



Seroprevalence of Schmallerberg Virus in Sheep from Southern Iraq

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Abstract | Abortion is an important problem causing high economic losses in ruminant animals. Schmallerberg virus (SBV) is one of the problems that causes abortion, worldwide distributed. The virus causes asymptomatic, subclinical, or nonspecific clinical signs, such as fever, diarrhea, and reduced milk yield, during the viremic phase; however, infection is mostly asymptomatic. The aim of the present study was to use an ELISA test to investigate SBV antibodies in sheep in southern Iraq, the present study included 90 ewes 47 serum samples of ewes from Basra province and 43 serum samples of ewes from Al Diwaniyah province) aged from 1-4 years, All the (90) ewes were suffered from reproductive problems included abortion, still birth and congenital defect. The ELISA results showed that 60 (66.6%) samples out of 90 samples were seropositive for Schmallerberg virus antibodies in both provinces Basra and Al Diwaniyah, In Basra province the ELISA test shows the 33 samples out of 47 samples were seropositive for antibodies of SBV while in Al Diwaniyah 27 samples of 43 samples were seropositive for antibodies of SBV. In total the results of ELISA test according to clinical signs of suspected infected animals show the ewes seropositive for SBV antibodies included 34 of the 61 stillbirth cases, 23 of the 26 abortion cases, and all 3 congenital defect cases, statistically no significant variation when compared them with each other. In conclusion, This study provides the first evidence of high SBV seroprevalence in sheep with reproductive disorders in southern Iraq.

Keywords | Schmallerberg Virus, Sheep, Abortion, insect-borne disease

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INTRODUCTION

Schmallerberg virus (SBV) is an infectious disease that infected mostly domestic and wild ruminant first discovered in the summer of 2011 in Northwestern Germany and Netherland after few months the virus had spread in western Europa countries such as France, Belgium, Swaziland and Austra (Yanase *et al.*, 2012; Sudhakar *et al.*, 2020). The virus belongs to the family Bunyaviridae, genus Orthobunyavirus. It is an enveloped,

negative-sense, segmented, single-stranded RNA virus, and is arthropod-borne. SBV transmitted by Culicoides midges, also SBV can be transmitted vertically via of placenta or from infected ewes of their offspring, in addition to the infected dams capable to transmitted virus to many females through semen, this make rams potent victor for rapidly spreading the virus with herd. This vertical transmission, which occurs during early stages of pregnancy, causes congenital anomalies (Hoffmann *et al.*, 2012; Bayrou *et al.*, 2014; Peperkamp *et al.* 2015).

Schmallenberg virus infection is classified as asymptomatic in goats and sheep with more severe clinical findings typically seen in cattle. Clinical signs characterized by pyrexia, decrease of milk production and in some cases, diarrhea while in pregnant sheep and goats clinical signs manifest as stillbirth, abortion and congenital defects in fetal (Hoffmann *et al.*, 2012; Laloy *et al.*, 2015; Lechner *et al.*, 2017).

Some studies refers to many sheep and goats show only mild clinical signs included fever, diarrhea, depression, lethargy, and snotty nose while commonly abortion, stillbirth, and malformation can occur without any clinical signs (Helmer *et al.*, 2013; Wernike *et al.*, 2013). The reproductive disorder include still birth and abortion in permanent animals or congenital defects of fatuous such as mummified fetus, disproportionate metatarsus, premature birth, torticollis, brachygnathia inferior, flattened skull, ankylosis, fixed joint and bent limbs This collection of congenital abnormalities is called arthrogryposis-hydranencephaly syndrome (AHS) (Gelagay *et al.*, 2018).

The implications of Schmallenberg virus extend beyond immediate clinical symptoms, as the long-term effects on livestock productivity and reproductive health are significant concerns for farmers and the agricultural industry at large (Abi-Rizke *et al.*, 2017). The economic burden associated with SBV includes not only the direct costs of veterinary care and loss of livestock but also the potential for decreased milk yield and fertility rates in affected herds, which can lead to substantial financial losses over time (Azkur *et al.*, 2013). Additionally, the virus's ability to persist within populations and its arthropod-borne nature necessitate ongoing monitoring and surveillance to prevent future outbreaks, particularly as climate change may alter the distribution of Culicoides midges, potentially expanding the virus's range (Stavrou *et al.*, 2017; Larska *et al.*, 2023). Understanding these broader implications is crucial for developing effective management strategies and vaccines to mitigate the impact of SBV on ruminant health and agricultural productivity. Moreover, targeted research and collaboration among veterinary and agricultural sectors are essential to enhance prevention strategies and improve response mechanisms against future SBV outbreaks (De Reege, 2016; Mahdavi *et al.*, 2023). In Iraq, very few studies have been conducted on the presence and impact of Schmallenberg virus, therefore the present study was aimed to investigate the presence of SBV antibodies and assess their relationship with the clinical signs and reproductive problems observed in sheep samples collected from southern Iraq.

ETHICAL APPROVAL

The current study was conducted in accordance with the guidelines and rules issued by collage of Veterinary Medicine- University of Basrah. The experimental procedures were approved by the Committee of Animal Ethics in the College of Veterinary Medicine, University of Basrah, Iraq.

STUDY DESIGN

The current study was conducted on 90 ewes 47 serum samples of ewes from Basra province and 43 serum samples of ewes from Al Diwaniyah province) aged from 1-4 years, All the (90) ewes were suffered from varies clinical signs included abortion, still birth and congenital defect were obtained from ranchers, the study extended between October 2022 to March 2023 in Basrah and Al Diwaniyah Provinces in Iraq.

CLINICAL EXAMINATION

All the animals in the current study suffering from a history of abortion, stillbirth, and clinical signs were examined clinically, and vital signs were properly recorded and documented.

SERUM COLLECTION

Blood samples were collected from the jugular vein, using a Vacutainer tube without anti-coagulant. Subsequently, these samples were centrifuged for ten minutes at 1500 rpm to produce the serum, then the serum was kept in the cryotubes in a deep freezer at - 45 and it was stored in cryotubes in a deep freezer at -45°C until analysis.

ELISA TECHNIQUE

In the present study, we followed the manufacturer's protocols (Svanova ELISA is an indirect ELISA, where the added conjugate forms a complex with the antibodies) as described in our previous research (Naji *et al.* 2021). The indirect ELISA tested the antibodies with a conjugate to form the complex. Each sample was tested in duplicate on two separate wells: One well was coated with the control antigen, and the other with the SBV antigen. The coated microplate was read at optical density OD 450 nm, the value of optical density was adjusted through subtracts both wells' values of every single sample, then the positive percentage (PP) value was correlated with the positive control value.

PP = ODCorr (sample or NC / ODCorr (PC) × 100.

PP = Percent Positivity, OD Corr = Corrected Optical Density, NC = Negative Control

PC = Positive Control

PP < 10 was considered as negative and PP > 10 as positive.

STATISTICAL ANALYSIS

All the data in current study analyzed with Chi square test (Pearson Chi Square and Fishers exact test), for evaluating the seropositive status and the percentage the clinical signs of Schmallerberg virus in sheep in southern Iraq.

RESULTS

A total of 90 ewe samples, all from animals with a history of clinical signs, were analyzed. These signs included 61 (67.78%) stillbirth, 26 (28.89%) suffered from abortion and (3.3%) 3 congenital defect (Table 1), In Basra out 47 ewes 30, 14 and 3 ewe suffered from stillbirth, abortion and congenital defect respectively, while in Al Diwaniyah out 43 ewes were 31 stillbirth and 12 ewes suffered from abortion (Table 2).

Table 1: Number and percentage of the samples according to the clinical signs.

Clinical signs	No. and Percent
Still birth	61 (67.78%)
Abortion	26 (28.89%)
Congenital defect	3 (3.33 %)
Total	90 (100%)

Table 2: Number of the samples according to the clinical signs of both provinces.

Province	Clinical signs	No.	Total
Basra	still birth	30	47
	Abortion	14	
	Congenital defect	3	
Al- Diwaniyah	still birth	31	43
	Abortion	12	
	Congenital defect	0	
Total	90		

Table 3: Total the positive and negative sample for elisa test in both provinces.

Province	Positive sample	Negative sample	Total
Basra	33	14	47
Diwaniyah	27	16	43
total	60 (66.67%)	30 (33.33%)	90

In the present study ELISA analysis shows 60 (66.6%) samples out 90 samples were seropositive for schmallerberge virus antibodies in both provinces Basra and Al Diwaniyah, In Basra province the ELISA test shows the 33 samples out 47 samples were seropositive for antibodies of SBA while in Al Diwaniyah 27 samples of 43 samples were seropositive for antibodies of SBV (Table 3). In total the results of ELISA test according to clinical

signs of suspected infected animals show the ewes were seropositive for antibodies of SBV included 34 out 61 cases stillbirth, 23 out 26 ewes suffered from abortion and 3 out 3 from congenital defects, statically show significant variation when compared them with each other (Table 4).

Table 4: Seropositivity for sbv antibodies according to clinical signs.

Clinical signs	Positive sample	Negative samples	Total
Still birth	34 ^a	27	61
Abortion	23 ^b	3	26
Congenital defect	3 ^c	0	3
total	60	30	90

Different small letters Vertically referred to significant differences ($p < 0.05$)

According to clinical signs in each provinces the ELISA analysis shows 19 samples out 30 still birth samples, 11 samples out 14 of abortion samples and 3 samples out 3 of congenital defects were seropositive for SBV in Basra Province while in Al Diwaniyah province the results of ELISA test show 15 samples out 31 still birth and 12 samples out 12 of abortion samples were seropositive for SBV antibodies, statically no significant variation when compared provinces with each other (Table 5).

Table 5: Seropositivity for sbv antibodies according to clinical signs in basra and al diwaniyah province.

Province	Clinical sings	Positive (n)	Negative (n)	Total
Basra	Still birth	19	11	30
	Abortion	11	3	14
	Congenital defect	3	0	3
Al Diwaniyah	Still birth	15	16	31
	Abortion	12	0	12
	Congenital defect	0	0	0

DISCUSSION

Schmallenberg virus infection is also classified as one of the emerging infectious diseases of ruminants, with the transmission mediated by Culicoides midges. (Pawaiya *et al.*, 2013), The ELISA results show test show seropositive samples for SBV antibodies were 33 samples out 47 in Basra and 27 out 43 samples in Al Diwaniyah provinces. These results indicated that SBV is more prevalent in Basra than in Al Diwaniyah. These variations between two cities may occur due to many reasons, firstly epidemiological factors including difference size, differences in climatic conditions between two provinces, such as humidity and temperature, which are conditions suitable for Culicoides

spp., the primary transmission vector for SBV. Secondly difference in livestock management factors, including deference of husbandry practices, herd movement, and trade. Finally, herd immunity of animals as suggested by Naji *et al.* (2021).

The pooled prevalence of SBV was significantly associated with publication time, detection techniques, and species of animals. The highest pooled prevalence of SBV was reported in cattle (59%), followed by sheep (37%) and goats (18%) (Dagnaw *et al.*, 2024).

Further, Naji *et al.* (2024) conducted a more comprehensive serological study on 78 dairy cattle exhibiting clinical signs in Basrah and Al-Qadisiyah Provinces. The study reported an overall SBV seroprevalence of 84.6%, with Basrah showing slightly higher positivity (87.1%) compared to Al-Qadisiyah (83.0%). Cattle appear to have a higher predisposition to infection with SBV than sheep for many reasons, First, cattle are more attractive to biting midges which transport the virus, in addition to their large body size and different behavior patterns leading to more frequent bites from *Culicoides* spp. Second, cattle have a longer lifespan than sheep which make cattle more exposure to the v SBV via multiple transmission seasons. Another serological investigation conducted in Nineveh (2018–2019) tested 400 imported calves using cELISA, with 21% testing positive (Al-Baroodi, 2021). The study noted a higher prevalence among older calves and during the spring season. The researchers hypothesized recent SBV introduction into Iraq and emphasized the risk posed by cross-border animal movement and inadequate quarantine protocols.

The results showed a higher seropositivity rate in abortion cases (23/26, 88.5%) compared to stillbirth cases (34/61, 55.7%). this may be occur due to the timing of fatal infection during ingestion, in addition to the virus have ability to cross the placenta then infect fetus a finding that agrees with Al-Barway (2018). reported the abortion in highest rate in infected animals in Dohuk province in Iraq.

Naji *et al.* (2021) Observed different clinical symptoms included decreased milk production, reduced appetite, and reproductive failures such as abortions and stillbirths.

This study represents the first confirmed evidence of SBV antibodies in southern Iraq's sheep population and suggests a need to investigate its potential economic impacts. Increased awareness and research on Schmallenberg virus in Iraq are essential to establish effective control measures. Increased collaboration between researchers, veterinarians, and farmers will be vital in addressing the knowledge gaps.

CONCLUSION

This study provides the first evidence of SBV circulation among sheep in southern Iraq, demonstrated by a high seroprevalence (66.6%) in a cohort of animals with reproductive disorders. This high rate warrants a larger, controlled study to determine if SBV infection is a significant risk factor for these conditions.

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NOVELTY STATEMENT

The appearance of clinical signs such as still birth, abortion and congenital defect in several sheep flocks in southern Iraq, combined with the absence of a definitive etiological diagnosis, Due to the fact that SBV is the primary suspect among the above causes. This study represents the first investigation of SBV antibodies in sheep in southern Iraq, thereby supporting efforts to control the disease in these areas.

AUTHOR'S CONTRIBUTIONS

Conceptualization: HAN. Data Curation: ZAHS and TAA. Formal analysis: HAN and WMMS. Investigation: HAN, ZAHS, and TAA. Funding acquisition: HAN. Methodology: ZAHS and WMMS. Project administration: HAN. Visualization: HAN and WMMS. Writing original draft: TAA. Writing review and editing: HAN.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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