Original Article

Bovine respiratory syncytial virus: First identification in eastern Algeria based on direct immunofluorescence and ELISA tests

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Abstract

Bovine respiratory syncytial virus (BRSV) is the main agent of the bovine respiratory disease complex (BRD). Its circulation among the Algerian bovine population has been strongly suspected but never confirmed. This study investigated the prevalence of BRSV in cattle in eastern Algeria. From 2022 to 2023, a total of 225 serum and 102 lung tissue samples were collected from farms and slaughtered cattle of different ages. Samples were tested for BRSV antibodies by Enzyme-Linked Immunosorbent Assay (ELISA) and BRSV antigens by Direct Immunofluorescence Test (DFAT). Histopathological examination was also performed on lung tissue sections. BRSV-specific antibodies were detected in 91.11% (205/225) of serum samples, while only 27.45% (28/102) of lung tissue sections harbored the BRSV antigen. Only a few BRSV-positive cattle lungs had syncytial cells. BRSV positivity rates by ELISA and DFAT were not age-related ($P>0.05$). On the contrary, semi-quantitative ELISA titers were significantly higher ($P<0.01$) in adult cattle (82.25%) than in young cattle (73.00%). Finally, for the 102 cattle concurrently tested by ELISA and DFAT, the BRSV positivity rate yielded by ELISA was higher (95% vs 27.45%). This discrepancy highlights variations in the temporal dynamics of BRSV infection within the studied cattle population. The high seroprevalence of BRSV in subclinically infected cattle in eastern Algeria suggests that the virus is endemic in the study region's cattle populations. This represents the first epidemiological study on the prevalence of BRSV in Algeria. Further research is needed to better determine how the virus spreads throughout the country.

Keywords: Algeria, bovine respiratory syncytial virus, direct immunofluorescence test, enzyme-linked immunosorbent assay, endemic, seroprevalence

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Introduction

Bovine respiratory syncytial virus (BRSV) is a significant pathogen that affects the upper and lower bovine respiratory tract, causing serious economic losses to the livestock industry. The virus is a component of the bovine respiratory disease complex (BRD) along with parainfluenza and herpesviruses as well as *Pasteurella multocida*, *Mannheimia haemolytica*, and *Mycoplasma bovis* (Sacco *et al*., 2014). The clinical manifestation of the disease is marked by respiratory signs, which are particularly severe in peripartum cows and young naïve calves (Hägglund *et al*., 2022). The virus spreads through horizontal transmission, facilitated by close animal contact, with infections frequently occurring during the winter housing period *(Stott et al., 1980; Hägglund et al*., 2006). Reinfections may occur during the summer (Baker *et al*., 1997; Bidokhti *et al*., 2012), indicating viral endemicity and persistence, likely due to the presence of immunologically naive animals (young calves) in the herd that help maintain viral infection. Thanks to viral gene sequencing, Larsen (2000) and Bidokhti *et al*. (2012) demonstrated that the cases involve new infections rather than viral persistence.

BRSV is an enveloped virus with a negative sense single-stranded RNA (Valarcher and Taylor, 2007). Its structure includes surface glycoproteins critical for viral attachment and entry into the respiratory epithelial cells, promoting the formation of multinucleated syncytia (Arbiza *et al*., 1992). These giant cells allow the virus to evade the extracellular environment where immune components and antiviral drugs are active (Cifuentes-Muñoz and Dutch, 2019). The development of immunity to BRSV is a complex process involving both humoral and cellular immune responses. Neutralizing antibodies, primarily targeting the viral fusion (F) and attachment (G) glycoproteins, are critical for preventing viral spread and reducing disease severity (Maina *et al*., 2023). Concurrently, activation of T lymphocytes contributes to clearing virus-infected cells (Taylor *et al*., 1995).

The labile nature of BRSV makes its detection through isolation a real challenge (West *et al*., 1998), prompting increased interest in serological methods such as viral neutralization test (VNT), complement fixation (CF), enzyme-linked immunosorbent assay (ELISA) or alternatively fluorescent antibody test (FAT) as a viral antigen detection method. These tests can be coupled with histological examination of lung tissue, even though the microscopic lesions caused by respiratory viruses are indistinguishable, and the syncytial cells characterizing BRSV infection may be associated with other viral infections such as bovine parainfluenza virus (BPIV), and human respiratory syncytial virus (HRSV), which primarily infects humans, but can also infect cattle and cause respiratory disease (Thomas *et al*., 1998). Recently, BRSV was successfully detected using near-infrared aquaphotomics, a technique based on biochemical modifications in exhaled breath condensate occurring during BRSV infection (Santos-Rivera *et al*., 2022).

ELISA offers the advantage of being simple, rapid, and well-adapted for large samples. It is suitable for determining seropositivity but does not distinguish between recent and past infections. FAT, based on an immunodetection of BRSV antigens, can detect nonviable viruses not isolated by a culture method (Johnston and Siegel, 1990), making the technique less dependent on transport and storage conditions. FAT is suitable for diagnosing acute infections and is limited by the small quantity of viral antigens remaining in infected tissue from past infections. Since its first isolation in Switzerland in 1970 (Paccaud and Jacquier, 1970) and in the USA 4 years later (Rosenquist, 1974), BRSV is found worldwide and is endemic in many cattle-producing regions. Cases of BRSV infections have long been suspected in Algeria. This suspicion, based on signs of pneumonia or lung lesions observed at the slaughterhouse, has never been confirmed. The main objective of the present study was to detect BRSV in cattle in the eastern region of Algeria, utilizing two immunological tests, namely the indirect ELISA technique and the direct immunofluorescent antibody test (DFAT). Additionally, histological examination was performed to study the microscopic lung lesions associated with this viral infection.

Materials and Methods

Study population and area: The studied bovine population hails from Batna and Setif provinces, situated in eastern Algeria at elevations of 1045 meters and 1100 meters above sea level, respectively. This region has a cold, semi-arid climate and is home to over 116,000 cattle (Hadef *et al*., 2022). For the study's purpose, 225 cattle were selected using stratified random sampling. This population includes non-BRSV-vaccinated animals of both sexes and different ages. Sampling spanned two consecutive years (2022-2023) during the winter and spring seasons. The sampling period has been determined to align with the weather conditions of the temperate climate in the eastern region of Algeria, which is conducive to BRSV infection. The 225 cattle used for sampling were sourced from farms (123 cattle) and the local slaughterhouse (102 cattle). The slaughterhouse, situated in the province of Batna, receives cattle not only from the province but also from the neighboring limotrophic provinces (Constantine, Khenchela, Setif). Conversely, all farms are located in the municipalities of Batna (Djerma, El-Madherr, Ain Yagout) and Setif (Bir El Arch, and Bazer Sakhra) provinces. The map illustrating the study area (Fig. 1), was generated using QGIS® (QGIS Desktop 3.28.10).

Blood sampling: Blood sampling was carried out systematically, encompassing both symptomatic and asymptomatic animals, and no selection criteria were applied to cattle sampled either at the slaughterhouse or on farms. Blood samples were collected from the jugular vein or mammary vein using 4 mL and 9 mL Veterinary Vacutainer dry tubes with vacutainer collection needles. Subsequently, these samples were transported to the laboratory under refrigeration within two hours. Sera were obtained through centrifugation at 6000g for 15 minutes at 4°C. All serum samples were then stored in 1.5 ml Eppendorf tubes at –80°C until performing ELISA test.

Tissue sampling: For immunofluorescence testing, lung tissue samples were collected from 102 slaughtered cattle irrespective of the presence or absence of clinical signs suggestive of respiratory disease. However, only lungs exhibiting evident macroscopic lesions were sampled for the histological study (42 samples). Sampling was specifically limited to the cranial lobe of the right lung. The sampled tissues, ranging from 3 to 4 cm in size, were either stored at −80°C or conserved in 10% neutral buffered formalin (NBF), depending on whether they were intended for immunofluorescence testing or histological examination.

ELISA test: The detection of BRSV-specific antibodies was performed using an indirect ELISA kit BIO K 061/5® (BioX Diagnostics, Belgium), consisting of 96-well polyvinyl chloride microtiter plates coated with specific BRSV F protein monoclonal antibodies (mAB). The reported sensitivity and specificity of the commercial kit were 93% and 87%, respectively. The odd columns of the plate contained the viral-specific protein, while even columns held a control antigen, which helped minimize false positives. The test was conducted according to the manufacturer's instructions. The optical density (OD) value was detected using an ELISA plate reader set at 450 nm (BioTek 800 TS Absorbance Reader). The corrected optical density (cOD) of samples, positive controls, and negative controls is calculated by subtracting the optical density (OD) value of the even-numbered columns from the OD value of the odd-numbered columns of the plate (cOD=ODodd columns −ODeven columns). The ELISA sample-to-positive (S/P) ratio was calculated as follows: cOD positive sample/cOD positive control. The S/P ratio cutoff was set at 0.2 as indicated by the manufacturer. After test validation, semi-quantitative interpretation was carried out as shown in Table 1.

Direct immunofluorescent antibody test (DFAT): DFAT was performed to detect specific BRSV antigens in frozen lung tissue sections using a commercial kit Bio 032® (BioX Diagnostics, Belgium), which consists of fluorescein isothiocyanate-labeled mouse anti-BRSV monoclonal antibody. The test was conducted according to the manufacturer's instructions. Briefly, cryo-tissue sections cut at 4 µm were promptly placed on X-tra adhesive positively charged surface slides (Leica®). Subsequently, they were fixed in an Acetone-PBS solution for 10 minutes, washed with PBS-Evans Blue solution, air-dried, and treated with fluorescently labeled antibodies, previously diluted 20-fold in PBS-Evans Blue solution (1:20). After 1-hour incubation at room temperature, the slides were rinsed, mounted with glycerol buffer, coverslipped, and examined for fluorescence.

Histopathological examination: To look for lung microlesions, 4-5 µm thick sections were sliced from paraffin-embedded tissues using a rotary microtome (Leica RM 2125 RTS). Hematoxylin and eosin (H&E) staining were then performed following the standard procedure.

Statistical analysis: The results from ELISA and immunofluorescence tests were subjected to statistical analysis using IBM SPSS Statistics 25. The chi-squared test was executed to determine whether the age of the animals had any effect on the BRSV seropositivity rate obtained by ELISA or DFAT. A *P*-value of less than 0.05 was considered statistically significant**.**

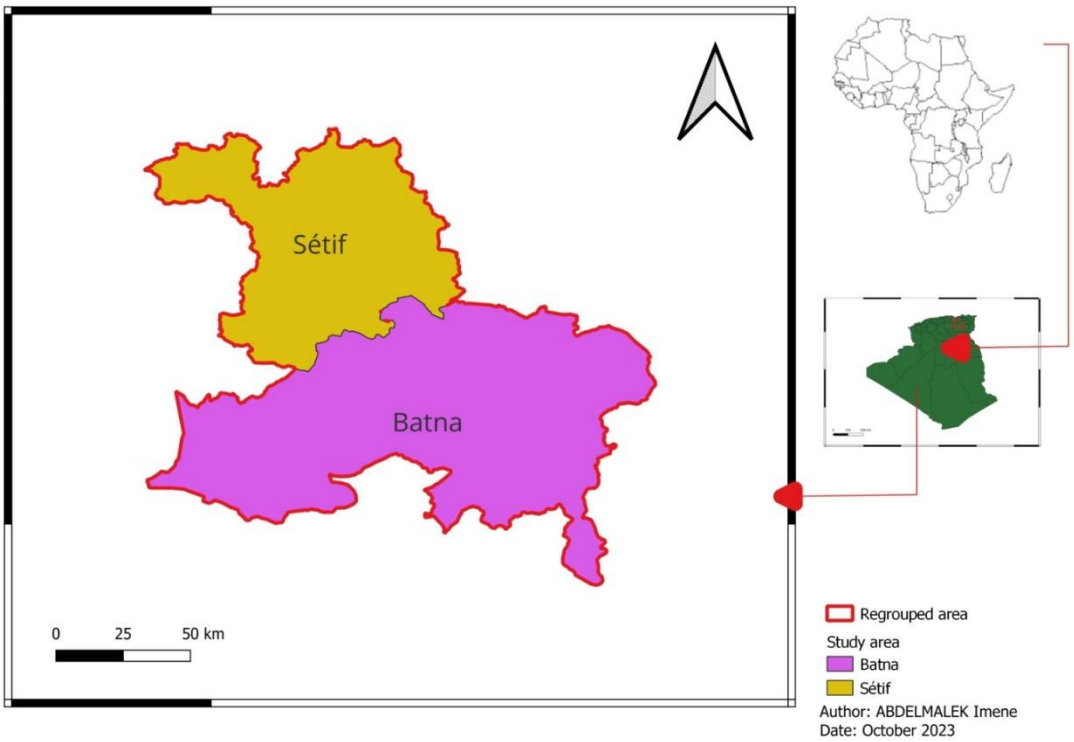

Figure 1 Study area in the eastern region of Algeria.

Table 1 Interpretation of ELISA test.

S/P Value	0<Val≤ 0.2	0.2<Val≤0.4	0.4<Val≤0.6	0.6<Val≤0.8	0.8<Val≤1	1<Val
Interpretation	Negative	+	++	+++	++++	+++++
Immunity level	Zero	Low	Medium		High	

Results

Indirect ELISA test: Of the 225 sera collected from farms and the slaughterhouse, 205 (91.11%) were positive for BRSV, showing varying levels of antibodies: low (15.56%), medium (43.56%), and high (32%). The seroprevalence of BRSV exhibited variation among different age groups. While all (30/30) of the oldest animals (more than 6 years) were seropositive, the positivity rates were 90.62%, 89.06%, and 91.42% for animals 2-6 years, 1-2 years, and less than 1 year, respectively (Table 2). The difference in seroprevalence among the 4 age groups was not statistically significant (P>0.05). However, a significant difference in antibody levels among seropositive animals based on their age was observed (P<0.01) (Fig. 2).

Direct immunofluorescent antibody test (DFAT): Out of the 102 samples, 28 (27.45%) tested positive for BRSV antigens through DFAT (Table 2). Statistical analysis showed no age-related effect on BRSV positivity (P>0.05). Fluorescence microscopy of positive specimens showed diffuse reticular fluorescence (Fig. 3a, b) in most of the infected lung tissues, with more intense fluorescent areas located in the bronchiolar epithelium (Fig. 3c, d) and occasionally in the alveolar epithelium, as shown in Figure 3.

Histological findings: Macroscopic examination revealed widespread lesions of interstitial pneumonia and bronchopneumonia in the cranioventral lobes of the sampled lungs, featuring brown consolidation associated with enlarged lymph nodes. The pleura exhibited thickness and cloudiness in some cases, with interlobular adhesions and deflation of the diaphragmatic lobes in two cases. Advanced stages of emphysema were observed in many cases. Microscopically, the lesions were characterized by syncytial cell formation, thickening and fibrosis of the interalveolar septa, and interstitial hemorrhage. Edema and emphysema lesions were also observed in many cases (Fig. 4).

Table 2 Rates of BRSV positivity as determined by ELISA and DFAT in the different age groups.

Age group	ELISA				DFAT	
	Negative	Positive			Negative	Positive
		Low	Medium	High		
0 < ... < 1	8.57% (3/35)	20% (7/35)	48.57% (17/35)	22.86% (8/35)	71.43% (10/14)	28.57% (4/14)
1 ≤ ... < 2	10.94% (14/128)	15.63% (20/128)	36.72% (47/128)	36.72% (47/128)	72.09% (62/86)	27.91% (24/86)
2 ≤ ... < 6	9.38% (3/32)	18.75% (6/32)	46.88% (15/32)	25.00% (8/32)	/	/
6 ≤ ...	0.00% (0/30)	6.67% (2/30)	63.33% (19/30)	30.00% (9/30)	100.00% (2/2)	0.00% (0/2)
Total	8.89% (20/225)	15.56% (35/225)	43.56% (98/225)	32.00% (72/225)	72.55% (74/102)	27.45% (28/102)
			91.11% (205/225)			

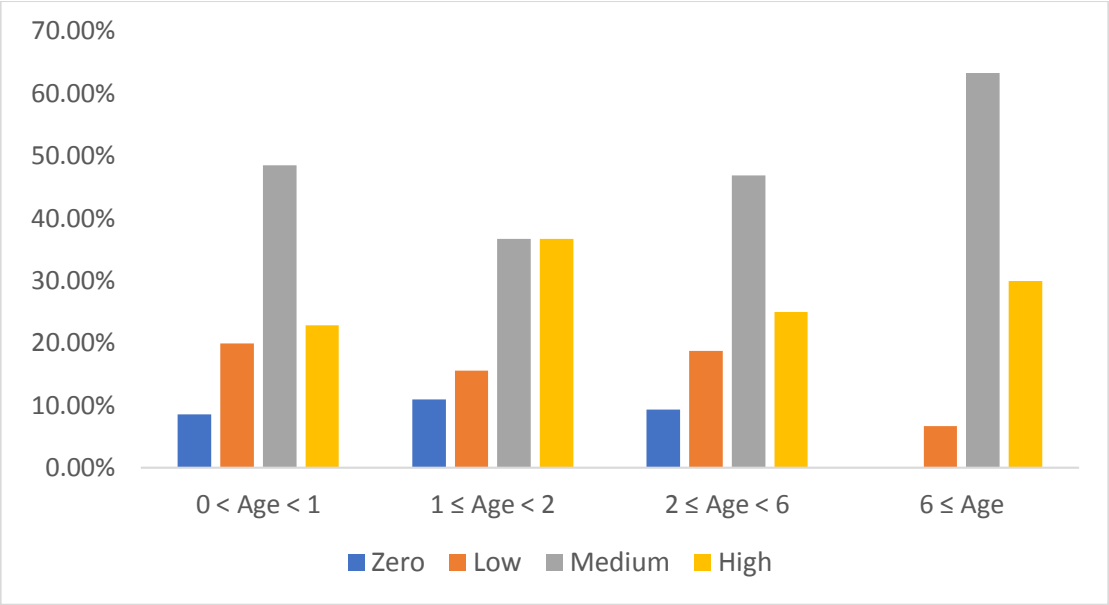

Figure 2 Levels of immunity to BRSV in the 4-age groups.

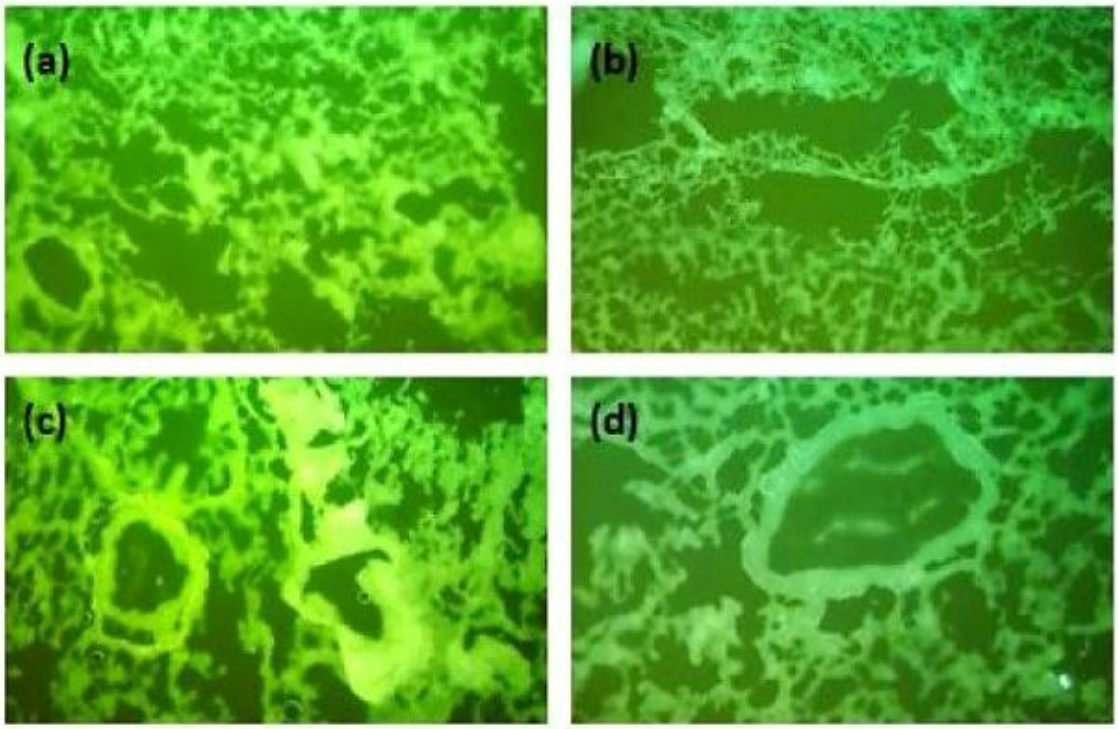

Figure 3 Representative immunofluorescent images of frozen lung sections from calves diagnosed positive for BRSV antigens. (a, b) BRSV fluorescent staining was noted as reticular diffuse fluorescence in the lung epithelium; (c, d) BRSV fluorescent staining was localized to bronchiolar epithelial cells.

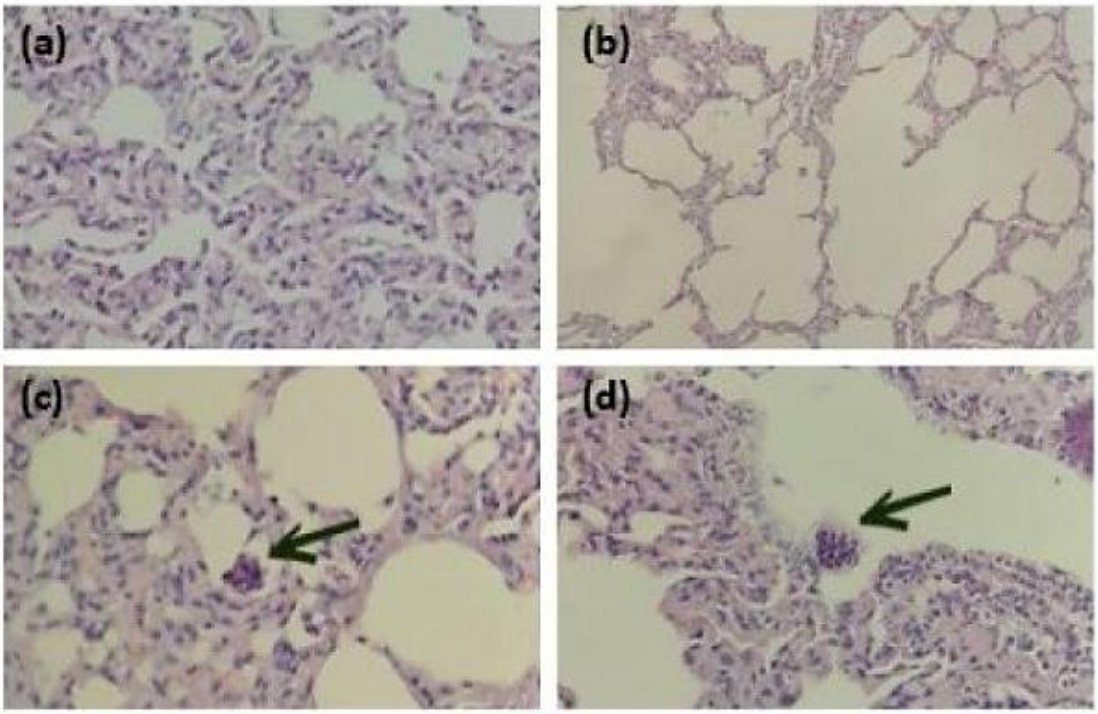

Figure 4 Histopathology of BRSV pneumonia (a) inflammation of the lung parenchyma with interstitial hemorrhage; (b) advanced stage of emphysema with destruction of the interalveolar septa; (c, d) formation of syncytia (giant cells) (black arrows) due to fusion of epithelial lung cells.

Discussion

This is the first survey to focus on the circulation of BRSV in cattle in Algeria. The study was carried out using immunological tests supplemented by histological examination. The indirect ELISA test revealed a high seroprevalence of 91.11%. Similar rates, indicating endemic circulation of BRSV, have been reported in England (Paton *et al*., 1998), Venezuela (Obando *et al*., 1999), Iraq (Hussain *et al*., 2019), United States of America (Collins *et al*., 1988), Mexico (Solís-Calderón *et al*., 2007), and Ethiopia (Woldemeskel *et al*., 2000) with BRSV antibody-positive rates of 100%, 85%, 83%, 95%, 99% and 92% respectively. The reliability of the seroprevalence found in our study as an indicator of virus circulation is further enhanced by the fact that vaccination against BRSV is not practiced in Algeria. Furthermore, only two of the calves sampled in this study were less than 8 months old. Consequently, the antibodies detected in the studied animals are neither vaccine-induced nor maternally derived, ensuring that the high seroprevalence authentically reflects the natural exposure and circulation of BRSV within the bovine population.

The age of cattle may influence the seroprevalence of BRSV infection, with adult cattle having a higher prevalence compared to younger animals (Bidokhti *et al*., 2009; Ferella *et al*., 2018). However, some studies present contradictory findings. Hussain *et al*. (2019) recorded a significantly higher seroprevalence in cattle aged 7-18 months compared to older animals, while 70% of calves in England were seropositive at 9 months of age (Stott *et al*., 1980) and 50% of cattle tested seropositive in France were calves under 12 months of age (Perrin *et al*., 1979). In our study, no significant difference in seropositivity was observed between the 4 age groups (P>0.05). The lower seropositivity in young calves has previously been attributed to two potential factors: i) reduced exposure to the virus in early life stages and ii) inadequate immune response to natural infection, possibly due to the suppressive effect of Maternal-Derived Antibodies (MDA) or the immaturity of the immune system in very young calves (Guzman *et al*., 2015). In our study, only 2 of the 30 calves sampled were less than 8 months old, the age at which calves are fully immunocompetent (Cortese, 2009) and have no trace of MDA (Uttenthal *et al*., 2000; Ferella *et al*., 2020). Such calves are well able to seroconvert in response to a possible natural infection. Additionally, close contact between animals and crowding have been identified as crucial risk factors for BRSV transmission (Baker *et al*., 1997; Ferella *et al*., 2018). On cattle farms in Algeria, it is common for post-weaning calves to be exposed to newly integrated adult bovines whose health status is not well-documented, thereby increasing the risk of BRSV transmission to calves before they are one year old. Although age-related seropositivity remains controversial, it is now well established that young calves are clinically more affected than adults (Hägglund *et al*., 2022). In fact, calves are considered naïve, and studies do not universally agree on the full protective role of MDA against BRSV infection (Kimman *et al*., 1988; Baker *et al*., 1993; Van der Poel *et al*., 1994; Baker *et al*., 1997). Moreover, MDA may exacerbate the clinical expression of the disease in calves via an antigen-antibody response (Ogilvie *et al*., 1981; Kimman *et al*., 1988). When compared, the semi-quantitative ELISA titers obtained in our study were significantly higher (P<0.01) in adult animals than in

those less than 2 years of age, with medium-to-high antibody levels estimated at 82.25% and 73%, respectively. This finding was expected and reflects the boosting effect of natural reinfections on the immune response of adults, which are more frequently infected than younger animals (Van der Poel *et al*., 1993). The DFAT was performed on 102 frozen tissue sections from young cattle less than 2 years of age. Only 2 older cattle (over 6 years) were tested and found negative. Of the 102 samples tested, 28 were positive (27.45%). Using the same test, Ceribasi *et al*. (2014) have found similar rate (27.94) in 247 cattle in Turkey. The positive rates in the 0-1 year and 1–2-year age groups were 28.57% and 27.91%, respectively, with no significant difference between the 2 age groups ($P>0.05$). Specific fluorescence was diffused throughout the lung tissue in 85.71% (24/28) of positive samples, with the remainder (14.28%) having fluorescence confined to the bronchiolar epithelium.

When considering the 102 samples concurrently tested with ELISA and DFAT, ELISA yielded a higher positivity rate (95%) compared to the rate revealed by DFAT (27.45%). This could be attributed to the different temporal dynamics of BRSV infection within the tested cattle. Some animals may have been infected for some time, hence having circulating antibodies without harboring viral antigens in their lung tissue. This argument is supported by the fact that none of the seronegative cattle tested positive for DFAT. In other words, all the antibody-free animals (5/102) had no viral antigen in the lungs. Under experimental conditions, (Trigo *et al*., 1984), reported no positive fluorescence in lungs from days 6 to 12 after inoculation, while BRSV-specific serum IgG persists for at least 3 months following initial infection (Kimman *et al*., 1989). This relatively rapid viral clearance makes DFAT suitable primarily for diagnosing acute or recent infections. The observed discrepancy in positivity rates between ELISA and DFAT may also be due to the limited size of the lung tissue fragments analyzed in the immunofluorescence assay (4 to 5 cm), potentially leading to the omission of localized viral particle concentrations within the lung lesions.

Inconsistencies between ELISA and DFAT results have been previously recorded. In India, a study conducted in 2020 reported a BRSV detection rate by DFAT of only 3% (Kamdi *et al*., 2020), significantly lower than the seroprevalence of 47% and 50% reported in studies conducted in the same country 2 and 1 year earlier, respectively (Goswami *et al*., 2017; Katoch *et al*., 2017). Lung tissue samples from DFAT-positive cattle were examined microscopically. Microscopic lesions previously associated with BRSV infection, such as fibrosis, interstitial hemorrhage, thickening of inter-alveolar septa, and emphysema (Ide *et al*., 1969; Pirie *et al*., 1981; Kimman *et al*., 1989; Narita *et al*., 2000; Cashwell and Williams, 2007), were observed in our BRSV-positive specimens. The specific, but not exclusive, histologic modification associated with respiratory syncytial viruses, i.e., epithelial syncytia formation, has been noted in a few cases. It is well established that the level of multinucleated syncytial cell production by the virus determines the course and severity of BRSV infection (Taylor, 2017; Jessie and Dobrovolny, 2021). Their production is particularly high during the acute phase of the infection, which explains their absence in most of the samples examined since the animals sampled did not show any symptoms consistent with acute infection at antemortem examination.

In conclusion, the present study confirms the long-suspected circulation of BRSV in Algeria. Interestingly, the high seroprevalence found was associated with the subclinical form of the infection, suggesting that BRSV is endemic in the study area. Seropositive cattle carrying the virus subclinically are an important source of the virus and contribute significantly to the spread of infection. These findings highlight the shortcomings of health management in Algerian cattle farms.

Conflicts of interest: There were no conflicts of interest that may have biased the work reported in this study.

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References

Arbiza J, Taylor G, López JA, Furze J, Wyld S, Whyte P, Stott EJ, Wertz G, Sullender W and Trudel M 1992. Characterization of two antigenic sites recognized by neutralizing monoclonal antibodies directed against the fusion glycoprotein of human respiratory syncytial virus. J Gen Virol. 73: 2225–2234.

Baker JC, Ames TR, Belknap EB, Dubovi EJ, Bryson DG and Kelling CL 1993. BRSV (Bovine respiratory syncytial virus) infection: its pathogenesis, diagnosis, prevention and treatment. Vet Med. 88: 880–906

Baker JC, Ellis JA and Clark EG 1997. Bovine respiratory syncytial virus. Vet Clin North Am Food Anim Pract. 13: 425–454.

Bidokhti MR, Tråvén M, Fall N, Emanuelson U and Alenius S 2009. Reduced likelihood of bovine coronavirus and bovine respiratory syncytial virus infection on organic compared to conventional dairy farms. Vet J. 182: 436–440.

Bidokhti MR, Tråvén M, Ohlson A, Zarnegar B, Baule C, Belák S, Alenius S and Liu L 2012. Phylogenetic analysis of bovine respiratory syncytial viruses from recent outbreaks in feedlot and dairy cattle herds. Arch Virol. 157: 601–607.

Cashwell JL and Williams K 2007. The respiratory system. In: Jubb KVF, Kennedy PC, Palmer N (Eds.), Pathology of Domestic Animals, 5th ed. Maxie MG (Eds.), Saunders Elsevier, Edinburgh, UK, 594-622.

Ceribasi AO, Ozkaraca M, Ceribasi S, Ozer H 2014. Histopathologic, immunoperoxidase and immunofluorescent examinations on natural cattle pneumonia originated from Parainfluenza type 3, Respiratory Syncytial virus, Adenovirus type 3 and Herpesvirus type 1. Rev Med Vet. 165: 201–212.

Cifuentes-Muñoz N and Dutch RE 2019. To assemble or not to assemble: The changing rules of pneumovirus transmission. Virus Res. 265: 68–73.

Collins JK, Teegarden RM, MacVean DW, Smith GH and Frank GR 1988. Prevalence and specificity of antibodies to bovine respiratory syncytial virus in sera from feedlot and range cattle. Am J Vet Res. 49: 1316–1319.

Cortese VS 2009. Neonatal immunology. Vet Clin North Am Food Anim Pract. 25: 221–227.

Ferella A, Aguirreburualde MSP, Margineda C, Aznar N, Sammarruco A, Santos MJD and Mozgovoj M 2018. Bovine respiratory syncytial virus seroprevalence and risk factors in feedlot cattle from Córdoba and Santa Fe, Argentina. Rev Argent Microbiol. 50: 275–279.

Ferella A, Aguirreburualde MSP, Sammarruco A, Parreno V, Santos MJD and Mozgovoj M 2020. Dynamics of neutralizing antibodies against Bovine respiratory syncytial virus in a dairy herd from Santa Fe Province, Argentina. Rev Argent Microbiol. 52: 293–297.

Goswami P, Banga HS, Mahajan V, Singh ND, Deshmukh S and Brar RS 2017. Detection of multiple antibodies and risk factor association of common respiratory viruses in the state of Punjab, India. Int J Curr Microbiol App Sci. 6: 567–577.

Guzman E and Taylor G 2015. Immunology of bovine respiratory syncytial virus in calves. Mol. Immunol. 66: 48–56.

Hadef A, Righi S, Boucheikhchoukh M and Bouzid CE 2022. Pattern and major reasons of cattle red offal condemnation in the slaughterhouse of the Arid Region of El Oued (Algeria). Agriculture. 12: 1377.

Hägglund S, Svensson C, Emanuelson U, Valarcher JF and Alenius S 2006. Dynamics of virus infections involved in the bovine respiratory disease complex in Swedish dairy herds. Vet J. 172: 320–328.

Hägglund S, Näslund K, Svensson A, Lefverman C, Enül H, Pascal L, Siltenius J, Holzhauer M, Delabouglise A and Österberg J 2022. Longitudinal study of the immune response and memory following natural bovine respiratory syncytial virus infections in cattle of different age. Plos One. 17: e0274332.

Hussain KJ, Al-Farwachi MI and Hassan SD 2019. Seroprevalence and risk factors of bovine respiratory syncytial virus in cattle in the Nineveh Governorate, Iraq. Vet World. 12: 1862.

Ide PR, Thomson RG and Ditchfield WJB 1969. Experimental adenovirus infection in calves. Can J Comp Med. 33: 1.

Jessie B and Dobrovolny HM 2021. The role of syncytia during viral infections. J Theor Biol. 525: 110749.

Johnston SL and Siegel CS 1990. Evaluation of direct immunofluorescence, enzyme immunoassay, centrifugation culture, and conventional culture for the detection of respiratory syncytial virus. J Clin Microbiol. 28: 2394–2397.

Kamdi B, Singh R, Singh V, Singh S, Kumar P, Singh KP, George N and Dhama K 2020. Immunofluorescence and molecular diagnosis of bovine respiratory syncytial virus and bovine parainfluenza virus in the naturally infected young cattle and buffaloes from India. Microb Pathog. 145: 104165.

Katoch S, Dohru S, Sharma M, Vashist V, Chahota R, Dhar P, Thakur A and Verma S 2017. Seroprevalence of viral and bacterial diseases among the bovines in Himachal Pradesh, India Vet World. 10: 1421.

Kimman TG, Zimmer GM, Westenbrink F, Mars J, Van Leeuwen E 1988. Epidemiological study of bovine respiratory syncytial virus infections in calves: influence of maternal antibodies on the outcome of disease. Vet Rec. 123: 104–109.

Kimman TG, Straver PJ and Zimmer GM 1989. Pathogenesis of naturally acquired bovine respiratory syncytial virus infection in calves: morphologic and serologic findings. Am J Vet Res. 50: 684–693.

Larsen LE 2000. Bovine respiratory syncytial virus (BRSV): a review. Acta Vet Scand. 41: 1–24.

Maina TW, Grego EA, Broderick S, Sacco RE, Narasimhan B and McGill JL 2023. Immunization with a mucosal, post-fusion F/G protein-based polyanhydride nanovaccine protects neonatal calves against BRSV infection. Front Immunol. 14: 1186184.

Narita M, Kimura K, Tanimura N, Arai S, Tsuboi T and Katsuda K 2000. Immunohistochemical characterization of calf pneumonia produced by the combined endobronchial administration of bovine herpesvirus 1 and Pasteurella haemolytica. J Comp Pathol. 123: 126–134.

Obando RC, Hidalgo M, Merza M, Montoya A, Klingeborn B and Moreno-Lopez J 1999. Seroprevalence to bovine virus diarrhoea virus and other viruses of the bovine respiratory complex in Venezuela (Apure State). Prev Vet Med. 41: 271–278.

Ogilvie MM, Santhire Vathenen A, Radford M, Codd J and Key S 1981. Maternal antibody and respiratory syncytial virus infection in infancy. J Med Virol. 7: 263–271.

Paccaud MF and Jacquier CL 1970. A respiratory syncytial virus of bovine origin. Arch Für Gesamte Virusforsch. 30: 327–342.

Paton DJ, Christiansen K, Alenius S, Cranwell MP, Pritchard GC and Drew TW 1998. Prevalence of antibodies to bovine virus diarrhoea virus and other viruses in bulk tank milk in England and Wales. Vet Rec. 142: 385–391.

Perrin B, Dannacher G and Solsona M 1979. Mise en evidence des anticorps contre le virus respiratoire syncytial chez les bovins Francais. Rev Med Vet. 155: 465-471.

Pirie HM, Petrie L, Pringle CR, Allen EM and Kennedy GJ 1981. Acute fatal pneumonia in calves due to respiratory syncytial virus. Vet Rec. 108: 411–416.

Rosenquist BD 1974. Isolation of respiratory syncytial virus from calves with acute respiratory disease. J Infect Dis. 130: 177–182.

Sacco RE, McGill JL, Pillatzki AE, Palmer MV and Ackermann MR 2014. Respiratory syncytial virus infection in cattle. Vet Pathol. 51: 427–436.

Santos-Rivera M, Woolums AR, Thoresen M, Meyer F and Vance CK 2022. Bovine respiratory syncytial virus (BRSV) infection detected in exhaled breath condensate of dairy calves by near-infrared aquaphotomics. Molecules. 27: 549.

Solís-Calderón JJ, Segura-Correa JC, Aguilar-Romero F and Segura-Correa VM 2007. Detection of antibodies and risk factors for infection with bovine respiratory syncytial virus and parainfluenza virus-3 in beef cattle of Yucatan, Mexico. Prev Vet Med. 82: 102–110.

Stott EJ, Thomas L, Collins AP, Crouch S, Jebbett J, Smith GS, Luther PD and Caswell R 1980. A survey of virus infections of the respiratory tract of cattle and their association with disease. Epidemiol Infect. 85: 257–270.

Taylor G, Thomas LH, Wyld SG, Furze J, Sopp P and Howard CJ 1995. Role of T-lymphocyte subsets in recovery from respiratory syncytial virus infection in calves. J Virol. 69: 6658–6664.

Taylor G 2017. Animal models of respiratory syncytial virus infection. Vaccine. 35: 469–480.

Thomas LH, Cook RS, Wyld SG, Furze JM and Taylor G 1998. Passive protection of gnotobiotic calves using monoclonal antibodies directed at different epitopes on the fusion protein of bovine respiratory syncytial virus. J Infect Dis. 177: 874–880.

Trigo FJ, Breeze RG, Evermann JF and Gallina AM 1984. Pathogenesis of experimental bovine respiratory syncytial virus infection in sheep. Am J Vet Res. 45: 1663–1670.

Uttenthal Å, Larsen LE, Philipsen JS, Tjørnehøj K, Viuff B, Nielsen KH and Nielsen TK 2000. Antibody dynamics in BRSV-infected Danish dairy herds as determined by isotype-specific immunoglobulins. Vet Microbiol. 76: 329–341.

Valarcher J-F and Taylor G 2007. Bovine respiratory syncytial virus infection. Vet Res. 38: 153–180.

Van der Poel WHM, Kramps JA, Middel WGJ, Van Oirschot JT and Brand A 1993. Dynamics of bovine respiratory syncytial virus infections: a longitudinal epidemiological study in dairy herds. Arch Virol. 133: 309–321.

Van der Poel WHM, Brand A, Kramps JA and Van Oirschot JT 1994. Respiratory syncytial virus infections in human beings and in cattle. J Infect. 29: 215–228.

West K, Bogdan J, Hamel A, Nayar G, Morley PS, Haines DM and Ellis JA 1998. A comparison of diagnostic methods for the detection of bovine respiratory syncytial virus in experimental clinical specimens. Can J Vet Res. 62: 245.

Woldemeskel M, Kebede E, Yigezu L and Potgieter LN 2000. Prevalence of bovine respiratory syncytial virus (BRSV) and bovine herpesvirus-4 (BHV-4) in cattle from Ethiopia. Dtsch Tierarztl Wochenschr 107: 464–466.