

Serodiagnosis of schmallerberg virus infection in sheep in Nineveh governorate, Iraq

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Article information

Article history:

Received September 18, 2022
Accepted November 28, 2022
Available online December 09, 2022

Keywords:

Schmallenberg virus
cELISA
Nineveh governorate
Sheep

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Abstract

Schmallenberg virus (SBV) is an emerging pathogen of sheep. It is transmitted through arthropods vertically from dams to fetuses through the placenta. As a result, it can cause prenatal defects that can result in abortion or death of the fetus, as well as sometimes the death of mothers due to complications following an abortion. The study was conducted on 92 sera of local breed sheep (67 aborted ewes and 25 rams). All sera were tested for Schmallenberg virus antibodies using a competitive enzyme-linked immunosorbent kit. Results indicated that the overall prevalence of the Schmallenberg virus was 79.3%. The highest percentages of SBV seropositive animals were found in Mosul city, Wana, and Tel Usqouf at 100%, whereas Badoush reported the lowest percentage at 40%. Additionally, there was a significant difference between the percentage of aborted ewes 85.07% and rams 64%. The most frequent infection type was the severe one 55.43%, followed by the moderate 18.47% and mild 5.43% types. In conclusion, the findings highlight the existence of a novel virus that significantly impacts ovine abortions. So, these preliminary data can guide livestock producers in designing appropriate prevention and control measures.

DOI: [10.33899/ijvs.2022.136029.2557](https://doi.org/10.33899/ijvs.2022.136029.2557), ©Authors, 2022, College of Veterinary Medicine, University of Mosul.
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Introduction

Schmallenberg virus (SBV) infection is a newly ruminant infectious disease that was spread throughout Europe from 2011 to 2012. SBV belongs to the Simbu serogroup of the family *Bunyaviridae*, genus *Orthobunyaviruses*, and is closely linked to other arthropod-borne viruses that mostly infect ruminants, such as Akabane, Sathuperi, Aino, and Shamonda virus. All of these viruses are spread by mosquitoes and biting midges, and they can cause congenital abnormalities in newborns if a pregnant dam is infected during the first trimester of pregnancy (1-3). Loss of appetite and body condition, hyperthermia, diarrhoea, and a decrease in milk yield are all clinical indications of SBV infection in adult animals (4). Diagnostic tools for SBV infections were soon evolved and disseminated to the affected areas. SBV can be diagnosed via genomic and antibodies identification

of the virus (5,6). The RT-PCR can be used to identify the SBV genome in the brain tissues of newborns with congenital anomalies or in the blood of viraemic animals. Additionally, SBV can be isolated on cell lines from insects, hamsters, or African green monkeys (3,7). Virus neutralization test (VNT), indirect immunofluorescence assays, and commercially ELISAs are a diagnostic method used to detect antibodies (8,9). The VNT has a very near to 100% sensitivity and good specificity. However, it takes a significant amount of time (4 to 6 days) and cannot be automated (10). Furthermore, indirect immunofluorescence tests may be carried out on plates that include antigen matrix made up of BHK-21 cells that have been infected with SBV. However, this approach might not be the most sensitive for identifying SBV antibodies (9). Therefore, in order to diagnose SBV infection and determine SBV seroprevalence in infected areas, a diagnostic test that enables serological

testing of a large number of samples, which is not possible by VNT, was required (11,12). Competitive Enzyme-linked immunosorbent assay was developed, based on a recombinant SBV nucleoprotein antigen, and currently provides a quick and affordable technique for serological diagnosis (9).

Despite the discovery and spread of SBV as an important economic illness in European nations, its potential for cross-border dissemination and impact on the ovine sector in Nineveh governorate, Iraq. In our governorate, where suspected clinical cases involving the reproductive system of ruminants frequently present, there is no information directly related to SBV infection in ovine. As a result, this investigation was carried out to confirm the existence of antibodies to Schmallenberg virus in the sera of ovine with a history of abortion and births of deformed fetuses.

Materials and methods

Ethical approve

The Institutional Animal Care and Use Committee, College of Veterinary Medicine, University of Mosul, accepted the sample collection methods on August 23, 2021, with approval issue number UM.VET.2021.27.

Study period and areas

The study was conducted between September 2021 and January 2022 from several areas in Nineveh governorate, including Mosul, Wanna, Telskuf, Ba'ashiqah, Tel Kaif, Al-Hamdaniya, Nimrud, Bartella, and Badush.

Blood sampling

A total of 92 blood samples from both sexes, greater than 3 years old, and from various farms with a history of abortion were taken (Table 1). Five milliliter of blood sample were collected from the jugular vein and the serum was separated into 2ml eppendorf tube and preserved at -20°C until analysis.

Table 1: Number of blood sampled sheep per area

Area	No. blood samples		Total
	Females	Males	
1 Mosul	6	2	8
2 Wanna	5	2	7
3 Telskuf	17	9	26
4 Ba'ashiqah	5	2	7
5 Tel Kaif	21	4	25
6 Hamdaniya	2	1	3
7 Nimrud	2	1	3
8 Bartella	6	2	8
9 Badush	3	2	5
Total	67	25	92

Detection of anti-Schmallenberg virus antibodies

The commercial serological ID vet® SBV multispecies ELISA test kit (Montpellier, France) for the detection of antibodies against SBV nucleoprotein was used. The SBV antibodies detection from sheep sera was carried out as described in prior research (6,13). The assay was prepared, incubated (Mempert®, Germany), and washed (Biochek, USA), in accordance with manufacturer's instructions. The optical density at 450 nm was measured using an ELISA Microplate Reader (Biochek, USA), and each sample's S/P value was computed in accordance with the recommendations of the manufacturer.

Statistical analysis

The chi-square test was used for comparing the frequencies of the competitive ELISA results, taking into account the full number of the studied observations. The statistical analysis and significant differences at the level of significance ($P \leq 0.05$) were done by SPSS V25 program (14).

Results

Percentages of SBV antibodies based on the study's areas

The total percentage of SBV antibodies in sheep was 79.34%, and ranged from 40 to 100% for the areas studied. The highest percentage 100% was recorded in Mosul city, Wanna and Telskuf, while the lowest percentage 40% was recorded in the Badush, and the rest of the percentages ranged between these two percentages with significant differences between the areas at the level of significance (Table 2).

Table 2: Percentages of SBV antibodies in sheep based on the study areas

Area	No. tested	No. positive	Percentage
Mosul	8	8	100 a
Wanna	7	7	100 a
Telskuf	26	26	100 a
Ba'ashiqah	7	5	71.42 b
Tel Kaif	25	17	68 b,c
Hamdaniya	3	2	66.66 b,c
Nimrud	3	2	66.66 b,c
Bartella	8	4	50 c,d
Badush	5	2	40 d
Total	92	73	79.34

A vertical difference of a, b, c, and d indicates a significant difference at level of $P \leq 0.05$.

Percentages of SBV antibodies based on the animal's sex

With regard to the sex risk factor, the results showed that the percentage of SBV antibodies was significantly higher in females 85.07% than that for males 64% (Figure 1).

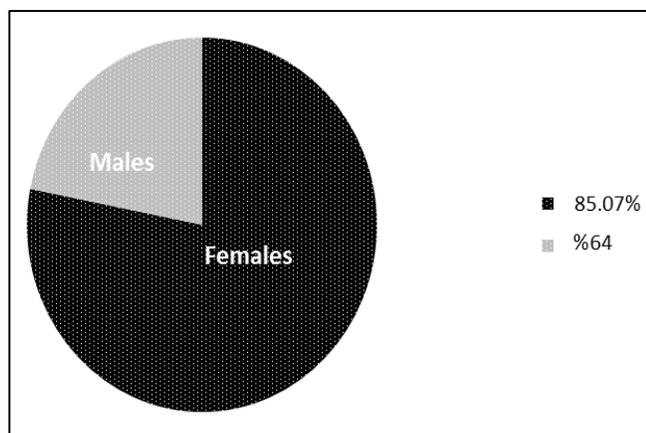


Figure 1: Percentages of SBV antibodies in sheep based on the animal's sex

Percentages of SBV antibodies based on the severity of infection

The results showed that the severe Schmallenberg virus infection was present in 51 out of 92 samples examined, with a percentage of 55.43%, the highest compared to the other two types of infection. However, only 17 and 5 samples, respectively, with percentages of 18.47 and 5.43%, revealed infection with the moderate and mild types (Figure 2).

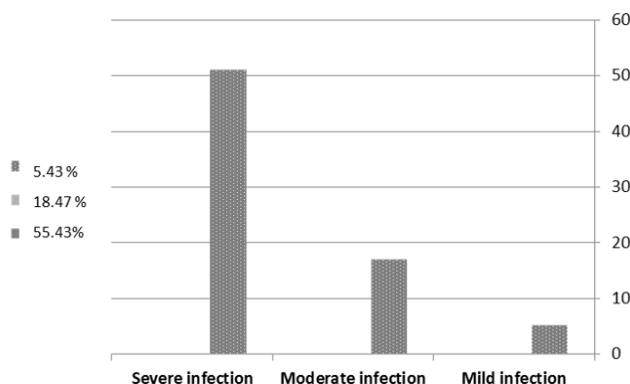


Figure 2: Percentages of SBV antibodies in sheep based on the severity of infection

Discussion

The Schmallenberg virus was initially identified in dairy cattle in Germany in the winter of 2011. Then, it was discovered in deformed lambs, kids, and calves (3,15). The transmission of SBV to neighboring countries seemed quite probable given how quickly it moved across Europe in 2011 and 2012. The results of the current study revealed a significant seropositivity of SBV antibodies in ovine in Nineveh Governorate between September 2021 and January 2022, indicating widespread exposure to the virus during the

summer and autumn seasons when biting insects are most active.

Seventy-three ovine sera were tested positive for the SBV virus. Our results were interestingly higher than those from Turkey, where the prevalence of SBV in sheep and goats was reported to be 39.8 and 1.6%, respectively (16), as well as higher than those previously noted in small ruminant flocks in Germany 53.3% (6). Furthermore, our findings lower than what had previously been observed in Belgium (over 80% of the herds) (17). Another study used cELISA to determine the seroprevalence of Schmallenberg virus in ovine, bovine, and caprine sera in Mozambique; the results revealed that 90% of these animals were infected (18). The variations in farm management systems and quarantine measures, the history of the disease in each country, and the source of the animals just are the explanations for the differences in disease distributions between the countries. Other explanations include differences in distributions and infestations of arthropod vectors (19). From this study, it is clear that the total percentage of SBV antibodies in sheep varied based on the area where samples were taken, with Mosul city, Wanna, and Telskuf recording the highest percentages 100% and Badush the lowest percentages 40% respectively. Similar results were found in a prior study, which found that the northeast, northwest, southwest, and southeast regions of Germany each have seen a varied seroprevalence rate of SBV (6).

Animals' breed, sex, and species are genetically controlled intrinsic or endogenous characteristics that are the main causes of variation in disease susceptibility or resistance in each population (20). In this study, the percentage of SBV antibodies was significantly greater in females, and there was a correlation between gender and SBV seropositive status of individual animals. According to this finding, there may be a higher risk of exposure to arthropod vectors in the field because of prolonged contact with infected animals as most female animals stay on the farm longer for breeding purposes. In addition, female animals tend to be more restless and move far and wide while grazing in search of breeding partners, which increases their exposure to arthropod vectors on pasture. Moreover, SBV has been found in the male genital tracts of sheep, which raises the likelihood of genital transmission (7,21). The findings also revealed that the severity of infection was severe in adult males and females, with a rate of 55.43%, which was the highest when compared to the moderate and mild types. Compared to other species of ruminants, sheep are more susceptible to viral infection. This is in agreement with results from Europe, where infection prevalence and severity were shown to be higher in sheep than in goats. In addition to the fact that the virus' seroprevalence in young ruminants rises with age, older animals have a higher possibility of being exposed to risk factors than younger ones (22), which is consistent with the study's findings given that the samples were taken from adult sheep. This result is

consistent with past research that showed that SBV seropositivity increased with age in cattle, sheep, goats (23), and wild animals (24,25). Consequently, older animals are more likely to be exposed to risk factors, which increase their likelihood of becoming infected and having a persistent infection.

Conclusion

This study proved for the first time that the Schmallenberg virus is highly prevalent in Nineveh Governorate and this virus is responsible for a large proportion of ovine abortions and stillbirths.

Acknowledgment

College of Veterinary Medicine, University of Mosul, Mosul, Iraq, provided funding for this research.

Conflict of interest

There are no conflicts of interests in the publishing of this work, according to the authors.

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والى موت الأمهات أحيانا نتيجة للمضاعفات الناتجة عن الإجهاض. أجريت الدراسة على ٩٢ مصلا من سلالة الأغنام المحلية (٦٧ نعجة مجهزة و ٢٥ كبش). تم فحص جميع الأمصال للتحري عن الأجسام المضادة الخاصة بفيروس شمالنبرغ باستخدام عدة اختبار الاليزا التنافسي. أشارت النتائج إلى أن معدل الانتشار الكلي لفيروس شمالنبرغ كان ٧٩,٣٪. حيث سجلت أعلى نسبة للحيوانات الموجبة مصليا في مدينة الموصل ووانه وتل أسقف ١٠٠٪، بينما سجلت بأدوش أقل نسبة ٤٠٪. بالإضافة إلى ذلك، لوحظ فرق معنوي بين النسبة المئوية لتواجد الأضداد في النعاج المجهزة والتي كانت الأعلى ٨٥,٠٧٪ بالمقارنة مع الأكباش ٦٤٪. كما سجلت الإصابة من النوع الشديد أعلى نسبة بلغت ٥٥,٤٣٪ بالمقارنة مع النوعين الأخرين المتوسط ١٨,٤٧٪ والخفيف ٥,٤٣٪. يستنتج من النتائج على انتشار فيروس جديد له دور مهم لحالات الإجهاض في الضأن، لذا فإن هذه البيانات الأولية يمكن أن توجه منتجي الثروة الحيوانية في تصميم تدابير الوقاية والسيطرة المناسبة.

التشخيص المصلي للإصابة بفيروس شمالنبرغ في الضأن في محافظة نينوى، العراق

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الخلاصة

يعد فيروس شمالنبرغ أحد مسببات الأمراض الطارئة في الضأن، إذ ينتقل عبر المفصليات فضلاً عن انتقاله عمودياً من الأمهات الى الأجنة عبر المشيمة مسبباً تشوهات جنينية قد تؤدي إلى موت الجنين وإجهاضه