

Seroprevalence of *Schmallenberg virus* infection in sheep in Kars province

Research Article

ABSTRACT

Schmallenberg virus (SBV) infection is an infectious disease transmitted by species vectors as *Culicoides sp.* and characterised by fever, anorexia, decreased milk yield, loss of condition, abortion and birth of offspring with arthrogryposis hydranencephaly syndrome. The aim of this study was to determine the prevalence and presence of SBV infection in sheep in private farms in Kars province. For this purpose, blood serum samples were taken from 376 (301 females + 75 males) healthy-looking Akkaraman sheep raised in small-scale family-type farms in five central villages of Kars province (Kümbetli, Çakmak, Dikme, Subatan, Cumhuriyet) and evaluated for SBV-specific antibodies with a commercial ELISA kit. The animals included in the study were 1-4 years old. Of the sampled animals, 1.1% (4/376) were positive and 1.1% (4/376) were suspected. Seropositivity rates were determined between 0% and 2.53% according to the settlements. 1.33% (4/301) of ewes were antibody positive and all rams were antibody negative (0/75). Of the four animals with suspected antibodies, two were sheep (2/301, 0.66%) and two were rams (2/75, 2.66%). It was shown that there was no statistically significant variation in seropositivity rates amongst age groups, genders, and villages ($P>0.05$). This study examined the seroprevalence of SBV in sheep grown in the province of Kars. The presence of the infection was serologically demonstrated for the first time, and it was determined that the seroprevalence rate of SBV infection was low.

Keywords: ELISA, Kars, *Schmallenberg virus*, seroprevalence, sheep, Türkiye

INTRODUCTION

Schmallenberg virus (SBV) is a member of the *Peribunyaviridae* family and belongs to the genus *Orthobunyavirus*. (ICTV, 2018) and there are three serogroups in this genus including animal and human viruses. As a result of genetic analysis, SBV was found to be similar to *Aino*, *Akabane* and *Shamonda viruses* and was classified in the Simbu serogroup (Garigliany et al., 2012b; Goller et al., 2012; Hoffmann et al., 2012). SBV, as other Simbu serogroup viruses, spreads mainly by biting *Culicoides* midges. Additionally, SBV is transmitted vertically as well. (De Regge et al., 2014; Garigliany et al., 2012b; Lehmann et al., 2012). There is no data showing that there is transmission from animal to animal by direct contact (Pawaiya & Gupta, 2013; Wernike et al., 2014). SBV was found in cattle, sheep, goats and bison. In addition to these animals, the presence of antibodies has been demonstrated in roe deer and fallow deer and natural infection was thought to have occurred. Dogs and wild boars have also been found to have SBV antibodies. (Brülisauer et al., 2017; Kauffold et al., 2021; Keşik-Maliszewska et al., 2018; Sailleau et al., 2013; Wensman et al., 2013). The disease in cattle progresses with fever, loss of appetite, decrease in milk yield, loss of

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condition, miscarriage and calf birth with arthrogryposis hydranencephaly syndrome, and congenital malformations characterized by stillbirth in sheep and goats. (Bilk et al., 2012; Hoffmann et al., 2012; Muskens et al., 2012; Van den Brom et al., 2012).

SBV has been reported on the European continent so far. Cases that started first in Germany and the Netherlands were later seen in establishments in Belgium, England, France, Italy, Spain and Luxembourg (Conraths et al., 2013). As a result, SBV seriously impairs domestic ruminant productivity. SBV is also observed in several locations of Türkiye, and seroprevalence tests in goats, sheep, cattle, and Anatolian buffaloes have shown low levels of SBV (Azkur et al., 2013; Elmas et al., 2018; Macun et al., 2017; Tonbak et al., 2016; Varol, 2022). The seroprevalence of SBV has not been investigated in any ruminant species in Kars region of Türkiye until now. Reverse transcription-polymerase chain reaction (RT-PCR), virus neutralization test (VNT), immunofluorescence assay test (IFAT), and enzyme-linked immunosorbent assay (ELISA) are frequently utilized for SBV diagnosis (Garigliany et al., 2012a; Garigliany et al., 2012b; Garigliany et al., 2012c; Kessell et al., 2011; Tarlinton et al., 2012). The SBV nucleocapsid protein formed the basis for the development of the currently available ELISA kit. This test is known to be rapid and simple to run, but it can also identify antibodies to other orthobunyaviruses (Breard et al., 2013). VNT is regarded as the SBV gold standard test (Van der Poel et al., 2014).

In this study, SBV antibodies were detected, and Indirect ELISA (I-ELISA) was used to measure SBV seroprevalence in sheep for the first time in the Kars region of Türkiye. The seroprevalence rate of SBV infection in sheep in the Kars province is low, according to these seroepidemiological data.

MATERIALS AND METHODS

Serum Samples

Blood samples were randomly gathered from 376 Akkaraman sheep (301 sheep + 75 rams) without clinical signs from small-scale family-type farms in five villages of Kars province (Kümbetli, Çakmak, Dikme, Subatan, Cumhuriyet). The samples were collected randomly from small-scale family type farms (less than twenty sheep), not on a herd basis. There is no information on the case history of abortions or births with congenital malformations in the sampled animals. The ages of the animals range from 1 to 4 years old (age of 1, n= 52; age of 2, n= 129; age of 3, n= 152; age of 4, n=43), and the samples were collected between November 2022 and March 2023 (Table 1, Figure 1). Blood samples were taken from the animals' jugular veins. Blood samples taken into silicone tubes were centrifuged at 2000 rpm for 10 minutes and serums were separated. Then serum samples taken into stock tubes were preserved at -20°C until tested.

Table 1. Distribution of the number of samples in the study according to gender and location.

Location	Number of animals sampled (n)		
	Sheep	Ram	Total
Kümbetli	48	9	57
Çakmak	68	11	79
Dikme	50	10	60
Subatan	65	20	85
Cumhuriyet	70	25	95
Total	301	75	376



Figure 1. Geographical positioning of the Kars province in which the study was performed.

Indirect Enzyme Linked Immunosorbent Assay (I-ELISA)

The commercial ELISA method (IDEXX Schmallenberg Ab Test®, IDEXX, Switzerland Cat No: 99-412-59) applied to identify antibodies against SBV was carried out in accordance with the manufacturer's instructions. For each tested sample and the positive serum, the percent inhibition was calculated (%inhib) by means of the following formula:

$$\% \text{ S/P} = [(\text{OD}_{\text{sample}} - \text{OD}_{\text{neg}}) \div (\text{OD}_{\text{pos}} - \text{OD}_{\text{neg}})] \times 100$$

S/P percentages were evaluated as follows; < 30 % negative, ≥30 % and <40 % suspect, ≥40 % positive

Statistical Analysis

The Statistical Package for Social Sciences software (IBM SPSS 21 Software, USA) was

applied to do statistical analysis. The statistical significance of the differences between SBV seropositivity rates determined according to sampling regions, sex (rams and ewes) and age groups was evaluated using the chi-square (chi-square χ^2) test. A significant difference was defined as a p-value less than 0.05 (P<0.05).

RESULTS

According to the results, four (1.1%, 4/376) blood samples from the study's animals contained antibodies specific to SBV. In 4 animals (1.1%, 4/376), the test result was suspectable for antibody positivity. Two of the four positive animals were Çakmak (2.53%); while two of them were detected in Cumhuriyet villages (2.10%); One of the four suspicious animals was found in Kümbetli (1.75%), one in Subatan (1.17%) and two in Çakmak (2.53%). No positivity was detected in Dikme village (Table 2).

Table 2. SBV seroprevalence rates according to sampling locations.

Sampled Locations	Number of samples (n)	Number of positive samples (%)	Number of suspicious samples (%)	X ²	P-value
Kümbetli	57	0 (0)	1 (1.75%)	8.338	0.401
Çakmak	79	2 (2.53%)	2 (2.53%)		
Dikme	60	0 (0)	0 (0)		
Subatan	85	0 (0)	1 (1.17%)		
Cumhuriyet	95	2 (2.10%)	0 (0)		
Total	376	4 (1.1%)	4 (1.1%)		

In this study, 1.33% (4/301) of the ewes were antibody positive and all of the rams were antibody negative (0/75). Of the four animals

with suspicious antibodies, two were ewes (2/301, 0.66%) and two were rams (2/75, 2.66%) (Table 3).

Table 3. SBV seroprevalence rates by gender.

Gender	n	Positive (%)	Suspicious (%)	Negative (%)	X ²	p-value
Ram	75	0 (0)	2 (2.66%)	73 (97.33)	0.140	0.588
Sheep	301	4 (1.33%)	2 (0.66%)	295 (98.0)		
Total	376	4 (1.1%)	4 (1.1%)	368 (97.8)		

Two of the animals determined as positive for SBV specific antibodies were in the age group of 2 and two of them were in the age group of 3, while two of the animals determined as suspicious were in the age group of 2 and two of them were in the age group of 3 (Table 4). In this study, the data on SBV positivity collected from

villages in Kars province were statistically analysed. It was found that the differences in SBV positivity rates among different age groups (p>0.05) (age groups $\chi^2= 0.544$, p=0.762), females and males (p>0.05) (Male/female $\chi^2= 0.140$, p=0.588), and villages (p>0.05) ($\chi^2= 8.338$, p= 0.401) were statistically insignificant.

Table 4. SBV seroprevalence rates according to age groups.

Age (years)	n	Positive (%)	Suspicious (%)	Negative (%)	X ²	p-value
1	52	0 (0)	0 (0)	52 (100)	0.544	0.762
2	129	2 (1.55%)	2 (1.55%)	125 (96.89%)		
3	152	2 (1.31%)	2 (1.31%)	148 (97.89%)		
4	43	0 (0)	0 (0)	43 (100)		
Total	376	4 (1.1%)	4 (1.1%)	368 (97.8%)		

DISCUSSION

SBV, one of the arboviruses carried by *Culicoides* biting midges, causes serious economic losses in sheep, goats and cattle due to factors such as death, abortion and abnormal offspring births, as well as animal movements, restriction of the use of embryos, semen and other animal products in regions where this infection is endemic (Lievaart-Peterson et al., 2015).

There are few studies on SBV in sheep raised in Türkiye. In one of these studies, ELISA test was performed on 307 serum samples collected between 2006-2012 and the seroprevalence of the disease in sheep was found to be 1.6% (5/307) (Azkur et al., 2013). In another study, Kızıltepe et al. (2023) detected 4.4% SBV positivity in blood samples taken from 180 sheep in the province of Iğdır. In a study conducted in Kırıkkale province, a total of 1038 sheep were sampled and 0.38% (4/1038) of the sampled animals were found positive for SBV-specific antibodies (Macun et al., 2017). In a study in Sivas province, a total of 368 animals (250 sheep, 118 rams) belonging to the Kangal Akkaraman breed were sampled and seropositivity was detected in only 1 sheep (0.27%) (Elmas et al., 2018). In a seroprevalence study performed in Hatay, the seropositivity rate in sheep was found to be 23.60% (Doğan, 2018). However, in this investigation, only 1.1% seropositivity was detected in blood samples collected from 376 Akkaraman sheep aged between one and four years, which were raised in small-scale production units located in the villages of Kars province. The rate observed in

our study is higher than the rates reported in previous studies (Elmas et al., 2018; Macun et al., 2017) and lower than the rates reported by Azkur et al., (2013), Kızıltepe et al., (2023) and Dogan et al., (2022).

Seropositivity rates were especially high in animals in the 2 and 3 age groups. According to this result, it is thought that there may be an age-related difference in susceptibility to infection in animals. In their study, Wernike et al., (2018) reported that the presence of seronegative young animals in the herd may decrease herd immunity, potentially leading to higher yield losses as a result of increased virus circulation and susceptibility to infection.

In our study, statistical comparison of antibody positivity and negativity according to the sex of the animals did not reveal any significance ($P>0.05$). Based on these results, it can be concluded that sex does not play a significant role in the distribution and susceptibility to infection, as indicated by statistical comparisons made according to the sex of the animals.

According to the study's findings, which indicate the presence of SBV infection, the infection holds significance for the Northeastern Anatolia Region, and preventive measures should be taken. In this study, a low seropositivity rate was detected in sheep raised in private family farms in Kars province. It is thought that this may be due to the sampling time when the vector population was low. Additionally, the low number of animals sampled may have also contributed to this. Kars

province has a harsh continental climate, so the population of *Culicoides* vector insects is low in this region, which may have led to a low SBV seroprevalence rate. Considering the current climate of the Kars region, the high prevalence of SBV in studies conducted in Hatay (Dogan et al., 2022) and Samsun (Azkur et al., 2013) provinces, which have a warmer and more humid climate, supports this situation.

On the other hand, the construction of dams and irrigation areas in the Kars region in recent years has led to milder winter months compared to previous years and an increase in humidity rates. This is thought to result in an increase in both mosquito population and flight activity. The expressed changes have a significant impact on the epidemiology of SBV infection.

In their publication, Doğan et al. (2022) reported that measures such as controlling breeding animals and semen, managing vectors, and implementing early warning systems may be important in combating SBV infection. Currently, there is no vaccine for SBV in our country. However, given that this disease has posed a risk in recent years, the development of a vaccine against SBV may be under consideration.

Vector control and vaccination should be applied together to control SBV infection. There are various methods of prevention and control of SBV infection. These methods include control strategies to reduce larval/adult habitat, drainage of swamps and dirty water ponds (Carpenter, 2008); various insecticides, biorational pesticides (the use of hormones or microbial agents such as *Bacillus thuringiensis* that limit the development of *Culicoides*), repellent applications in animals and fly traps can be considered as alternative methods (Mullens et al., 2004).

CONCLUSION

As a result of this study, the prevalence of SBV infection in Kars province was determined and

the presence/prevalence of the infection was revealed serologically. According to the results of this study, SBV appears to be circulating in the Kars region. It should be considered that SBV may play a role as an etiological agent in abortions, premature births, and congenital malformations, which are common in the region, similar to infections such as *Bovine Viral Diarrhoea Virus* (BVDV), *Border Disease Virus* (BDV), *Bluetongue Virus* (BTV), and *Akabane Virus* (AKAV), which cause abortions and congenital malformations in pregnant sheep. We believe that testing sheep for SBV antibodies can be helpful in determining whether the disease is present in the region, identifying potential financial losses and selecting the best control programme for disease prevention. It is also thought that this study may shed light on epidemiological studies that can be conducted across the region/country from a broader perspective. In our study, only serum samples were tested and it is a preliminary study. In future studies, the aim is to determine antigen positivity by screening samples taken from animals exhibiting clinical findings, such as whole blood, semen, and abortion material.

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Ethical statement or informed consent: This research was conducted after the approval of Kafkas University Animal Testing Local Ethics Council (Approval Number: KAÜ-HADYEK-2019-135).

Consent: Serum samples were obtained from herds in small-scale family-type enterprises in five villages of Kars province. This research was conducted after the

approval of Kafkas University Animal Experiments Local Ethics Committee (Approval Number: KAU-HADYEK-2019-135) and the permission of the Ministry of Agriculture and Forestry.

Author contributions: Conceptualization, VY; investigation, VY, NC, ŞK; validation and formal analysis, VY, NC, EK; writing—original draft preparation, VY, writing-review and editing, VY, NC, EK, supervision, VY. All authors have read and agreed to the published version of the manuscript.

Availability of data and materials: The datasets presented in this study are publicly available.

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