SHORT COMMUNICATION

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First detection of Schmallenberg virus antibody in cattle population of eastern Iran

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Abstract

Schmallenberg virus (SBV) is an emerging single-stranded RNA virus being classified under *Simbu* serogroup of *Bunyaviridae* family. This study aimed to detect antibodies against SBV in cattle for the first time in three eastern provinces of Iran. Blood samples were randomly collected from jugular veins of 273 cattle, from 19 farms in Razavi Khorasan, South Khorasan and Sistan and Baluchestan provinces. Separated sera were analyzed to find SBV antibody using ID vet® SBV indirect multi-species enzyme-linked immunosorbent assay test kit. From a total of 273 serum samples analyzed for SBV presence, 12.45% (n = 34) were positive for SBV antibody. Risk factors including breed, age and geographic area showed a statistically significant relationship with the virus prevalence. In conclusion, the seroprevalence of SBV is not high; but it is considerable in the studied parts of Iran. This is the first study regarding SBV seroprevalence in cattle population of eastern Iran and further studies about the virus epidemiology are recommended.

Introduction

Schmallenberg virus (SBV) is an emerging single-stranded RNA virus and based on its segmental size and genome characteristics, it is classified under *Simbu* serogroup of *Orthobunyavirus* genus from the *Bunyaviridae* family.[1,2] This virus was first detected in the cattle in 2011 at the eastern regions of The Netherlands and some farms of Germany and now it is spread to most European countries, including Italy, Sweden and France.[3] Later animals blood analyses in Germany led to recognition of this new virus and showed that it does not only affect cattle but also affects sheep and goats. However, it has been shown that SBV has mild effects on adult animals.[4] Clinical signs of infection include fever, decrease in milk yield, diarrhea and stillbirth in cows; while, congenital malformations such as scoliosis, hydrocephalus, arthrogryposis, cerebellar hypoplasia and an enlarged thymus are the most common symptoms in newborns.[5] Like other *Orthobunyavirus*, the usual way of transmission is by arthropod vectors and hematophagous midges (*Culicoides* spp.).[1,6]

The gold standard method for the diagnosis of SBV is reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The PCR can detect the genetic materials of SBV, even if the virus is present in extremely small amounts. However, RT-PCR has many limitations such as false negative results, changes in diagnostic accuracy during the disease period and expensive test materials. Serological tests can be considered as alternatives for RT-PCR because they might be cheaper and easier to setup.[7] Considering the fact that there is a lack of specific knowledge regarding SBV epidemiology in Iran, this study aimed to detect antibodies against SBV in cattle for the first time in three eastern provinces of Iran.

Materials and Methods

In winter and spring of 2019, blood samples were collected from cattle population of three eastern provinces of Iran including Razavi Khorasan, South Khorasan and Sistan and Baluchestan. Two hundred and seventy three cattle from 19 farms were tested. Blood samples were

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collected randomly from jugular vein. Tubes were then centrifuged at 10,000 rpm for 10 min, and separated serum was stored at – 20.00 ˚C until further use. Samples were analyzed for SBV antibody detection using ID vet® SBV indirect multi-species enzyme-linked immunosorbent assay (ELISA) test kit (ID Screen SBV multispecies ELISA kit; ID vet®, Montpellier, France). Reagents were allowed to come to the room temperature (21.00 ± 5.00 ˚C) before use. All reagents were then homogenized by vortex. Ninety microliter of dilution buffer 14 (IDvet) was added to each micro-well. Then 10.00 µL of positive and negative controls were added to A1 and B1 micro-wells, respectively. Finally, other micro-wells were filled with 10.00 µL of samples and the micro-plate was shaken manually for reaching a complete mixture. Incubation was performed for 45 min at 21.00 ± 5.00 ˚C. Micro-wells were washed three times with 300 µL of washing solution. Drying between each washing step should be avoided. One hundred microliter of 1x conjugate was added to each well and incubation was performed for 30 min at 21.00 ± 5.00 ˚C. Micro-wells were washed three times with 300 µL of washing solution again. One hundred microliter of substrate was added to each micro-well and incubation was done for 15 min at 21.00 ± 5.00 ˚C. Finally, 100 µL of stop solution was added to each well. The ELISA reader (Anthos, Salzburg, Austria) was used to determine optic density values (450 nm). According to the manufacturer's instructions, the cut off values were considered S/P ≤ 40.00% for positive, 40.00% < S/P ≤ 50.00% for doubtful and S/P > 50.00% for negative samples. Risk factors such as sex, age, breed, origin and the area of sampling were also considered. Statistical analysis was conducted using SPSS Software (version 18.0; IBM Corp., Armonk, USA) and chi-square test was used to evaluate the associations between suggested risk factors and SBV seropositivity.

Results

According to ID vet kit protocol, samples were categorized in three groups including positive (calculated percentage > 60.00%), negative (calculated percentage ≤ 50.00%) and doubtful (calculated percentage > 50.00% and inferior or equal to 60.00%). From a total of 273 serum samples analyzed for detection of antibody against SBV, 12.45% (n = 34) of total samples were positive for SBV antibody and 87.55% (n = 239) were negative (Table 1). There was no sample with doubtful results. There was a significant statistical relationship between age, breed and area risk factors ($p < 0.05$); while, there was no relationship between sex and origin ($p > 0.05$).

Discussion

Schmallenberg virus was first detected in central Europe at 2011, spread rapidly throughout the majority of European countries and now is a worldwide issue. No study has been conducted regarding detection of the antibody against the virus in ruminants of Iran. The only study about virus antibody detection was carried out among Iranian equine population, and it has been reported that 5.00% of samples have antibodies against SBV.[1]

According to the present study, the seroprevalence of disease in cows is higher than horses (result of the previous study in equine population). Higher prevalence of the virus in cow population in comparison with other examined species has been reported in other articles. A research done in Turkey suggested that cattle have the highest seroprevalence (39.80%) among other species such as goat, sheep and buffalo.[8] Another study performed in China during 2012 - 2015 showed a high seroprevalence (30.00 ~ 100%) of SBV in cattle; while, there was a low prevalence (8.30 - 19.00%) in buffalo and goat.[2] These studies may support the idea that cows are

Table 1. Risk factors of Schmallenberg virus infection in 273 cattle.

Risk factors		Positive (n = 34)	Negative (n = 239)	Total (n = 273)		*p*-value
		No. (%)	No. (%)	Positive (%)	Negative (%)	
Sex	Male	6 (6.98)	80 (93.02)	2.19	29.30	0.063
	Female	28 (14.97)	159 (85.03)	5.48	52.24	
Age	< 2 years	3 (3.12)	93 (96.88)	1.09	34.06	0.02
	2 - 6 years	23 (18.70)	100 (81.30)	8.42	36.63	
	> 6 years	8 (14.81)	46 (85.19)	2.93	16.84	
Breed	Holstein-Friesian	24 (22.68)	75 (77.32)	8.79	27.47	0.00
	Sistani	8 (4.97)	153 (95.03)	2.93	56.04	
	Other breeds	2 (13.33)	13 (86.67)	0.73	4.76	
Area	Sistan and Baluchestan	13 (7.30)	165 (92.70)	4.76	60.43	0.03
	South Khorasan	7 (15.22)	39 (84.78)	2.56	14.28	
	Razavi Khorasan	12 (24.49)	37 (75.51)	4.39	13.55	
Type	Imported	12 (11.43)	93 (88.57)	4.39	34.06	0.068
	Non-imported	22 (13.10)	146 (86.90)	8.05	53.47	

the most susceptible host for the virus; but, more studies must be done and current data aren't enough to make a definite conclusion. Based on the results of the present study, the seroprevalence of SBV in Iranian cow population is relatively lower than the prevalence of disease in other countries, which may be due to differences in sample size, the seasons when sampling was performed, regional climate and prevalence and activity of arthropods vectors.[6-11]

The seroprevalence was shown to be affected by the age, breed and area being consistent with the results of former studies conducted in Ethiopia and Poland.[12,13] As the age increases, the rate of infection grows which can be due to the greater exposure of the aged animals to the virus during their lifetime. On the other hand, in a study conducted in The Netherlands, the seroprevalence was shown not to be affected by the age;[9] therefore, more information is needed about the relation between age and the rate of infection. Elbers *et al.* have shown that there is a significant relationship between geographical area and the presence of virus and seroprevalence is significantly lower in the northern and southern regions in comparison with central-eastern part of The Netherlands, which is consistent with the results of the present study indicating that there is a relationship between geographical area and seroprevalence.[9] Different seroprevalence patterns in different geographical regions may be due to differences in climate, humidity and vectors prevalence. Kęsik-Maliszewska *et al.* have reported that gender affects seroprevalence, which is in line with our study and expresses that the sensitivity of both males and females are equal to this virus.[13] The present study indicated that there is a significant association between cow breed and infection with the SBV. Based on the results of this study, it seems that the Holstein cattle are more susceptible than the native Sistani cattle (22.68% *versus* 4.97%). On the other hand, Sibhat *et al.* have shown that local breeds have a higher prevalence than Holstein and Jersey breeds in Ethiopia.[12] These results showed that not all local breeds are equally resistant to the virus. In this study, ID vet® SBV indirect multi-species ELISA test kit was used for the detection of SBV antibody. According to the kit information, the wells are covered with the recombinant nucleocapsid protein of SBV; so, cross-reactions with other related *Simbu* serogroup such as *Aino* (AINV) and *Akabane* (AKAV) viruses should be considered. *Akabane* virus has been detected in the livestock of Khuzestan and Semnan provinces of Iran; however, according to recent studies, AKAV and AINV antibodies will not interfere with test results, as they do not cause protective immunity to SBV.[14-16] Furthermore, other *Simbu* serogroups (except AKAV being only reported in two studies) have not been reported in the ruminant population of Iran until the present date. On the other hand, false negative probability of ELISA test due to the technical laboratory error, recent infection and hypogammaglobulinemia should be taken into consideration. Western blotting, viral neutralization test, PCR and indirect imunofluorecent reduce the chance of false positive and negative results.[17] The disease has already been reported in the livestock population of neighboring countries, including Turkey and Azerbaijan.[8,18] Thus, based on the potential ability of the virus to transmit via arthropod vectors and wild hosts crossing the border, the possibility of virus originating from mentioned countries should not be underestimated. The transmission of SBV, like other members of *Bunyaviridae*, occurs by midges of the genus *Culicoides.*[19] However, according to the previous epidemics of the virus, the dissemination and transmission rates of SBV are higher than other members of *Bunyaviridae*, proposing that there may be other means of transmission than *Culicoides spp* needing additional studies focusing on potential transmission ways. This is the first study of SBV seroprevalence in the cattle population of eastern Iran. As a newly emerging disease, the prevalence of disease (12.45%) in the cattle population of eastern Iran is considerable. However, more concerns are associated with the western areas of country, where the neighbor countries like Turkey with relatively high prevalence of the disease are in close geographical contact. So far, no studies have been conducted regarding the epidemiology of SBV in Iran's eastern neighbors (Afghanistan and Pakistan). Due to the spread of the disease through arthropod vectors and livestock trafficking, the presence of virus in these countries seems possible. The seroprevalence was shown to be affected by the age, area and breed. Major histocompatibility complex (MHC) encodes the molecules that bind processed peptide antigens and presents them to T-cells, activating a specific immune response. The MHC genes are important because they are associated with genetic resistance and susceptibility to a wide range of diseases such as mastitis, bovine leukemia virus-induced lymphoma, dermatophilosis and avian influenza. As a result, the association of this gene cluster of different breeds with Schmallenberg disease should not be underestimated and further studies regarding how MHC polymorphism contributes to this disease resistance and susceptibility are a necessity.[20,21] Also, additional studies focusing on other risk factors such as seasons, climate and vectors are needed to elucidate SBV profile. Exposure to SBV may become widespread in Iran due to the infection of various species of livestock (horses and cattle so far) and the presence of virus in the neighboring countries. Current data in Iran are not enough to define a complete profile for SBV infections, and additional studies are necessary for different regions and different animal hosts. In addition, the need for more accurate tests such as PCR and viral neutralization tests should not be neglected.

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Conflict of interest

The authors declare no potential conflicts of interest.

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