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# Seroprevalence and risk factors for bluetongue virus Infection in ruminants in Northeastern Algeria

# Seroprevalencia y factores de riesgo de la infección por el virus de la lengua azul en rumiantes del noreste de Argelia

**Racha OUACHTATI[1]* ,Hassane BENSEGHIR[2] ,Abdennour AZIZI[3] ,Leila AOUN[1] , Mourad ZEGHDOUDI[1] , Samir LAIB[1] , Dounia OUACHTATI[4]**

*[1]Chadli Bendjedid University, Faculty of Natural and Life Sciences, Laboratory of Epidemiological surveillance, health, production and reproduction, experimentation and cell therapy of domestic and wild animals, PO Box 73,36000 El-Tarf, Algeria.*
*[2]University of Batna 2, Faculty of Natural and Life Sciences, Department of Microbiology and Biochemistry, 05078, Algeria.*
*[3]University of Batna 1, Veterinary Sciences and Agricultural Sciences Institute, Department of Veterinary Science, 05000, Algeria*
*[4]Mohamed-Cherif Messaadia University - Souk Ahras, Institute of Agricultural Sciences and Veterinary Sciences, BP 41000, Souk-Ahras, Algeria*
*Correspondence author: ouachtati-racha@univ-eltarf.dz*

## ABSTRACT

Bluetongue disease is a vector-borne viral infection caused by bluetongue virus, an Orbivirus of the Reoviridae family. Bluetongue virus primarily affects domestic and wild ruminants, with sheep showing the highest clinical susceptibility, followed by goats and cattle. The virus is transmitted by hematophagous Culicoides midges. This cross-sectional study assessed bluetongue virus seroprevalence and identified associated risk factors in sheep, goats, and cattle across three provinces (wilayas) in northeastern Algeria. Between March 2024 and April 2025, serum samples (n = 380) were collected and analyzed using competitive enzyme-linked immunosorbent assay. The overall bluetongue virus seroprevalence was 54.74 % (208/380). Species-specific seroprevalence rates were highest in goats (73.91 %, 68/92), followed by cattle (73.27 %, 74/101) and sheep (35.29 %, 66/187). Multivariable logistic regression analysis revealed significant associations between bluetongue virus seropositivity and animal species ($P < 0.001$), geographic location ($P < 0.05$), and sampling season ($P < 0.05$). Compared to sheep, cattle showed seven-fold higher odds of seropositivity (OR = 7.746, 95 % CI: 3.621-16.571), while goats demonstrated nine-fold higher odds (OR = 9.044, 95 % CI: 4.440-18.422). Animals sampled during winter exhibited significantly higher seropositivity rates (OR = 3.400, 95 % CI: 1.526-7.574). These findings indicate endemic bluetongue virus circulation in northeastern Algeria, with significant species and spatial variation requiring targeted surveillance and control strategies.

**Key words:** Algeria; bluetongue; seroprevalence; ELISA; risk factors

## RESUMEN

La lengua azul es una infección viral transmitida por vectores causada por el virus de la lengua azul, un orbivirus de la familia Reoviridae. El virus de la lengua azul afecta principalmente a rumiantes domésticos y silvestres, siendo las ovejas las más susceptibles clínicamente, seguidas de las cabras y el ganado vacuno. El virus se transmite por mosquitos hematófagos del género Culicoides. Este estudio transversal evaluó la seroprevalencia del virus de la lengua azul e identificó los factores de riesgo asociados en ovejas, cabras y bovinos en tres provincias (Wilayas) del noreste de Argelia. Entre marzo de 2024 y abril de 2025, se recogieron muestras de suero (n = 380) y se analizaron mediante un ensayo inmunoabsorbente ligado a enzimas competitivo. La seroprevalencia global del virus de la lengua azul fue del 54,74 % (208/380). Las tasas de seroprevalencia específicas por especie fueron más altas en las cabras (73,91 %, 68/92), seguidas de los bovinos (73,27 %, 74/101) y las ovejas (35,29 %, 66/187). El análisis de regresión logística multivariable reveló asociaciones significativas entre la seropositividad al virus de la lengua azul y la especie animal ($P < 0,001$), la ubicación geográfica ($P < 0,05$) y la temporada de muestreo ($P < 0,05$). En comparación con las ovejas, el ganado vacuno presentó una probabilidad siete veces mayor de seropositividad (OR = 7,746, IC del 95 %: 3,621-16,571), mientras que las cabras presentaron una probabilidad nueve veces mayor (OR = 9,044, IC del 95 %: 4,440-18,422). Los animales muestreados durante el invierno mostraron tasas de seropositividad significativamente más altas (OR = 3,400, IC del 95 %: 1,526-7,574). Estos hallazgos indican la circulación endémica del virus de la lengua azul en el noreste de Argelia, con una variación significativa entre especies y espacios geográficos, lo que requiere estrategias específicas de vigilancia y control.

**Palabras clave:** Argelia; lengua azul; seroprevalencia; ELISA; factores de riesgo

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## INTRODUCTION

Bluetongue (BT) is an economically important vector-borne viral disease affecting domestic and wild ruminants worldwide. The causative agent, bluetongue virus (BTV), belongs to the genus Orbivirus within the Reoviridae family and is primarily transmitted by hematophagous Culicoides midges [1, 2, 3]. While all ruminant species are susceptible to infection, sheep typically exhibit the most severe clinical manifestations, whereas cattle and goats often remain asymptomatic despite developing protective immunity [4, 5, 6].

Serological surveillance represents a cornerstone of BTV epidemiological studies, particularly in endemic and high-risk regions. Competitive enzyme-linked immunosorbent assays targeting the highly conserved VP7 protein provide reliable detection of group-specific antibodies, serving as indicators of prior viral exposure across multiple bluetongue virus serotypes [7].

The World Organisation for Animal Health classifies bluetongue as a notifiable disease due to the disease's ability to cause major economic losses across the livestock sector, such as reduced production of meat and milk alongside disruptions to international trade [8].

A 2024 meta-analysis of African studies further emphasized regional disparities, with ruminant seroprevalence ranging from 36 to 54% depending on diagnostic methods and ecological zones [7]. Risk factor analysis, encompassing variables such as host species, geographic location, and temporal patterns, provides crucial insights into transmission dynamics and facilitates identification of epidemiological hotspots [9]. Recent mathematical modelling techniques have been established to define BTV transmission dynamics, facilitating more precise predictions of epidemic patterns and guiding evidence-based control strategies [10].

Historically, BTV distribution was restricted to tropical and subtropical regions. However, climate change, increased livestock movement, and vector adaptation have facilitated viral spread into previously unaffected temperate zones, including extensive areas of Europe and North Africa [11].

The recent outbreaks in Europe from 2023 to 2024, especially in the Netherlands, Germany, and Belgium, highlight the virus's potential for rapid geographic spread and indicate the necessity of constant surveillance measures [12, 13]. Mediterranean countries have experienced multiple BTV outbreaks, resulting in substantial economic losses, trade restrictions, and increased surveillance costs [14, 15].

The 2020–2025 period underscores bluetongue virus's expansion into new ecological niches, driven by rising temperatures and changing precipitation patterns. Goats consistently exhibit higher susceptibility than sheep, necessitating species-specific surveillance [16, 17].

Despite the documented presence of bluetongue virus in Algeria, comprehensive updated epidemiological data essential for effective surveillance and control programs remain limited [9].

Critical knowledge gaps regarding current bluetongue virus epidemiology across Algerian ruminant species persist, hindering evidence-based intervention strategies. This study aimed to determine bluetongue virus seroprevalence in domestic ruminants: sheep (Ovis aries), goats (Capra hircus), and cattle (Bos taurus) across three provinces in northeastern Algeria while simultaneously identifying potential risk factors associated with bluetongue virus seropositivity at both individual animal and herd levels.

The findings will inform evidence-based surveillance strategies and control measures for bluetongue virus in Algeria and regions with similar epidemiological characteristics.

## MATERIAL AND METHODS

### Ethical statement

The animals were studied according to the ethical principles of animal experimentation and international guidelines for animal welfare (Terrestrial Animal Health Code 2018, section 7. Art 7.5.1) and national executive decree No. 95-363 of November 11, 1995 of Algeria.

### Study design and area

This cross-sectional serological survey was conducted between March 2024 and April 2025 across three Algerian provinces (wilayas): El Tarf, Batna, and Khenchela. These regions were selected based on their diverse ecological characteristics and elevated risk for vector-borne diseases due to favorable climatic and geographic conditions (FIG. 1).

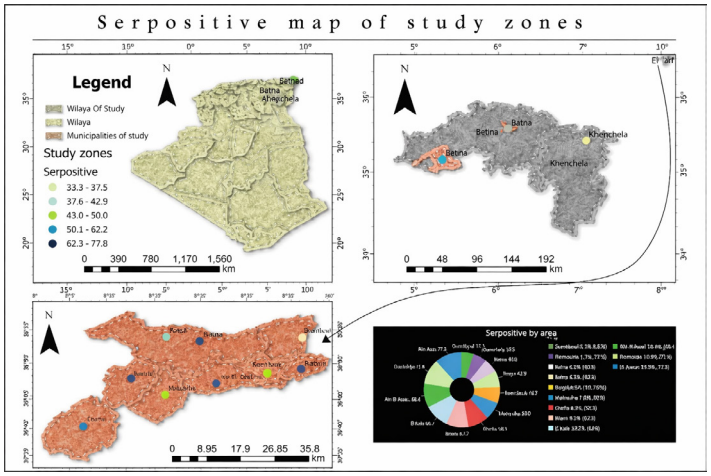


**FIGURE 1.** Geographic distribution of Bluetongue virus seroprevalence among ruminants in northeastern Algeria using Arc GIS software.

### Sample collection and processing

The sample size was determined using Thrusfield's formula for infinite populations: $n = z^2p(1-p)/m^2$. With z = 1.96 (95% confidence interval), P = 0.5 (conservative estimate in the absence of preliminary data), and m = 0.05 (5 % precision), this yielded a minimum requirement of 385 individuals. However, based on an anticipated prevalence of 24 % reported by Madani *et al.* [18], the calculated sample was reduced to 281 animals.

The population of ruminants in the study region was estimated based on data from agricultural statistics from Algeria's Ministry of Agriculture and Rural Development which

reported approximately 1.7 million sheep, 384,042 goats, and 164,037 cattle across the three study provinces.

Sample allocation was deliberately adjusted to ensure adequate numbers of each species for robust species-level comparison of bluetongue seroprevalence, as susceptibility and epidemiology differ among ruminant species. Sampling units were selected from 46 representative herds within each administrative district, with 5-10 animals sampled per herd. A total of 380 apparently healthy animals at the time of sampling were ultimately included to ensure adequate statistical power, comprising sheep (n = 187), goats (n = 92), and cattle (n = 101).

Blood samples (5-10 mL) were collected from the jugular vein using sterile vacutainer tubes without anticoagulant. Samples were maintained in cold chain (Condor CRDN570ZX, Algeria) (4-8 °C) during transport and processed within 24 hours (h) of collection. Serum was separated by centrifugation (TDZ4-WS, Bioridge, Shanghai, China) and stored in sterile cryotubes (Sarstedt CryoPure, France) at -20 °C (Congélateur CFH-T13GM03, Algeria) until analysis [17, 19].

Bluetongue virus -specific antibodies were detected using a commercial competitive ELISA kit (ID Screen® Bluetongue Competition, IDvet, France) targeting the conserved VP7 protein. The assay was performed according to the manufacturer's instructions. Briefly, serum samples were added to microplate wells pre-coated with VP7 antigen, incubated with conjugate, and developed using chromogenic substrate. Optical density was measured at 450 nm by an ELISA reader (Biotek Instruments Inc, USA), and results were expressed as sample-to-negative control percentage (S/N %). Samples were classified as: positive (S/N % ≤ 35 %), doubtful (35 % < S/N % ≤ 45 %), or negative (S/N % > 45 %).

To quantify the association between the risk factors and ruminal outcomes, the statistical analysis derived odds ratios (OR) alongside their corresponding 95 % confidence intervals.

## Data collection

For each animal, epidemiological data were recorded including species, sex, age group (< 3 years, ≥ 3 years), sampling season (spring, summer, autumn, winter), and geographic location (province and commune). The study encompassed 13 sites: Batna, Bitam, Khenchela, El Tarf, Bouteldja, Chafia, El Aioun, El Kala, Aïn El Assel, Matrouha, Oum Tboul, Raml Souk, and Tonga.

## Statistical analysis

Statistical analyses were performed using SPSS version 20.0. Seroprevalence was calculated with 95 % confidence intervals. Chi-square tests were used for univariable analysis of potential risk factors, with variables showing P ≤ 0.05 retained for multivariable analysis. Multivariable logistic regression was employed to identify independent risk factors, with model fit assessed using Hosmer-Lemeshow goodness-of-fit test [20].

Variables showing high collinearity (correlation coefficient > 0.95) were excluded to prevent statistical interference. Results are presented as odds ratios (OR) with 95 % confidence intervals, with statistical significance set at P < 0.05.

# RESULTS AND DISCUSSION

## Overall seroprevalence

Among 380 serum samples tested, 208 were seropositive for BTV antibodies, yielding an overall seroprevalence of 54.74 % (95 % CI: 49.7-59.7 %). Species-specific analysis revealed marked differences, with goats showing the highest seroprevalence (73.91 %, 68/92), followed by cattle (73.27 %, 74/101) and sheep (35.29 %, 66/187) (TABLE I).

**TABLE I**
***Seroprevalence of bluetongue virus in domestic ruminants by species as determined by competitive ELISA***

| Species | Tested (n) | Positive (n) | Negative (n) | Seroprevalence (%) | 95 % CI |
|---|---|---|---|---|---|
| Overall | 380 | 208 | 172 | 54.74 | 49.7 - 59.7 |
| Cattle | 101 | 74 | 27 | 73.27 | 63.6 - 81.5 |
| Goats | 92 | 68 | 24 | 73.91 | 63.9 - 82.3 |
| Sheep | 187 | 66 | 121 | 35.29 | 28.4 - 42.6 |

Note: n = number of animals; CI = confidence interval. Seroprevalence was determined using competitive enzyme-linked immunosorbent assay (cELISA) targeting the BTV VP7 protein

These findings reveal a BTV seroprevalence of 54.74 % in northeastern Algeria, substantially higher than previously documented rates in Algeria, 24 % in 2011 and 16.44 % in 2016 [9, 18]. This increase likely reflects either intensified viral circulation in recent years or considerable regional variation in exposure patterns across Algeria. When examining regional differences, Morocco shows the lowest prevalence at 41.7 %, while Libya and Egypt both exceed 48 %, with Egypt at about 48.37 % and Libya slightly higher at 48.4 % [16, 21, 22].

However, Ethiopia reports a substantially elevated prevalence of 84.5 % [17], nearly double the rates observed in North African region. This striking geographic gradient reflects distinct epidemiological drivers: Ethiopia's markedly higher prevalence is driven by expanded vector biodiversity. Researchers have identified twelve Culicoides species in Northwest Ethiopia, including eight previously unrecorded in the country. African meta-analyses document ruminant seroprevalence ranging from 36–54 %, with southeastern regions exhibiting the highest rates, and competitive ELISA tending to yield higher values than alternative methods [7].

Outside Africa, Peru shows markedly lower prevalence (20.34 % in cattle, 7.63 % in sheep, 8.58 % in goats [23]). These patterns underscore the dramatic geographic variation in BTV distribution globally and regionally. Peru's substantially lower seroprevalence is primarily attributable to geographic protection through altitude: regions above 3,000 meters above sea level (masl) showed reduced BTV seroprevalence, while increased maximum temperatures exceeding 30 °C were associated with greater prevalence.

Notably, seroprevalence was low in southern Peru (< 10 %), varied in central and northern regions, but high (> 30 %) in the eastern Amazon rainforest region. This suggests that Peru's extensive Andean highlands provide natural protection against vector-mediated transmission despite suitable conditions existing in lower-elevation tropical zones.

Across Asia, seroprevalence rates tend higher: Bangladesh documents 39.3 % in small ruminants with grazing management and water body proximity emerging as significant risk factors

[24] while Pakistan reports 52 %, particularly among sedentary farming systems [25]. A large-scale seroepidemiological study conducted in Iran revealed that 56.13 % of animals testing positive for Bluetongue virus antibodies [26]. Chinese cattle meta-analysis (1988-2019) reveals pooled seroprevalence of 12.2 %, with substantial regional variation linked to Culicoides species diversity [27].

These global patterns suggest that seroprevalence variations reflect multiple interconnected factors: methodological considerations including sample size and sampling design, ecological variables such as spatiotemporal context and seasonal vector activity, and epidemiological dynamics including vaccination strategies and viral evolution [27].

This multivariable analysis identified animal species as the strongest predictor of bluetongue virus seropositivity. Compared to sheep, cattle demonstrated seven-fold higher odds of infection (OR = 7.746, 95 % CI: 3.621-16.571), while goats showed nine-fold higher odds (OR = 9.044, 95 % CI: 4.440-18.422).

These marked differences reflect fundamental variations in host-pathogen interactions. Cattle and goats maintain higher antibody prevalence through subclinical infections and prolonged antibody persistence, whereas sheep typically exhibit lower seroprevalence potentially due to higher case-fatality rates during acute outbreaks or management practices that reduce vector exposure [5, 7].

Notably, our observed goat seroprevalence of 73.9 % aligns with reports from South Asia and Africa, which similarly document elevated viropositivity in goats compared to sheep, reinforcing their significance as viral reservoirs in endemic regions [16, 17] (TABLEII).

**TABLE II**
***Univariable and multivariable analysis of risk factors associated with bluetongue virus seropositivity in ruminants***

| Variable | Category | Univariable Analysis | | | Multivariable Analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | | Positive/Total (%) | $\chi^2$ | P-value | OR (95 % CI) | Wald | P-value | |
| **Species** | Sheep | 66/187 (35.3) | 47.89 | < 0.001 | 1.00 (Reference) | 46.998 | < 0.001 | |
| | Cattle | 74/101 (73.3) | | | 7.746 (3.621 -16.571) | 27.837 | < 0.001 | |
| | Goats | 68/92 (73.9) | | | 9.044 (4.440 -18.422) | 36.795 | < 0.001 | |
| **Sex** | Female | 133/237 (56.1) | 0.486 | 0.486 | | NS | | |
| | Male | 75/143 (52.4) | | | | | | |
| **Province** | Tonga | 9/21 (42.9) | 12.45 | 0.006 | 1.00 (Reference) | 15.959 | 0.068 | |
| | Aïn El Assel | 13/19 (68.4) | | | 2.056 (0.498 -8.491) | 0.991 | 0.319 | |
| | Batna | 20/50 (40.0) | | | 0.908 (0.333 -2.474) | 0.036 | 0.850 | |
| | Bitam | 28/45 (62.2) | | | | | | |
| | Bouteldja | 15/21 (71.4) | | | 3.447 (0.815 -14.586) | 2.828 | 0.093 | |
| | Chafia | 21/36 (58.3) | | | 2.504 (0.755 -8.301) | 2.253 | 0.133 | |
| | El Aioun | 14/18 (77.8) | | | 13.191 (2.660 -65.421) | 9.969 | 0.002 | |
| | El Kala | 44/66 (66.7) | | | 3.367 (1.092 -10.379) | 4.466 | 0.035 | |
| | Khenchela | 18/48 (37.5) | | | | | | |
| | Matrouha | 16/32 (50.0) | | | 1.924 (0.528 -7.017) | 0.982 | 0.322 | |
| | Oum Tboul | 3/9 (33.3) | | | 3.556 (0.610 -20.747) | 1.988 | 0.159 | |
| | Raml Souk | 7/15 (46.7) | | | 0.877 (0.186 -4.132) | 0.028 | 0.868 | |
| **Wilaya** | Batna | 48/95 (50.5) | 8.98 | 0.011 | | NS | | |
| | El Tarf | 142/237 (59.9) | | | | | | |
| | Khenchela | 18/48 (37.5) | | | | | | |
| **Season** | Autumn | 48/94 (51.1) | 7.89 | 0.048 | 1.00 (Reference) | 17.137 | 0.001 | |
| | Spring | 33/63 (52.4) | | | 1.025 (0.466 -2.255) | 0.004 | 0.951 | |
| | Summer | 85/164 (51.8) | | | 0.642 (0.341 -1.209) | 1.883 | 0.170 | |
| | Winter | 42/59 (71.2) | | | 3.400 (1.526 -7.574) | 8.968 | 0.003 | |
| **Age Group** | <3 years | 132/235 (56.2) | 0.51 | 0.475 | | NS | | |
| | ≥3 years | 76/145 (52.4) | | | | | | |

Note. OR = odds ratio; CI = confidence interval; NS = not significant. Ref = reference category

The elevated seroprevalence in cattle and goats contrasts sharply with observations from the 2023 BTV-3 outbreak in the Netherlands, where cattle served as key amplifying hosts while sheep experienced more severe clinical disease with mortality rates up to 15.5 times higher than baseline [13].

This discrepancy helps explain regional patterns: sheep's higher clinical susceptibility can result in increased mortality during acute outbreaks, thereby reducing the proportion of animals surviving to develop detectable antibodies.

Species-specific patterns also vary by geography: southern Italian studies documented adult cattle and water buffalo seroprevalence of 43.6 %, with temperature identified as the primary climatic predictor [15]. These trends underscore species-specific immunological responses and varying exposure risks tied to husbandry practices, extending beyond simple susceptibility differences.

Geographic location emerged as a significant risk determinant. Animals from El Aioun demonstrated substantially higher infection odds (OR = 13.191, 95 % CI: 2.660-65.421) compared to Tonga, while El Kala similarly showed elevated risk (OR = 3.367, 95 % CI: 1.092-10.379). Both border areas exhibited notably elevated seroprevalence.

El Aioun at 77.8 % and El Kala at 66.7 % and share common characteristics: proximity to Tunisia and lower altitude. These patterns likely reflect enhanced viral circulation facilitated by cross-border livestock movement and shared ecological conditions favoring vector populations.

The Mediterranean climate and dense vegetation in coastal regions provide optimal breeding conditions for Culicoides species, establishing these areas as epidemiological hotspots. Molecular identification studies in Algeria have documented *C. imicola, C. obsoletus,* and *C. pulicaris*, all with known or suspected vector competence [28].

Altitude consistently influences bluetongue virus risk through vector distribution: African high-risk zones occur below 1500 meters, where elevated temperatures and humidity support midge breeding [7].

Winter sampling was associated with significantly higher seropositivity odds (OR = 3.400, 95 % CI: 1.526-7.574) compared to autumn, a pattern that contrasts with typical temperate zone dynamics where peak transmission occurs during warmer months.

This finding aligns with observations from Egypt, where non-hot seasons showed elevated seroprevalence, potentially reflecting Mediterranean climate patterns where mild winters maintain vector activity while summer heat may reduce transmission efficiency [22].

Since serological surveys detect cumulative exposure rather than active infection, winter sampling may capture antibodies from autumn transmission peaks when Culicoides activity reaches maximum levels in Mediterranean climates [29]. Climatic factors directly influence vector-borne transmission: African studies correlate seroprevalence with ecosystems supporting Culicoides activity at temperatures of 12–32 °C [30].

Sex and age showed no significant associations with BTV seropositivity in our analysis, contrasting with some previous reports [15]. This pattern suggests that in endemic settings, demographic factors exert limited influence as all population segments experience uniform exposure. However, African studies documented higher susceptibility in adult versus juvenile small ruminants and in indigenous versus crossbred animals, reflecting cumulative exposure and potential genetic susceptibility differences [16, 17]. Breed susceptibility further complicates risk profiles, with indigenous breeds in Africa and Asia showing

higher seroprevalence than cross-breeds, likely due to genetic adaptation gaps [7, 25].

These cross-sectional design prevents determination of infection timing or temporal trends, and geographic focus limits broader generalizability. Wide confidence intervals for some odds ratios (particularly El Aioun) indicate considerable uncertainty. This is primarily attributable to the limited number of animals available for sampling in El Aioun, a sparsely populated border region with Tunisia where ruminant populations are smaller compared to other study areas.

Despite this limitation, the elevated seroprevalence observed in this region (77.8 %) highlights its epidemiological importance as a cross-border zone with shared disease dynamics with Tunisia. Future studies in border regions would benefit from larger sample sizes and coordinated cross-border surveillance to better characterize bluetongue transmission in these epidemiologically critical areas.

The competitive ELISA method, while highly sensitive and specific for detecting group-specific BTV antibodies, cannot differentiate between recent and historical infections or identify specific viral serotypes. This limitation is particularly relevant for understanding serotype replacement dynamics and vaccination program impacts.

## CONCLUSION

This study demonstrates widespread BTV circulation among ruminants in northeastern Algeria, with significant species and geographic variation in seroprevalence. The elevated infection rates in cattle and goats compared to sheep, combined with high prevalence in border regions, highlight the complex epidemiology of BTV in this Mediterranean setting. The identification of El Aioun and El Kala as high-risk areas, coupled with seasonal transmission patterns, provides crucial information for designing targeted surveillance and control strategies.

These findings underscore the need for enhanced cross-border collaboration, particularly with Tunisia, and implementation of risk-based surveillance focusing on high-prevalence areas and susceptible species. Future research should incorporate longitudinal studies and molecular characterization to better understand viral circulation dynamics and serotype distribution in this region.

### Conflict of interest

The authors declare that they have no conflict of interest.

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