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### SEROLOGICAL INVESTIGATION OF SCHMALLENBERG VIRUS IN CATTLE IN MOSUL CITY, IRAO

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#### **ABSTRACT**

Schmallenberg virus (SBV) is a new Orthobunyavirus that infects animals and results in pregnancy loss, congenital deformity, and decline milk supply. The serological research for evidence of SBV was highly beneficial for management and early warning. Thus, the study aimed to determine SBV antibodies in the sera of cows and buffaloes with a history of reproductive issues, to determine the relationship between the percentage of infection and certain risk factors, and to find out the severity of infection. Between March 2023 and February 2024, the study was carried out in a number of districts in Nineveh governorate. Ninety-two blood samples from cows and buffaloes older than two years were collected. Schmallenberg virus antibodies were detected using the commercial ELISA kit. At a significance level of P≤0.05, the chi-square test was applied. Overall, the percentages of SBV antibodies in cows and buffaloes were 74.07% and 63.15%, respectively, with no significant differences between the districts included in the investigation. The results also showed that the highest percentages were found in cows (84.61%) and buffaloes (77.77%) that were three years of age or less. These percentages vary significantly, with those older than four years. Spring had the highest percentage of SBV antibodies (87.09%), and it differed significantly from summer (52.63%) and winter (47.05%). Serologically, the most prevalent infection type was severe (46.73%), which differed significantly from moderate (15.21%) and mild (7.6%). In conclusion, there is a high and widespread level of serological evidence of SBV in the studied areas.

Keywords: Nineveh Governorate, cELISA, Buffaloes, Cows

### **Highlights**

- 1. The serological evidence of Schmallenberg virus was highly prevalent in cows and buffaloes in Nineveh Governorate.
- 2. The percentage of Schmallenberg virus antibodies in cows and buffaloes varied significantly and noticeably with age and season of sampling.
- **3.** Compared to animals with moderate or mild infection, most of the investigated animals had severe infection.

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#### INTRODUCTION

The first detection of SBV occurred in Germany in November 2011 in blood samples taken from cattle showing acute clinical signs, such as decreased milk supply, elevated temperature, and diarrhea (Hoffmann et al., 2012). The virus belongs to the Simbu serogroup and is a negativesense single-stranded RNA that shares genetic similarities with the Akabane, and Shamonda viruses (Pawaiya and Gupta, 2013). SBV is an arbovirus, which means that hematophagous insect vectors, particularly Culicoides spp, are the primary mode of transmission (Carpenter et al., 2009; Carpenter et al., 2013). Transmission of virus through the placenta may also occur in ruminants during the early stages of pregnancy, and result in teratogenic consequences. In addition, several studies have confirmed presence of schmallenberg virus in cattle semen (Laloy et al., 2017). Following the initial outbreak in Germany Netherlands, the SBV rapidly spread throughout ruminant populations, with numerous reports of clinical symptoms and serological directories of the disease in various countries around the world, including Iraq (Beer et al., 2013; Tonbak et al., 2016; Zhai et al., 2017; Taha and Alhankawe, 2022). SBV infection in mature cattle is evident by clinical symptoms such as fever, diarrhea, lack of appetite and decreased milk production. It has also been shown that infection during a crucial stage of pregnancy will affect the neuro-musculoskeletal systems of fetus, leading to neonatal malformations, which are happening between days 47 and 162 of gestation (Hoffmann et al., 2012; Wernike et al., 2014). Consequently, SBV causes pecuniary losses and negatively impacts the cattle industry due to its potential to cause embryo distortionist, retained placenta, endometritis, abortion, and stillbirths (Lievaart-Peterson et al., 2015; Laloy et al., 2017). Diagnostic technologies have been developed for detecting SBV infection, and they posted speedily in afflicted areas. Viral genome analysis and antibody detection are approaches relied upon to diagnose SBV (Humphries and Burr, 2012; Gerhauser et al., 2014). Several tests are used for virus diagnosis, including reverse transcriptasepolymerase chain reaction. neutralization test (VNT), ELISA, and fluorescent-immunoassay test (Garigliany et al., 2012; Tarlinton et al., 2012). RT-PCR for viral RNA has been shown to be the most accurate in confirming acute SBV infection. However, the short duration of viremia (4-6 days) and the non-specific symptoms associated with SBV infection in mature animals propose that detection of SBV specific antibodies may be a more accurate diagnostic test than detection of the virus itself (De Regge et al., 2013). The virus neutralization test has high sensitivity and specificity. Moreover, it is not possible to be automated and takes a long time. In addition, indirect immunofluorescent assays may be done using plates that contain antigen matrix made of BHK-21 cells infected with SBV. However, this may not be the main perfect method for detecting antibodies of SBV (Loeffen et al., 2012; Breard et al., 2013). So that, it was essential to use a diagnostic test that would permit serological testing multiple samples for detection of the existence of SBV in affected regions (Van der Poel et al., 2014). A recombinant SBV nucleoprotein antigen served as the basis for the development of the competitive enzyme-linked immunosorbent test, which now offers a rapid, cost-effective method for serological diagnosis, is more sensitive and specific and can test for a greater number of samples at once (Breard et al., 2013). Although clinical cases involving the genital system are common in the Nineveh region, there is limited concrete evidence that cattle are infected with SBV. Therefore, this study was conducted to determine SBV antibodies in the sera of buffaloes with a history of cows and reproductive disorders, to determine the relationship between the percentage of infection and certain risk factors, such as

district, age, and sampling season, and to find out the severity of infection.

#### MATERIAL AND METHODS

### 1 Ethical approval

In March 2023, the sera collecting method was authorized by approval issue number UM.VET.2023.090.

### 2. Study time, districts and blood sampling

Between March 2023 and February 2024, the study was carried out in a number of districts in Nineveh governorate, which are listed in Table (1). Ninety-two blood samples were obtained from cows (54) and buffaloes (38), older than two years, from various farms. Five milliliters of blood were drawn, and the sera were then placed in a two-milliliter tube and stored at -20°C till testing.

### 3. Screening for Schmallenberg virus antibodies and infection severity

Schmallenberg virus antibodies were detected from cattle sera using commercial ELISA kit (ID vet, France), following the procedures outlined in the previous study (Taha and Alhankawe, 2023). According to the manufacturer's instructions, the S/P value of every sample was determined.

# 4. Classifying the disease severity according to the ELISA optical density (OD) data

The classification involved comparing the measured OD to predefined thresholds. The mean OD of negative controls plus two or three standard deviations was used to calculate a cut-off value, which was subsequently used to distinguish between positive and negative outcomes. Samples with OD values over this cut-off value were categorized as positive and placed into different severity groups. Mild cases have OD values that are 1.5–2 times higher than the cut-off, moderate cases have OD values that are 2-3 times higher, and severe

cases have OD values that are much higher (more than 3–5 times the cut-off).

### 5. Analytical statistic

The frequencies of competitive ELISA findings were compared using the chi-square test. The significance threshold (P≤0.05) was employed with the SPSS V25 program (Petrie and Watson, 2006).

**Table 1:** Number of blood samples collected from different districts in the Nineveh governorate

No.	region	Numbe sa	Total	
	8	Cows	Buffaloe	
1	Mosul	10	5	15
2	Ba'ashiqah	8	3	11
3	Badoush	6	7	13
4	AL-Shoura	5	2	7
5	TelKaif	6	6	12
6	Wanna	7	3	10
7	Al-Hamdaniya	5	5	10
8	Nimrud	3	4	7
9	Bartella	4	3	7
Total		54	38	92

#### RESULTS

## 1. The SBV antibody percentages according to district

Percentages of SBV antibodies in cows and buffaloes do not differ significantly, according to the districts. In cows, the overall SBV antibodies percentage was 74.07%. The Badoush and TelKaif districts had the highest percentage (83.33%). Whereas, Wanna had the lowest percentage (57.14%), and the remaining districts fell between these two percentages. The overall percentage of SBV antibodies in buffaloes was 63.15% and varied between 33.33% and 85.71% in the districts under investigation. Badoush recorded highest (85.71%), while percentage Ba'ashigah and Bartella recorded the lowest percentage (33.33%) (Table 2).

**Table 2:** District's percentage of SBV antibodies

No.	District	Cows		Buffaloes	
		Number of	*Percentage	Number of	*Percentage
		positive	%	positive	%
1	Mosul	8/10	80	3/5	60
2	Ba'ashiqah	6/8	75	1/3	33.33
3	Badoush	5/6	83.33	6/7	85.71
4	AL-Shoura	3/5	60	1/2	50
5	TelKaif	5/6	83.33	4/6	66.66
6	Wanna	4/7	57.14	2/3	66.66
7	Al-Hamdaniya	4/5	80	3/5	60
8	Nimrud	2/3	66.66	3/4	75
9	Bartella	3/4	75	1/3	33.33
	Total	40/54	74.07	24/38	63.15

<sup>\*</sup> The SBV antibody percentages do not differ significantly, according to the district

### 2. The SBV antibodies percentages according to age

The findings demonstrated that the percentage of SBV antibodies in two age groups for both species varies significantly ( $P \le 0.05$ ). In the age range of equal or less than three years, the highest percentages

were seen in both cows (84.61%) and buffaloes (77.77%). It differs significantly, with the lowest percentages occurring in cows and buffaloes older than four years, at 46.66% and 27.27%, respectively (Table 3).

**Table 3:** Percentages of SBV antibodies according to age.

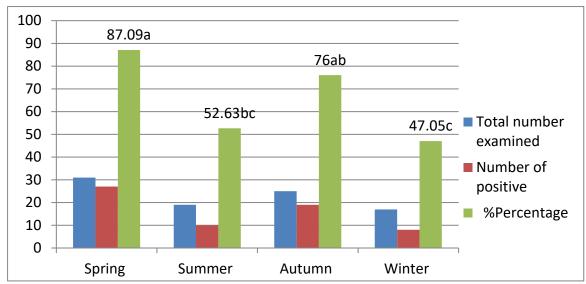
Species	Age	Total number examined	Number of positive	Percentage (%)
Cows	≤3 years	39	33	84.61 <b>a</b>
Cows	>4 years	15	7	46.66 <b>b</b>
Buffaloes	≤3 years	27	21	77.77 <b>c</b>
Dullaloes	>4 years	11	3	27.27 <b>d</b>

Vertical difference between letters (a, b, c, d) indicating significant difference at  $P \le 0.05$ .

### 3 The SBV antibody percentages according to season of sampling

The percentage was highest in the spring (87.09%; 27 out of 31), and it differed significantly ( $P \le 0.05$ ) from summer (52.63%) and winter (47.05%). There was

also a significant difference between autumn (76%) and winter (47.05%) at  $P \le 0.05$ . Whereas, spring and autumn, summer and winter, and summer and autumn did not vary significantly ( $P \le 0.05$ ) (Figure 1).



**Figure 1:** Percentages of SBV antibodies according to season of sampling. Difference between letters significant at  $P \le 0.05$ .

### 3.4 The SBV antibody percentages according to infection severity

The findings indicated 43 out of the 92 examined samples had a severe Schmallenberg virus infection (46.73%), which was significantly ( $P \le 0.05$ ) higher than

moderate (15.21%) and mild (7.6%) infections. Nevertheless, there were no significant variations between moderate (15.21%) and mild (7.6%) infections. (Figure 2).

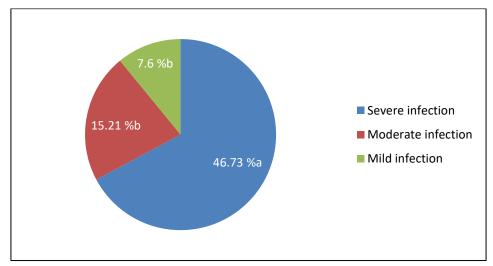


Figure 2: The SBV antibody percentages according to infection severity. Difference between letters significant at  $P \le 0.05$ .

### **DISCUSSION**

The results of this investigation provide the first indication of SBV disease in cows and buffaloes in Nineveh province. Since there is no vaccine present against SBV in the entire country, the finding of seropositive animals among the cattle under test indicates the animals were exposed to the virus. Overall, cows and buffaloes had

SBV antibody percentages of 74.07% and 63.15%, respectively, with no significant differences in percentages throughout the study's involved districts. These percentages were seen to be quite high, in comparison to other previous findings from our country. Al-Baroudi (2021) reported that 21% of imported calves carried antibodies to the Schmallenberg virus. Moreover, Naji *et al.* (2021) found 47.7%

of buffaloes in Basrah were infected with virus using cELISA. The results of this study are consistent with the results of former research conducted in other regions of the world, and the percentages were seen to be rather high. A study by sibhat et al. (2018) using cELISA showed that 56.6% of cattle in Ethiopia were infected with the virus. Additionally, a study in Mozambique indicated 90% of cattle were infected with SBV, employing cELISA to detect the viral antibodies in bovine serum (Blomstrom et al., 2014). There are several factors responsible for the variation in disease spread across regions countries, including the animal's origin, disease history in each nation, quarantine laws, variations in farm management techniques, and the existence of arthropods (Pawaiya and Gupta, 2013; Al-Baroodi et al., 2022). Moreover, lack of effective mosquito control programs in Iraq may lead to an increase in the seroprevalence proportion. It is also possible that the high levels of SBV antibodies in local Iraqi cows and buffaloes compared to cattle in other nations, such as Turkey and China, consequence of sampling techniques, or that animal movement is a major source of SBV infection (Schulz et al., 2014; Tonbak et al., 2016). The results also showed a significant variation in the percentages between cows and buffaloes with age, three-year-olds and younger animals had the highest percentages. On the other hand, the lowest percentages were seen in animals older than four years. This finding was at odds with Armin et al. (2012), who found there were no obvious age differences, indicating that the SBV is emerging from other regions. Whereas this finding is consistent with the findings of Meroc et al. (2013), who found that calves older than two years had a greater seroprevalence and proposed a link to the highest level of insect bite exposure in this The findings showed that the winter had the lowest percentage of SBV antibodies, while the spring had the highest. Also, the majority of the animals under examination had severe infections. The environment was more suited to the

transmission of vector-borne illnesses when temperatures increased, as opposed to fall. Moreover, the virus can spread during the winter months when there are fewer midges, but to a lesser degree (Rasmussen *et al.*, 2012; Dominguez *et al.*, 2014; Bessell *et al.*, 2014; Haider *et al.*, 2018).

#### CONCLUSION

In Nineveh province, cows and buffaloes have significant levels of SBV antibodies. It was extensively distributed over the studied areas. Age and sample season had a significant impact on the percentages, with the majority of the animals under examination having severe infections.

#### ACKNOWLEDGMENT

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### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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### التحري المصلي عن فيروس شمالنبرغ في الأبقار في مدينة الموصل ، العراق

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فيروس شمالنبرغ من فيروسات أورثوبونيا الحديثة الذي يصيب الحيوانات مؤدياً إلى فقدان الحمل والتشوهات الخلقية وإنخفاض إنتاج الحليب. البحث المصلي عن تواجد الفيروس ذو فائدة كبيرة كإنذار عن خطره و لإتخاذ التدابير اللازمة. بالتالي، هدفت الدراسة إلى التحري عن تواجد الأجسام المضادة لفيروس شمالنبرغ في مصول الأبقار والجاموس التي لديها تاريخ من الإضطرابات التناسلية، لتحديد العلاقة بين نسبة الإصابة وبعض عوامل الخطورة، ومعرفة شدة الإصابة. بين أذار 7.77 وشباط 7.77، شملت الدراسة عدد من المناطق في محافظة نينوى. تم جمع إثنان وتسعون عينة دم من الأبقار والجاموس التي يزيد عمرها عن عامين. تم الكشف عن وجود الأجسام المضادة لفيروس شمالنبرغ بإستخدام عدة إختبار مربع كاي عند مستوى دلالة 7.77. كانت نسب تواجد الأجسام المضادة الكلية لفيروس شمالنبرغ في الأبقار والجاموس هي 7.77. و 7.77. على التوالي، نسبة لوحظت في الأبقار (7.77) والجاموس (7.77) التي كانت أعمار هم ثلاث سنوات أو أقل, مع مع عدم وجود فروق ذات دلالة إحصائية مع التي تزيد أعمارهم عن أربع سنوات. أظهر فصل الربيع أعلى نسبة نواجد الأجسام المضادة لفيروس شمالنبرغ (7.77)، مع وجود فروق ذات دلالة إحصائية مع التي تزيد أعمارهم عن أربع سنوات. أظهر فصل الربيع أعلى نسبة سنوات أو أقل, مع أبطت في الصيف (7.77) والشتاء (7.77). مصلياً كانت الإصابة من النوع الشديد أكثر إنتشاراً سنوات أو أقل مستوى مرتفع ومنتشر من الأدلة المصلية لفيروس شمالنبرغ في المناطق المدروسة.

الكلمات المفتاحية: محافظة نينوى, الإليزا التنافسي, الجاموس, الأبقار