

Investigating serological evidence of Schmallenberg virus in cattle in eastern Algeria

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Abstract

Schmallenberg virus (SBV) is a newly identified virus belonging to the Orthobunyavirus genus, of the Bunyaviridae family, and transmitted by haematophagous arthropods in particular mosquitoes and biting midges of the Culicoides genus. The SBV is known to cause reproductive disorders in ruminants mainly abortions, stillbirths and congenital malformations (hydranencephaly and arthrogryposis syndromes). The aim of this study was to investigate the presence of SBV in dairy cattle in Algeria. Between September 2023 and December 2023, blood serum samples from 300 dairy cows from 75 dairy farms in north eastern Algeria were tested for SBV antibodies using a commercial indirect enzyme-linked immunosorbent assay kit. Individual seroprevalence was 38.33% (115/300; 95.00% confidence interval: 32.83 - 43.83), while herd seroprevalence was 41.33% (31/75; 95.00% confidence interval: 30.18 - 52.47). In addition, the results of this study revealed that SBV seroprevalence at individual or herd level was high and not negligible confirming the presence of SBV in the regions studied in Algeria. In conclusion, more in-depth studies are recommended concerning the molecular proof, origin and pathogenesis of SBV in ruminants mainly those linked to reproductive disorders as well as the study of the various associated risk factors.

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Introduction

Schmallenberg virus (SBV), an Orthobunyavirus, is an emerging pathogen affecting ruminants. First detected in November 2011 in the town of Schmallenberg (province of Rhineland, Germany) in plasma samples from dairy cattle showing fever, minimal milk yield and suffering from severe diarrhoea.¹ Subsequently, in 2012, the SBV spread to cattle herds in many European countries,^{2,3} and even to Asia and Africa.⁴⁻⁸ Based on these segmental genomic and phylogenetic characteristics, SBV is known to be highly related to the Akabane, Shamonda and Aino viruses. The virus is transmitted by hematophagous arthropods, particularly mosquitoes and biting midges of the Culicoides genus.^{9,10}

Clinically, SBV infection in cattle results in a transient fever (> 40.00 °C), reduction or even loss of appetite, deterioration in the animal general condition, drop in milk production (up to 50.00%) and the appearance of severe diarrhoea. However, previous studies highlighted serious signs including abortions, foetal mummification, stillbirths and the birth of malformed calves, arthrogryposis and hydranencephaly.¹¹

Several methods have been used to detect and confirm SBV infection. Initially, researchers at the FLI Friedrich Loeffler Institute used real-time reverse transcriptase polymerase chain reaction.^{1,12} Subsequently, several methods were developed and used for immediate diagnosis of infection including serological methods enzyme-linked immunosorbent assay (ELISA), neutralization methods and indirect immunofluorescence tests. The aim of the present research was to investigate the prevalence of exposure to SBV in dairy cattle in eastern Algeria at both individual and herd level using the ELISA test.

Materials and Methods

Study area. The study was carried out in the wilayas of Sétif, Bordj Bou Arreridj and Mila. The latter is located in northeastern Algeria which is considered as a large agricultural plain known for cereal and livestock production. In addition, the climate was another motive to choose the given area for the study. It is continental and semi-arid, hot and dry in summer, and rainy and cold in winter. The study took place from September 2023 to

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December 2023 and involved 300 blood samples from 300 cows belonging to 75 dairy cattle farms declared free of brucellosis and tuberculosis located in eastern Algeria and belonging to the wilayas of Sétif (n = 25), Bordj Bou Arreridj (n = 25) and Mila (n = 25).

Sample collection. To carry out this study, 5.00 ml of blood in a dry Vacutainer tube were taken and transported in a cool box at a temperature of 4.00 °C to the animal biotechnology laboratory at Blida 1 University, where they were centrifuged for 5 min at 3,000 revolutions per min. The sera collected using a micropipette were placed in 1.50 mL Eppendorf tubes and stored at - 20.00 °C until the serological test was carried out.¹³ All methods used in this study were applied respectively with current guidelines and norms. The veterinarians who handled the animals followed an appropriate practice in accordance with World Organisation for Animal Health (Formerly OIE) ethical guidelines and animal welfare regulations. This study is based essentially on scientific research which has not been the subject of a specific application to an ethics committee. All the methods used in this study were applied in accordance with the guidelines and standards in force relating to the use of animals in research and their welfare (chapter 7.8 of the World Organisation for Animal Health WOAHA Terrestrial Animal Health Code). The veterinarians who handled the animals followed appropriate practice in accordance with the ethical guidelines of the World Organisation for Animal Health. In addition, oral consent was obtained from all breeders who participated in the present study.

Serological analysis. The presence of *anti-SBV* antibodies was detected using an ELISA kit (IDVET, Montpellier, France). The kit used was the ID Screen® SBV competition multi-species kit using a recombinant SBV nucleoprotein. The diagnostic specificity and sensibility of this test announced by the manufacturer are 100%. Validation of the ELISA test used, interpretation of the results and calculation of the sample/negative control (S/N) serum percentages were carried out in accordance with the manufacturer's recommendations. The test used was validated on the basis of an optical density of the negative controls greater than 0.70, and a ratio between the mean of the positive controls and the mean of the negative controls of less than 0.30 (< 30.00%). When these two conditions were met, the optical densities of the samples tested were measured at a wavelength of 450 nm (DiaLab, Neudorf, Austria). The S/N percentages were calculated using equation 1 and interpreted according to the ELISA manufacturer's instructions (IDVET). Thus, a cow is positive if the S/N ≤ 40.00%, doubtful if the S/N% was

between 40.00% and 50.00% and negative if S/N% > 50.00%. Consequently, a farm was considered seropositive if at least one cow belonging to that farm was seropositive (S/N ≤ 40.00%). Seroprevalence was calculated by dividing the number of serologically positive sera by the total number of sera as it is shown below in which OD is optical density:

$$S/N\% = (OD \text{ sample} / OD \text{ negative control}) \times 100$$

Statistical analysis. The prevalence was calculated using XLSTAT Software (version 2023.2.0.1411; Lumivero, Denver, USA). Individual and herd seroprevalence rates are given with a 95.00% confidence interval.

Results

The results of the present study showed an individual seroprevalence of SBV of 38.33% (115/300; 95.00% confidence interval: 32.83 - 43.83). The herd seroprevalence rate was 41.33% (31/75; 95.00% CI: 30.18 - 52.47), (Table 1).

Discussion

The individual seroprevalence of SBV obtained was evaluated at 38.33% (95.00% CI: 32.83 - 43.83) (population of dairy cows). This result could be compared to other studies conducted in the same context (determination of SBV seroprevalence) and based on the use of the same serological diagnostic and investigated technique (ELISA). The seroprevalence reported in the current study is lower than those reported in various other studies, namely: 57.40% in China,⁴ 49.50% in Jordan,¹⁴ 70.60% in Spain,¹⁵ 45.50% in Kosovo,¹⁶ 40.70% in Turkey,¹⁷ and 69.90% in Ireland.¹⁸ Our findings were inconsistent with the previous one carried out in Jordan, Spain, China, Turkey and Kosovo, and the SBV seroprevalence obtained at the individual level was similar to that of 38.20% obtained in Italy.¹⁹ It means that the result were closely consistent to a study that was made in Italy. However, it was still much higher than those reported in Albania (11.10%),¹⁶ Iraq in imported calves (21.00%),²⁰ Iran (12.40%),²¹ and Brazil, where the SBV virus was not detected in any of the blood serum samples collected (0.00%).²²

The herd-wide seroprevalence of SBV obtained was estimated at 41.33%, a lower value than those found in China (100%),⁴ Tanzania (87.00%),⁷ Ireland (77.70%),¹⁸ and Italy (90.40%).¹⁹ However, it remains much higher than that reported in Brazil, where SBV was not detected in any herd (0.00%).²²

Table 1. Individual and herd seroprevalences as 95.00% confidence interval (min - max) of Schmallenberg virus.

Pathogen	Number of individuals			Individual prevalence rate	Number of herds			Herd prevalence rate
	Positive	Doubtful	Negative		Positive	Doubtful	Negative	
Schmallenberg virus	115	0	185	38.33% (32.83 - 43.83)	31	0	44	41.33% (30.18 - 52.47)

Given the fairly significant and not inconsiderable repercussions of SBV infection in cattle, particularly adults, which frequently takes the form of non-specific clinical signs, SBV infection transmitted by vectors (mosquitoes and biting midges) generally manifests itself as a mild, transient illness with anorexia, hyperthermia and, in some cattle, diarrhoea and a drop in milk production (by up to 50.00%).^{1,23} In this case, wind plays an important role in the transmission of the virus, as infected midges and mosquitoes are easily carried by air currents.²⁴ The rate of spread of SBV is estimated between 0.90 and 1.50 km per day.²⁵

In addition to the vector mode of SBV transmission, there is vertical transmission between the contaminated or infected mother and the foetus during the first and early second trimesters of gestation leading to abortion, stillbirths and the birth of malformed newborns.^{26,27} Finally, the horizontal or direct transmission of the SBV virus from infected cattle to healthy cattle by direct contact or via the oro-nasal or faeco-oral route has not been demonstrated.²⁸ The same applies to the transmission of SBV from infected bulls to females during natural mating or via artificial insemination and despite the detection of SBV in the semen of infected bulls, this mode of transmission has not yet been well studied.²⁹

The current study concluded that the SBV was indeed present in dairy cattle in Algeria, due to the high prevalences obtained at individual and herd level within herds in eastern Algeria. This exposure to SBV could be widespread in Algeria. Because of the importance and economic consequences of this disease, further studies are strongly recommended to determine the molecular specificities, pathogenesis and different risk factors associated with this exposure.

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Conflicts of interest

The authors declare no conflict of interest.

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