Original Article

Epidemiological Study of Bovine Parainfluenza 3 Virus in Sheep: Seroprevalence, Risk Factors, and Distribution in Two Regions of Algeria

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ABSTRACT

Background: Respiratory viral diseases, including the bovine parainfluenza 3 virus, cause significant economic losses in ruminants. There is no available data regarding the epidemiological situation of this virus in Algeria.

Objectives: The present study aims to determine the seroprevalence and the associated risk factors of bovine parainfluenza 3 virus (BPI3V) in sheep in two different climatic regions of Algeria.

Methods: A total of 108 serum samples were collected from sheep at different ages and tested for antibodies against BPI3V using an indirect enzyme-linked immunosorbent assay (ELISA). A real-time polymerase chain reaction (PCR) test was also performed on nasal swabs to detect the viral genome.

Results: At the animal level, out of 108 sera tested, 82 (75.93%, 95% CI, 66.75%, 83.63%) showed antibodies against BPI3V. At the herd level, all 23 herds tested (100%) had at least one animal with BPI3V antibodies. Our results showed no association between the presence of BPI3V antibodies and the region (P=0.72). However, at the herd level, risk factors such as flock size and predisposing factors like climate change, feed deficit, postpartum stress, and dust were identified. At the animal level, a highly significant association was found between BPI3V seroprevalence and the age of the animals (P<0.0001). Notably, the sheep group over 3 years was more susceptible than other age groups. Furthermore, a significant difference in BPI3V seroprevalence based on sex was observed (P<0.003). All collected nasal swabs were negative for BPI3V genome detection using real-time PCR.

Conclusion: This study is the first serological survey on BPI3V in Algeria, confirming its presence in sheep from two regions. The high serum prevalence of BPI3V observed in the study population highlights addressing this viral disease to mitigate economic losses in ruminants.

Keywords: Algeria, Bovine parainfluenza 3 virus, ELISA, Risk factors, RT-PCR

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Introduction

The small ruminant respiratory complex is one of the major causes of morbidity and mortality in sheep flocks. The contributing factors comprise exposure to adverse weather conditions, animal movement, overcrowding, and stress, which increase the susceptibility of animals to viral and bacterial infections (Scott, 2011). The prevalence of parainfluenza 3 (PI3) virus has been documented in various countries, including 2.2% in Egypt (Gafer et al., 2009), 95% in Iran (Shoukri et al., 2013), 10.53% in Turkey (Ceribasi et al., 2014), and 3.69% in India (Kamdi et al., 2020).

Respiratory viral infections have a severe economic impact on ruminants. Bovine parainfluenza 3 virus (BPI3V), along with other viruses such as bovine respiratory syncytial virus (BRSV), bovine herpes virus-1 (BHV-1), and small ruminant morbillivirus (SRMV), in conjunction with bacteria and mycoplasma (Mashhour et al., 2020; Ashrafi et al., 2022), contribute to the respiratory disease complex in ruminants, leading to severe illnesses (Ellis, 2010; Rasooli et al., 2023). The most important risk factors for ruminant respiratory disease comprise low environmental temperature and high humidity, increased animal density, stress, dust, poor ventilation, and parasites (Goodwin-Ray et al., 2008; Scott, 2011).

BPI3V is an enveloped, non-segmented, negative-sense, single-stranded RNA virus that belongs to the genus *Respirovirus* in the family Paramyxoviridae (Ellis, 2010; Newcomer et al., 2017). This widely distributed virus causes respiratory tract infection in cattle (Alkan et al., 2000), sheep (Gafer et al., 2009), goats (Eberle et al., 2015), and camels (Ma et al., 2021). Moreover, PI3V can be transmitted between different species (Brako et al., 1984). In areas where cattle are infected with PI3V, a similar infection rate is expected in small ruminants (Yesilbag & Gungor, 2009). BPI3V infection seems to predispose the host to secondary bacterial infections (Murphy et al., 1999) due to its immunosuppressive effects (Ellis, 2010), especially under stressful conditions (Haanes et al., 1997). The prevalence of this viral pathogen has been reported in several countries (Solis-Calderon et al., 2007; Betancur et al., 2017). However, we found no documented reports regarding the epidemiology of respiratory diseases caused by BPI3V in small ruminants in Algeria. Therefore, our study aimed to conduct a preliminary serological analysis and genome detection of the BPI3V on sheep and identify the risk factors associated with BPI3V seropositivity in two regions of Algeria.

Materials and Methods

Study areas

This study was carried out during the winter and spring seasons of 2018 in two different climatic regions of Algeria (Batna & Boumerdes). Batna is located in the eastern part of Algeria, located across the Aurès mountain range (4°7′ N, 35°36′ E). It has a semi-arid climate with an annual rainfall of 496 mm. The average temperature is 4°C in January and 35°C in July. Winter nights experience temperatures below freezing point with frequent frosts, while summer temperatures can reach 45°C in the shade. Batna has a sheep population potential of 1137361, including 638423 ewes (DSA, 2019).

Boumerdes is located on the central coast of Algeria (36°46′ North, 3°28′ East) and has a humid climate characterized by two distinct seasons: Mild, rainy winters and hot, humid summers, with an average annual rainfall of 672 mm. The sheep population potential in Boumerdes is 33942, including 13470 ewes (DSA, 2020).

The samples were taken from sheep farms located in 5 districts of Batna and 3 districts of Boumerdes (Figure 1).

Study population and sample size determination

The cross-sectional study focused on herds with a history of respiratory disease to assess the distribution of BPI3V. A total of 23 flocks were included in the study, 16 out of 23 being mixed flocks (promiscuity of sheep with cattle and goats), while 7 were sheep flocks. The flocks comprised 1127 crossbreed sheep, with an average flock size of 49 (10-150). From these flocks, 108 sheep (9.58%) with or without respiratory signs (seromucosal/mucopurulent discharge, cough, tachypnea, dyspnea, and fever) were selected for this study, of which 80 were females and 28 males, aged between 4 months and 6 years (mean age: 2.8 years). Notably, the sheep included in this study had not been vaccinated against PI3V.

Most of the sheep sampled were females, accounting for 80 out of the 108(74.07%). This imbalance in gender distribution can be attributed to the fact that the studied farms primarily focused on breeding programs, where lambs were raised until they reached 3 months of age.

The sample size was determined using a table for estimating prevalence in a large population with desired fixed-width confidence limits, following the sampling method provided by Thrusfield (2018). Based on an ex-

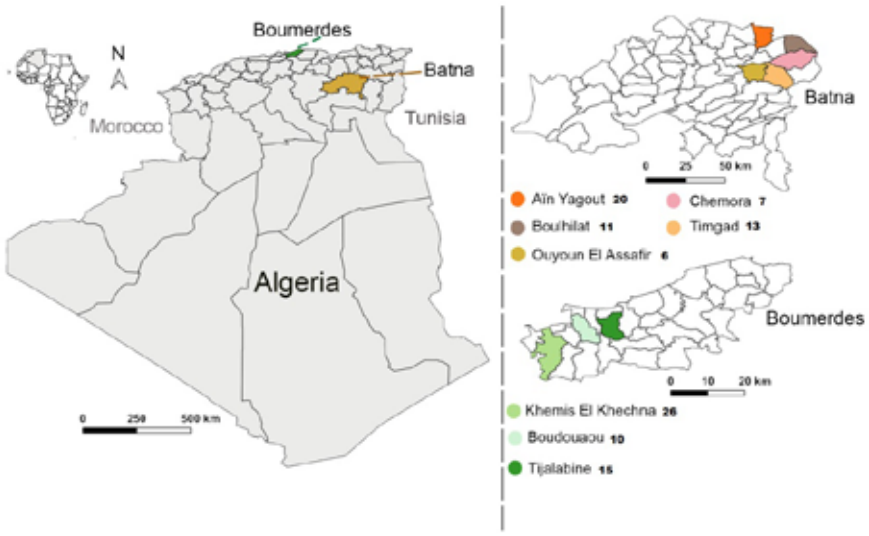

Figure 1. The geographical locations of Batna and Boumerdes regions, Algeria

pected prevalence of 10% (as reported by Saeed et al., 2016 in Sudan), a desired absolute precision of 5%, and a confidence level of 95%, the table indicated a sample size of 99 (Thrusfield, 2018). However, the sample size was increased to 108 as the remaining reactions in the second kit were used to repeat suspected reactions

Blood samples and data collection

Blood samples were collected by puncturing the jugular vein using vacuum tubes (5-mL Vacutainer®). The blood samples were labeled, transported to the laboratory, and left at room temperature until a clot formed. Afterward, sera were obtained by centrifugation at 3000 rpm for 10 min, transferred to sterile 1.5-mL tubes (Eppendorf), and stored at -20˚C until further examination. The samples were processed in the laboratory of the Biotechnology Research Centre in Constantine, Algeria.

Nasal swabs were taken from sheep showing clinical signs of pneumonia and placed in a viral transport medium (VTM) (Xpert®). Samples were identified and transported on ice to the laboratory and stored at -80˚C until analysis. Molecular analysis was performed at the laboratory of Pasteur Institute in Algeria for influenza and other respiratory viruses, Sidi-Fredj Unit.

Data at both the animal and herd levels were collected simultaneously with the serosurvey through interviews with willing farmers. To facilitate this process, a semi-structured questionnaire was prepared. The questionnaire primarily focused on gathering information related to animal biodata, such as age and sex. Additionally, it included questions regarding the region and time of the study, including climate and season, as well as herd management data, such as hygiene practices and herd size. These factors were categorized as follows: Age (<1 year, 1-3 years, >3 years), sex (male, female), study area (Boumerdes, Batna), hygiene level (dirty, fair, and clean), season (winter, spring), promiscuity with other animals (yes, no), the introduction of new animals into the herd (yes, no), transport (yes, no), herd size (10-50, 51-100, >100-150), and predisposing factors (climate change, feed deficit, postpartum stress, and dust).

Serological analysis

A commercial indirect ELISA kit developed by Bio-X Diagnostics, Jemelle Belgium (BIO K 239/2), was used to detect antibodies against BPI3V in sheep. The test was performed according to the manufacturer's instructions. Serum samples were diluted in PBS (1:100), volumes of 100 µL were dispensed into each well and incubated for one hour at 2±3˚C, then rinsed 3 times with wash buffer. A bovine immunoglobulin peroxidase conjugate solution was dispensed into each well and incubated for a further hour at 21˚C Bio-X Diagnostics, Jemelle Belgium 3˚C. After the second incubation, the plate was rewashed, and chromogen (tetramethylbenzidine) was added to each plate well and incubated for 10 min in the dark at room temperature. Supposing specific immunoglobulin is present in the test sera. In that case, the conjugate remains bound to the microwell containing the viral antigen, and the enzyme catalyzes the transformation of the colorless chromogen into a pigmented compound. The resulting blue color's intensity is proportional to the title of the specific antibody in the sample. To stop the reaction, 50 µL of stop solution (phosphoric acid) was added.

Finally, the optical density (OD) was measured at 450 nm by the EnSpire® multimode plate reader (Indirect ELISA, Bio-X Diagnostics, Jemelle Belgium [BIO K 239/2]).

Molecular analysis

Nucleic acid extraction

Viral RNAs were extracted with the QIAamp® Viral RNA Mini Kit (Qiagen) using 140 µL of each sample according to the manufacturer's instructions. Nucleic acids were eluted in a final volume of 60 µL and stored at -80°C until examination.

Real-time polymerase chain reaction (RT-PCR)

A total of 86 samples were tested by RT-PCR for bovine parainfluenza 3 virus RNA using the ViroReal® bovine parainfluenza 3 virus kit (DVEV02811). All reactions were performed in a total volume of 20 µL, containing 10 µL of sample eluate and 10 µL of Master Mix, which consisted of 2 µL of nuclease-free water, 5 µL of RNA reaction mix, 1 µL of bovine PI3 assay mix, 1 µL of IPC RNA assay mix, and 1 µL of IPC RNA target diluted at 1:500. Reactions were performed using the ABI PRISM® 7500 one-step reverse transcription real-time PCR thermocycler (Applied Biosystems). Samples were amplified in 47 cycles starting with the first step, which is the synthesis of the DNA strand complementary to the viral RNA by reverse transcriptase at 50°C for 15 min, followed by a denaturation step at 95°C for 25 seconds, and a final elongation step at 60°C for 1 min (ViroReal® bovine Parainfluenza 3 virus kit [DVEV02811]).

Statistical analysis

The apparent prevalence (AP) was obtained by dividing the number of positive animals by the number of animals tested.

Univariable statistical analyses of the present study were performed using R Statistical software, version 4.0.2). An initial explanatory analysis was performed using the chi-square and Fisher exact tests to assess the independence between risk factors and BPI3V seropositivity. Variables with a P<0.2 were deemed statistically significant and selected for multivariable analysis using a regression model. A binary logistic regression model was applied to measure the association between BPI3V seropositivity and risk factors using IBM SPSS software, version 22 (Armonk, NY, USA). The variables were considered risk factors if the odds ratio was >1 and the P≤0.05.

Results

Serological study of BPI3V

Seropositivity

When sampling, all the investigated herds had a history of respiratory diseases. Serological results are summarized in Table 1.

At the individual animal level, out of the 108 sheep sera tested, 82 (75.93%, 95% CI, 66.75%, 83.63%) were positive for BPI3V, while 26(24.07%) were negative.

At the herd level, all 23 out of 23 farms (100%) had at least one animal with antibodies against bovine parainfluenza virus type 3.

Risk factors of parainfluenza virus 3

At the individual animal level

Animal-related factors, such as age and sex, were significant in the explanatory analysis. The highest seroprevalence was observed in older sheep (>3 years) and in both study regions (93%). However, the lowest seropositivity rate was recorded in lambs (<1 year) (53%). Seropositivity for BPI3V was significantly higher in females than males (P<0.003; Table 1), with 84% and 54% rates, respectively. Age was included in the binary logistic regression model and was identified as a risk factor for BPI3V infection. Adult sheep (>3 years) had a higher susceptibility to developing antibodies against BPI3V compared to young sheep (<1 year) (P<0.0001; Table 1). Sheep over three years old exhibited a higher predisposition to developing antibodies against BPI3V than sheep under one year (OR=6.94; 95% CI, 1.16%, 41.44%; Table 2).

At the herd level

Among herd-related factors, only flock size and predisposing factors were significant in univariable analysis and were subjected to multivariable analysis (binary regression). The presence of BPI3V antibodies was significantly higher in sheep with feed deficit (17 out of 19, 89%) and those exposed to climate change (43 out of 50, 86%) (P<0.005; Table 1). There were significant differences in BPI3V seroprevalence based on flock size, with a lower chance of having a seropositive animal in larger flocks compared to smaller flocks (P<0.003; Table 1), with rates of 61% and 72%, respectively.

Table 1. Relative risk factors associated with the prevalence of BPI3V in sera collected from sheep using indirect ELISA test

Factors	Levels	No. of Positive/No. of Animals Tested (%)	P
Region	Boumerdes	40/51 (78)	0.72
	Batna	42/57 (74)	
Age (y)	<1	24/45 (53)	0.0001*
	[1-3]	20/22 (91)	
	>3	38/41 (93)	
Sex	Male	15/28 (54)	0.003*
	Female	67/80 (84)	
Season	Winter	53/68 (78)	0.64
	Spring	29/40 (72)	
Hygiene level	Dirty	17/22 (77)	0.08
	Fair	48/58 (83)	
	Clean	17/28 (61)	
Promiscuity with other animals	Yes	66/88 (75)	0.94
	No	16/20 (80)	
Favorable factor	Climate change	43/50 (86)	0.005*
	Food deficit	17/19 (89)	
	Postpartum stress	9/16 (56)	
	Dust	13/23 (57)	
Transport	Yes	12/15 (80)	0.94
	No	70/93 (75)	
Introduction of new animals into the herd	Yes	13/16 (68)	0.82
	No	69/92 (75)	
Herd size	10-50	33/46 (72)	0.003*
	51-100	30/31 (97)	
	>100-150	19/31 (61)	

*P<0.05.

However, other factors like hygiene, transportation, and introducing new animals were not significant. Although promiscuity with other animals, specifically cattle, on the farm was expected to be a significant risk factor for BPI3V seropositivity, this factor was not significant (P<0.94).

At the regional level

The seroprevalence of BPIV3 was similar between sheep from the Batna and Boumerdes regions, at 74% and 78%, respectively. The explanatory analysis revealed no association between the presence of antibodies to BPI3V and the different climatic regions studied (P<0.72, Table 1).

Table 2. Logistic regression analysis of factors associated with seropositivity to PIV3 in sheep

Factors	Levels	B	S.E	Odds Ratio	95% CI_{OR}	P
Constant		-	-	0.32	-	0.44
Age (y)	<1	-	-	Referent	-	-
	1-3	1.70	1.11	5.50	0.62-48.53	0.12
	>3	1.94	0.91	6.94	1.16-41.44	0.03
Sex	Male	-	-	Referent	-	-
	Female	0.43	0.87	1.54	0.27-8.54	0.622
Hygiene level	Clean	-	-	Referent	-	-
	Fair	0.16	0.83	1.18	0.23-6.01	0.120
	Dirty	-1.61	1.04	0.19	0.02-1.52	0.120
Favorable factor	Dust	-	-	Referent	-	-
	Climate change	2.04	1.72	7.67	0.26-224.14	0.236
	Food deficit	1.48	1.90	4.41	0.10-183.01	0.435
	Postpartum stress	-0.32	1.10	0.72	0.08-6.31	0.772
Herd size	10-50	-	-	Referent	-	-
	51-100	2.73	1.64	15.36	0.61-382.98	0.096
	>100-150	0.45	1.56	1.57	0.07-33.77	0.772

Notes: B: Coefficient; SE: Standard error; 95%CI_{OR}: 95% confidence interval of OR.

P<0.05.

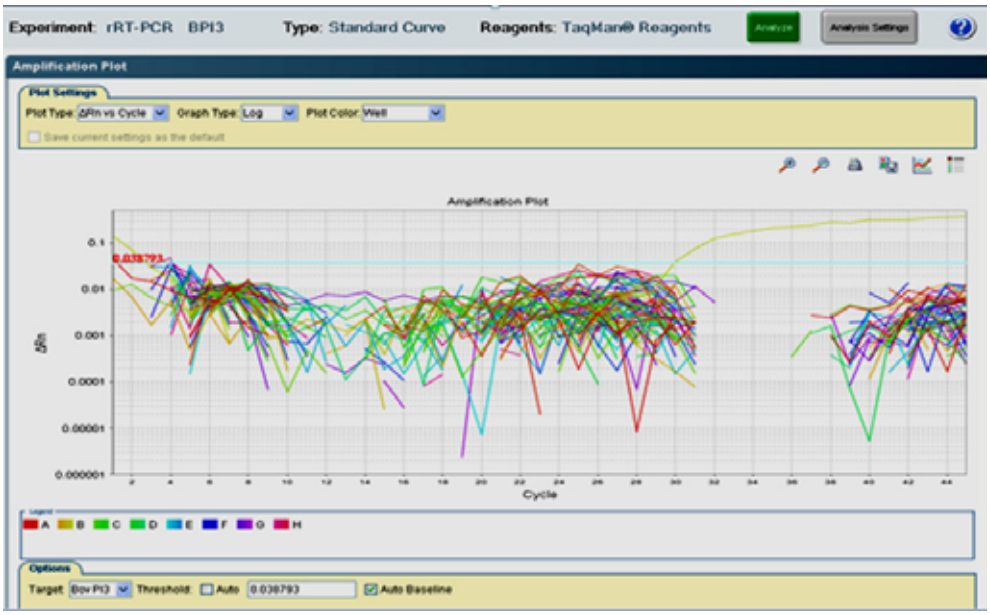

Figure 2. The logarithmic amplification curves of the samples tested and the positive control

Molecular study of BPI3V

All nasal swabs collected in this study were passed through real-time PCR to detect the genome of BPI3V. Of 86 examined swabs, no sample was found positive for the bovine Parainfluenza 3 virus, as shown in Figure 2.

Discussion

The prevalence of the PI3 virus in naturally infected flocks around the world is assessed using PCR, RT-PCR, culture, virus isolation, electron microscopy, direct fluorescent antibody test (DFAT), indirect fluorescent antibody test (IFAT), and immunoperoxidase (IP) techniques (Tiwari et al., 2016; Jarikre & Emikpe, 2017; Emikpe et al., 2019; Ma et al., 2021; Ren et al., 2023).

Several serological surveys conducted in many countries reported a wide distribution of the PI3 virus in sheep (Cabello et al., 2006; Gafer et al., 2009; Saeed et al., 2016). This study represents the first seroepidemiological survey of BPI3V in sheep flocks in two different climatic regions of Algeria. Our study confirmed the circulation of the BPI3V, with a seroprevalence of 75.93% (82 out of 108).

A higher prevalence was reported in Brazil, with rates of 82% (Aline et al., 2018) and 52.5% (Franco et al., 2020). However, several studies have reported lower prevalence rates: 16.7% in Grenada (Tiwari et al., 2016), 11.73% in Japan (Giangaspero et al., 2013), 8.8% in Turkey (Yesilbarg & Gungor, 2009), 62.2% in Iran (Hazrati et al.,1976) and in Sudan, BPI3V antigen was detected in 9.8% of sheep lung samples (Saeed et al., 2016).

The variation in prevalence estimates in sheep from different countries can be explained by diverse factors such as differences in geographical region, husbandry methods, and management conditions, flock size, type of farming, age of animals, disease management, disease control programs, type of samples taken and laboratory diagnostic methods (Mainar-Jaime et al., 2001; Hussain et al., 2019). After 6 to 8 weeks, the levels of mucosal antibodies against the virus decrease significantly, while serum antibodies remain present for 3 to 5 months (Makoschey & Berge, 2021). It is important to note that antibody detection in serum does not indicate recent illness, whereas detection of viral antigens requires samples in the acute phase of the disease.

Many risk factors predispose to respiratory disease complex. These factors include temperature changes, animal transportation, feed changes, high stocking density, the introduction of an animal into the herd, etc. Animal-related factors such as the age of the animal and its immune status also play a role (Figueroa-Chavez et al., 2012). In this study, despite the difference in environmental conditions, there were no significant differences between the prevalence of antibodies against BPI3V in the two regions studied. In contrast, a significant difference was found between animals from the uplands, Mexico City, and the tropic, Veracruz, Mexico (Contreras-Luna et al., 2017). Regarding the age of the animals, the prevalence of BPI3V was higher in adult sheep (>3 years) compared to those in other age groups (<1 year and 1-3 years) (Table 1). We reported a seroprevalence of 93% in adult sheep, which is higher than the prevalence reported in Peru (50%) (Cabello et al., 2006) and Mexico (81.4%) (Contreras-Luna et al., 2017). Unlike our study, the results obtained in Mexico showed no effect of age on the seroprevalence of BPI3V in sheep (Contreras-Luna et al., 2017). Adults tend to have a higher seroprevalence of BPI3V, which may be attributed to multiple previous infections at this age (Intisar et al., 2018). The risk of disease is higher in the medium-sized flock (97%) than in the large flock (61%). This finding contradicts the result reported in Mexico by Solis-Calderon et al. (2007). They indicated that BPI3V seropositivity was higher in large herds and suggested that exposure to other animals should be higher in extensive herds.

A significant association was found between BPI3V seroprevalence and promoting factors. The respiratory system of animals can be compromised by environmental factors such as inadequate feeding, early weaning, extreme temperatures (both low and high), lack of rest, and stress of transportation. Additionally, dust particles can act as irritants and increase the susceptibility of animals to respiratory diseases (Callan & Garry, 2002). Climate plays a major role in modulating the pathogen's virulence and reducing host defense, thereby increasing susceptibility (Rahal et al., 2014). As mentioned earlier, adults were more susceptible than young animals. Since the majority of the sampled females were elderly and the males were young, the seroprevalence of BPIV3 was higher in females compared to males. This finding can be attributed to the higher number of females sampled (as the selected farms were focused on breeding strategies). Still, animals raised longer are also believed to have a higher likelihood of contracting the disease.

Despite respiratory disease symptoms and antibodies to BPI3V in the tested subjects, no BPI3V genome was detected in nasal swabs by the RT-PCR. This finding is consistent with that of Carrillo Gaeta et al. (2018), who reported no viral genome was found in tracheobronchial

swabs of cattle with the respiratory disease, regardless of positive serology. However, several other studies have reported the detection of the BPI3V genome in nasal swabs, although in a limited number of cases. For instance, the viral genome was detected in 8 out of 119 samples tested in Serbia using RT-PCR (Veljovic et al., 2016), 11 out of 89 samples tested in Japan using RT-qPCR (Goto et al., 2023), 2 out of 127 nasal swabs tested in Turkey using RT-PCR (Timurkan et al., 2019), and 69.3% of sheep were found positive in Mexico (Contreras-Luna et al. 2017). Additionally, 16.59% of samples in China were detected as BPI3V-positive using RT-PCR (Ren et al., 2023).

Our investigation's absence of a viral genome could be attributed to two possible factors. Firstly, other pathogens might be responsible for the respiratory infections observed in the subjects. Secondly, the timing of sample collection may have been inappropriate, as it is difficult to determine when the animals contracted the disease. According to Grubor et al. (2004) and Ackermann (2014), PI3V and RSV typically disappear from the respiratory tract within 17- and 14-days post-infection in young experimentally infected lambs, respectively. Furthermore, the presence of antibodies to BPI3V and the lack of viral antigen detection could indicate that the animals have experienced a regressive infection and have developed specific immune responses (Carrillo Gaeta et al., 2018).

Conclusion

This study is the first serological survey conducted on BPI3V, confirming its presence in sheep populations across two regions of Algeria. The seroprevalence was detected in 75.93% of sampled sheep. Our findings indicate that age and sex significantly influence the seroprevalence of BPI3V, while herd-level factors such as predisposing conditions and flock size may also contribute as significant risk factors. However, the region of study does not affect the seroprevalence of BPI3V. These results provide valuable insights for future large-scale epidemiological studies, which can aid in developing effective prevention and control programs for respiratory diseases in sheep. It is worth noting that the BPI3V genome was not detected in any of the swabs using the RT-PCR test. Therefore, further in-depth investigations are recommended to explore the role of this virus in initiating respiratory diseases and investigating potential concurrent infections.

Ethical Considerations

Compliance with ethical guidelines

The study sheep belonged to private farmers who were fully informed about the research objectives. Certified veterinarians carried out the sampling process with the explicit consent of the sheep owners. All methods employed in this study adhered to the regulations set by Algeria regarding the handling and treatment of domestic animals, specifically Law 08-88 of January 26, 1988, on the activities of veterinary medicine and the protection of animal health.

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Authors' contributions

Conceptualization, data collection, data curation, and writing the original draft: Sameh Baghezza; Methodology: Sameh Baghezza and Alia Gradi; Statistical analysis: Azizi Abdennour; Formal analysis, validation and visualization: Alia Gradi; Review and editing: Azizi Abdennour and Khireddine Ghougal; Project administration: Bakir Mamache and Omar Bennoune.

Conflict of interest

The authors declared no conflict of interest.

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