al. (2016). There is no sex predilection for this virus and affects both sex.

The prevalence rates were also significantly lower in native cows than in other breeds. Generally, exotic breeds are more susceptible than indigenous breeds. Elhassan et al. an Ahi et al. showed that Akabane virus antibodies prevalence was highly associated with breeds (1, 22).

The prevalence rate was significantly lower in Shadegan and Dezful in comparison with other cities. It means that Akabane virus infection is associated with locality that agreement with the other reports (1, 16, 17, 22, 25). The difference between geographical area is related to biological pattern of this virus. Akaban virus is transmitted by mosquitos and these vectors are not active in all of the geographical situation. Akabane virus has been reported in a number of countries on the African continent, the Middle East, Southeast Asia and Australia. It is considered likely that Akabane virus or other Simbu viruses are also present in neighboring countries in these regions. Its presence is suspected in many other countries in the tropical and temperate zones, especially those where bluetongue is reported. Within a country, the distribution of the virus is absolutely restricted to that of the insect vector (12). In countries with a temperate climate, there is also a distinct seasonal pattern of virus transmission, coinciding with warm, moist summer and autumn months. This seasonal pattern is also a consequence of the abundance of the insect vector. There is a critical population density required before virus spread can occur. Vector numbers begin to increase in the late spring and early summer, usually peaking in early autumn. Even in tropical and subtropical regions, there is a tendency towards seasonal transmission, with the highest infection rates in the summer months. In temperate regions, transmission ceases with the onset of very low temperatures and the first frosts, while in tropical regions transmission rates decline with the onset of the periods of lower rainfall (6).

The prevalence rates were not significantly different between cows which had history of recently abortion and in cows which had delivered normally. This result didn't confirmed records of Jun et al., Ahi et al. and Elhassan et al. that discussed the associations between this virus and abortion experience. In the study of Liao et al. (1996), the virus was isolated not only from those affected calves but

also from those calves without clinical symptom in Taiwan. These might indicate that there were differences in the virulence of isolates. The presence of antibody against low pathogenic Akabane virus had been noted in cattle in Taiwan. They believed that the presence of viruses and vectors probably induced a minimal immune response or protection to the cattle from ensuing disease.

The present study clearly indicated the widespread prevalence of Akabane virus infection in cattle in Khouzestan province of Iran. Since a vaccination program for Akabane is not established in Iran, this seropositive result indicates the presence of Akabane virus infection in this area. 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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