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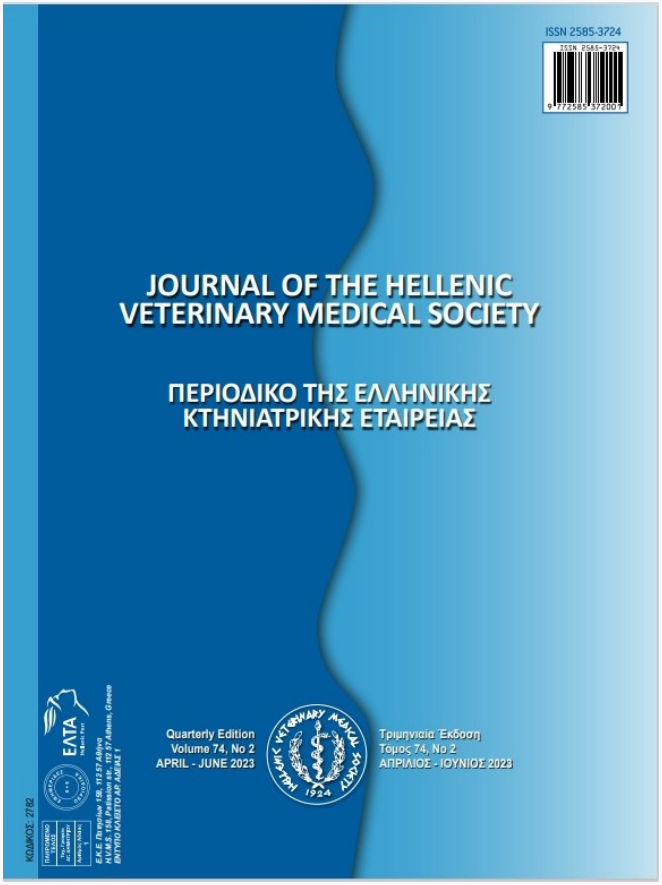

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Ερευνητικό άρθρο

Detection of Schmallenberg virus antibody in equine population of Kurdistan province, west of Iran

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ABSTRACT: Schmallenberg virus (SBV) is a newly emerging virus from the Simbu serogroup. It is an arbovirus (virus transmitted by arthropods) that causes various complications in the fetus after infecting pregnant animals. The clinical signs of infection to this virus are fever, loss of appetite, reduced milk yield and in some cases, diarrhea and in pregnant animals, congenital malformations in calves, lambs, and kid goats. The aim of the current study was to detect the Schmallenberg virus antibody in an equine population of Kurdistan province, in the western part of Iran. In this cross sectional study, blood samples from 184 horse from different rural areas of Kurdistan province were analyzed using an indirect ELISA test. Risk factors of each sample such as sex, age, breed, history of abortion and the geographic location were taken into consideration. From a total of 184 serum samples that analyzed for the presence of antibody against SBV, 14.68% (n=27) of total samples were positive for SBV antibody, and 2.17% (n=4) were doubtful, and 83.15% (n=153) were negative. Except for the geographical area, there was no statistically significant difference between risk factors ($p>0.05$). The positive results of the samples tested in this study indicate the high activity of Culicoides biting midges, their living conditions that allow midget infestation. The findings might also be understood in terms of illegal entry of infected livestock from neighbouring regions.

Keywords: Schmallenberg virus; Horse; ELISA; Iran

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INTRODUCTION

Schmallenberg virus (SBV) is a newly emerging RNA virus belonging to Simbu serogroup of Orthobunyavirus genus from the Bunyaviridae family. Similarly to disease caused by other Simbu serogroup viruses, SBV can induce congenital malformations, stillbirth, severe arthrogryposis, torticollis, brachygnathia, hydrocephaly, ataxia, paralysis, muscle atrophy, joint malformations, scoliosis, behavioral abnormalities, and blindness in fetuses and neonates, especially in ruminants. These malformations have been designated as arthrogryposis-hydranencephaly syndrome (Doceul et al., 2013; Haffmann et al., 2012; Rossi, 2012). So far, the virus has been found in a wide range of ruminant and non-ruminant hosts. Antibodies against Schmallenberg virus have been detected in cattle, sheep, goats, deer, buffaloes and camels. In addition, antibodies have been reported in dogs and some wildlife animals such as zebras, boars and elephants (Collins et al., 2019). Similar to other Simbu serogroup viruses, researchers have shown that SBV is suspected to be transmitted through Culicoides midges (Rasmussen et al., 2012; Regge et al., 2012; Veronesi et al., 2013). Diseases that have a reservoir in the wild or have the potential to infect wildlife are a major global health problem (Bakhshi et al., 2013). The mortality rate in herds infected with this virus varies from 20%-50% (Bayry., 2017). There is no evidence of human infection and zoonotic disease (Ducomble et al., 2012). The emergence of Schmallenberg virus in Germany and its spread throughout Europe and some other countries has had devastating economic effects on the livestock industry, and it showcases the importance of this disease. In Iran, and neighboring countries, studies have been conducted on the prevalence or presence of the virus in various livestock populations (Asadolahizoj et al., 2020). However, current data are not sufficient to determine the overall picture of Schmallenberg disease and further studies are needed in different regions and wider range of hosts. Antibody detection (serological method) in body fluids and milk may be used as an alternative (instead of molecular methods) because sometimes the viral genome is not detectable in all lambs or calves suspected of being infected (De Regge et al., 2013). The aim of the current study was to detect of Schmallenberg virus antibody in an equine population of Kurdistan province, west of Iran.

MATERIALS AND METHODS

Sample collection

This cross-sectional study, was performed from March to November 2020 in different farms of the rural area of Kurdistan province (including Sanandaj, Baneh, Kamyaran, Marivan and Saghez cities) located in west of Iran where there is a high equine population. Blood samples were collected from the jugular vein of 184 healthy horses and were centrifuged in plain heparin? tubes at 10,000 g for 10 min. Sera were separated and kept in −20°C for serological studies. Risk factors such as sex, age, breed, history of abortion and the geographic location were taken into consideration.

ELISA test to detect Schmallenberg virus antibody

Serum samples were analyzed for detection of SBV antibody using ID vet® SBV indirect multispecies ELISA test kit (ID Screen SBV multispecies ELISA kit; ID vet, France). An ELISA reader was used for determining optic density values. According to the manufacturer's instructions, the S/P percentage was calculated for each sample. Statistical analysis was conducted using IBM SPSS Statistics 25 software ($SPSS_{25}$ Chicago company, USA) and frequency comparisons were performed by Chi-Square and Fisher's exact tests. If there was a significant difference, the difference between the groups was determined by a Benferroni supplementary test.

RESULTS

A sample was considered positive if the calculated percentage was >60% (ID vet) and if ≤50% the samples were considered negative while >50% or ≤60% the samples were considered doubtful. According to this, from a total of 184 serum samples that were analyzed for the presence of antibody against SBV, 14.68% (n=27) of total samples were positive for SBV antibody, and 2.17% (n=4) were doubtful, and 83.15% (n=153) were negative (Table1). Due to lack of SBV infection reporting in Iran and as an expression of caution, the doubtful results of the data analysis were considered as negative. In this study, risk factors such as age, sex, breed, history of abortion and geographic location were examined, and except for the geographical area, no statistically significant difference was observed (p>0.05).

Table1. Risk factors for SBV in 184 horses.

Risk factors	N (%)		Total		P value
	Positive (n= 27)	Negative (n= 157)	Positive (%)	Negative (%)	
Sex					
Female	10 (12)	73 (88)	5.45	39.67	0.362
Male	17 (16.8)	84 (83.2)	9.23	45.65	
Breed					
Kurd	9 (9.5)	86 (90.5)	4.9	46.73	
Arab	2 (10.5)	17 (89.5)	1.1	9.23	0.052
Workhorse	16 (22.9)	54 (77.1)	8.7	29.34	
Age					
≤4 years	14 (19.4)	58 (80.6)	7.6	31.52	
5-8 years	10 (13.2)	66 (86.8)	5.43	35.86	
9-12 years	3 (10.7)	25 (89.3)	1.65	13.6	0.492
>12 years	0 (0)	8 (100)	0	4.34	
Area					
Sanandaj	4 (8)[a]	46 (92)	2.17	25	
Baneh	8 (12.7)[a]	55 (87.3)	4.35	29.9	
Kamyaran	7 (46.7)[b]	8 (53.3)	3.8	4.35	0.018
Marivan	4 (15.4)[ab]	22 (84.6)	2.17	11.95	
Saghez	4 (13.3)[ab]	26 (86.73)	2.17	14.14	
History of abortion					
With a history	1 (33.3)	2 (66.7)	0.55	1.1	0.381
No history	26 (14.4)	155 (85.6)	14.14	84.21	

SBV=Schmallenberg virus, a-b: within a column, values not sharing the same superscript letter were considered as significantly different ($p<0.05$).

DISCUSSION

SBV infection is a newly emerging infectious disease that may cause huge economic losses to livestock ruminants. Infection with SBV is studied in different species and is detected in cattle, sheep, goat, alpaca, red deer, and water Anatolian buffaloes (Azkur et al., 2013; Jack et al., 2012). In cattle, the acute onset of the disease is a typical form, characterized by obvious clinical signs of fever, diarrhea, and milk yield reduction that subsides with the animal recovering in 2-3 weeks. SBV infection among sheep and goats is almost without symptoms, and transmission of SBV to fetuses in pregnant animals is through placenta causing abortions, stillbirths and a variety of congenital malformations mainly involving the skeletal and nervous systems (Gibbens, 2012). In the current study, the ELISA test was used using ID vet® SBV indirect multispecies ELISA test kit for the detection of SBV antibody and 14.68% (n=27) of total samples was positive for SBV antibody. Based on manufacturer instruction, the kit wells are coated with the recombinant nucleocapsid protein of SBV, thus cross-reaction with other related Simbu serogroups such as Shuni (SHUV), Aino (AINV), and Akabane (AKAV) virus might be justified. Based on recent studies it was indicated that antibodies to AKAV and AINV will not provide protective immunity to SBV (Hechinger et al., 2013); hence, the cross-reaction of suspected viruses and resultant provoked antibodies is less probable. Furthermore, the related viruses have not been reported in the equine population of Iran to present date. This research was the first seroepidemiological study of SBV in the west of Iran. In a study in northern and northeast of Iran, it was reported that SBV antibody in equine population was 5% (Rasekh et al., 2018). In another systematic review on the spread of Schmallenberg virus in Iran and neighboring countries, it was reported that Turkey and Pakistan showed the highest percentages of positive serum samples in cattle (39.82%) and camel (86%) hosts, respectively (Asadolahizoj et al., 2020). In a research in Iraq, it was revealed that seroprevalence of SBV in sheep, in Duhok province, was 16.14% (AL-Barwary, 2018). In another study in Iraq, it was showed that seroprevelance of Schmallenberg virus infection as emerging disease in cattle was 21% (Al-Baroodi, 2021). The SBV is one of the arboviruses group, also known as the arthropod-transmitted diseases. The most important of these arthropods are certain species of Culicoides biting midge. Multiple potential factors such as climate change,

anthropogenic, and social factors and/ or the movement of vectors or their hosts which are infected by the virus will favor the emergence of these diseases (Johnson et al., 2014; Wernike et al., 2013). The disease was first described in the border region of Germany and the Netherlands, between August and October 2011 and extended to neighboring countries (Gibbens, 2012). On the other hand, another study which has been performed in Turkey between 2006 and 2013 indicated that the virus was present before its detection in 2011. As Culicoides midges can widely spread infection across national borders (Veronesi et al., 2013; De Regge et al., 2013), it is postulated that the diseases associated with arboviruses pre-existed in neighboring countries such as Iran. Presence of diseases associated with arboviruses such as African horse sickness and Bluetongue proves the high population and activity of the vector (Culicoides) in Iran (Rasekh et al., 2018). Although the true role of climate changes in causing the recent global expansion of the range and distribution of arboviruses remains conjectural (Elbers et al., 2013). Due to the proximity and livestock exchanges between Iran and Iraq and Turkey in the west and northwest of Iran, as well as between Iran and Pakistan in the southeast, precautionary measures are recommended by veterinary officials.

CONCLUSION

Despite the reasons mentioned above and detection of antibodies against the SBV, the authors of this article believe that the claim that the disease exists in Iran needs to be treated cautiously; however, a 14.68% serologic outbreak can significantly increase the risk of new emerging diseases in various animal populations. The necessity of a repetition of ELISA test to express the definite existence of the disease is inevitable and performing the molecular and more accurate serological tests such as PCR and Virus Neutralization test, respectively, should not be underestimated.

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CONFLICT OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/ or publication of this article.

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