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Paper

# Seroprevalence of small ruminant lentivirus infections (SRLV) in family farming goats from Alagoas semiarid region, Brazil

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## Abstract

Small ruminant lentiviruses (SRLVs) are chronic and progressive diseases caused by retroviruses that pose a serious threat to family farming. This study aimed to determine the seroprevalence and risk factors associated with SRLV infection in goats from family farming properties in Alagoas, Northeastern Brazil. In the semiarid region of Alagoas, seventeen family farming properties were visited, distributed across eleven municipalities. An epidemiological questionnaire was administered to collect information on animal management practices and to identify potential risk factors for SRLV. Serological diagnosis of SRLV was performed using the agar gel immunodiffusion (AGID) technique with an in-house kit. Logistic regression analysis was conducted to identify factors associated with seropositivity for SRLV. A total of 388 goat serum samples were tested using AGID, resulting in a prevalence of 9.3% (36/388; 95% CI [0.0677–0.1258]). Logistic regression revealed one significant risk factor: “the type of farming” ($p < 0.001$; OR = 5.13; 95% CI [2.26–11.62]). Given the risk factor identified, it is essential to emphasize prophylactic measures against SRLV during prolonged stabilization periods. The detection of SRLV in family-run livestock operations in socially and economically vulnerable areas of Alagoas underscores the need for public policies that support family farming.

## Keywords

Goat, Epidemiology, Virus, AGID

## Introduction

Small ruminant lentiviruses (SRLVs) are infectious and contagious diseases caused by viruses from the *Retroviridae* family, *Orthoretrovirinae* subfamily, and *Lentivirus* genus. Caprine Arthritis Encephalitis (CAE) and Maedi-Visna fall within this group (Dawson, 1980; Callado et al., 2001; Azevedo et al., 2017; Luz-Armendáriz et al., 2021; Carrozza et al., 2023). It was previously believed that the CAE virus (CAEV) infected only goats and that the Maedi-Visna virus (MVV) affected sheep exclusively. However, interspecies transmission has been confirmed through phylogenetic studies. Therefore, it is now more appropriate to classify these viruses as part of a heterogeneous group known as small ruminant lentiviruses (Castro et al., 1999; Shah et al., 2004; Carrozza et al., 2023).

These diseases are slow-developing, progressive, chronic, and persistent. The incubation period ranges from months to years, and most infected animals may remain asymptomatic. SRLVs primarily target macrophages and monocytes, showing a preference for tissues of the nervous and respiratory systems. They may also affect the mammary glands and joints. Thus, there are four primary clinical forms: articular, respiratory, mammary, and neurological (Azevedo et al., 2017; Luz-Armendáriz et al., 2021). SRLVs cause economic losses in goat and sheep farming due to animal culling, increased mortality, higher replacement rates, delayed offspring development, reduced meat and milk



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production, impaired reproductive performance and lactation, and greater predisposition to other diseases (Rizzo et al., 2018; Rocha et al., 2022). Milk quality may also decline due to decreased fat and protein content (Azevedo et al., 2017; Sousa et al., 2019). Moreover, herd devaluation and trade restrictions on products, breeding animals, and genetic material are indirect economic impacts (Pinheiro et al., 2001; Azevedo et al., 2017).

In this context, beyond representing a challenge to animal health, SRLVs represent an obstacle to livestock production among small-scale farmers, potentially threatening their livelihoods and weakening the fragile economies dependent on this sector. Therefore, this study aimed to determine the seroprevalence and to identify the risk factors associated with SRLVs in goats raised on family farms in Alagoas, Northeastern Brazil.

# *Matherials and methods*

## Study area

The state of Alagoas is located in the Northeast region of Brazil (Figure 1), covering an area of 27,830.661 km$^2$, with a population of 3,127,683 inhabitants and a Human Development Index (HDI) of 0.684 (IBGE, 2024). It lies between latitudes 8°48'12" and 10°29'12" South (S), and longitudes 35°09'36" and 38°13'54" West (W). It is bordered to the north and west by the state of Pernambuco, to the south by the states of Sergipe and Bahia, and to the east by the Atlantic Ocean (Souza et al., 2020). The goat population in Alagoas is estimated at 64,482 animals (IBGE, 2023). The state is divided into three mesoregions: Agreste, East, and Sertão. The semi-arid climate predominates in the Agreste and Sertão regions. For this study, a family farm was defined as one that: a) predominantly employs family labor; b) derives the majority of household income from livestock farming; and c) is managed by the family. These properties have limited infrastructure, are operated by low-income families utilizing low-tech production systems, and typically exhibit modest productivity (Buainain et al., 2004; Brasil, 2006).

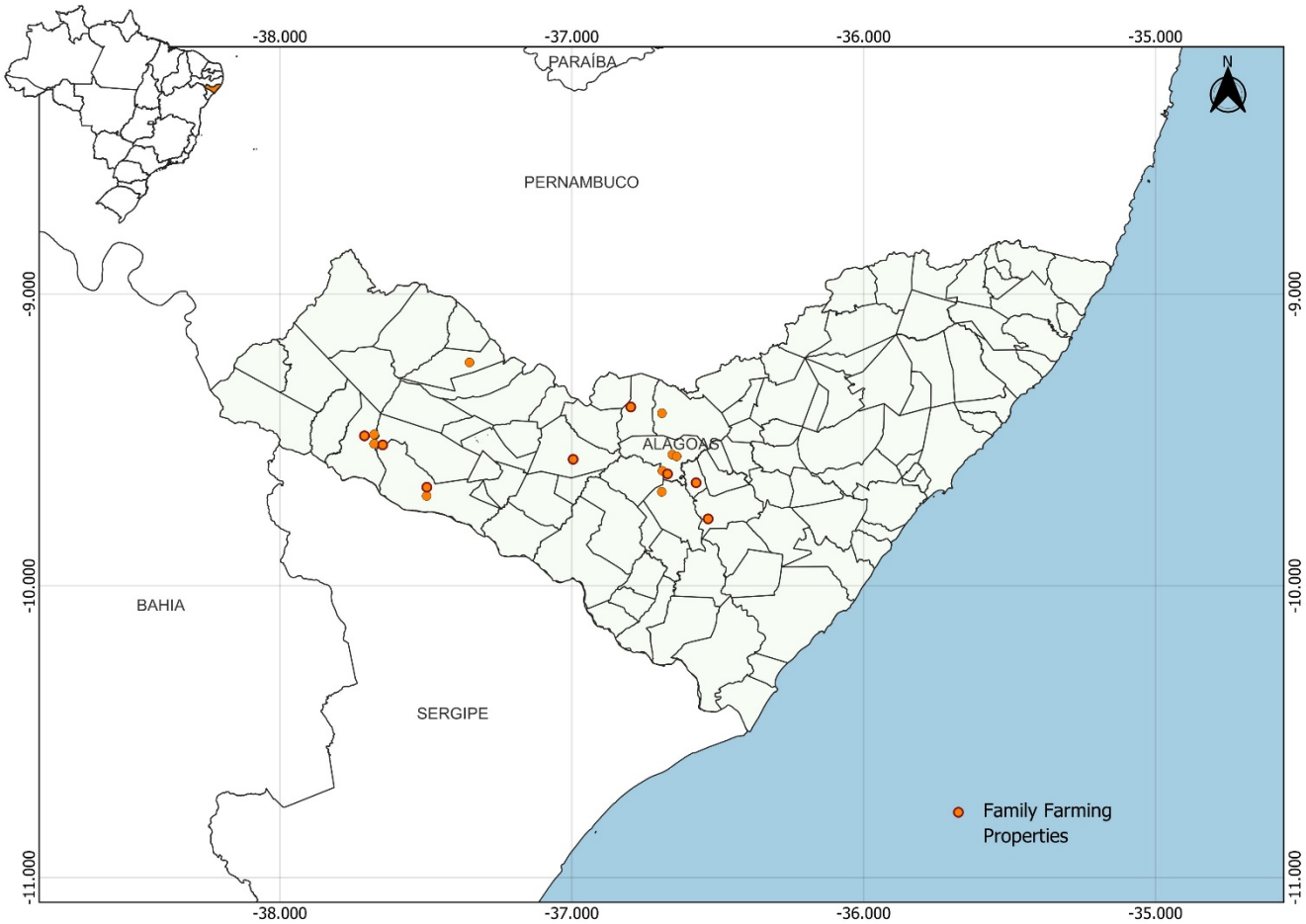


**Figure** 1. Location and geographic setting of the study area.



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## Sampling and Sample Collection

This research was approved by the Animal Use Ethics Committee of the Federal University of Alagoas (approval number: 04/2022). The minimum sample size was determined using the formula for simple random sampling, based on the following parameters: an expected prevalence of 50% (to maximize sample size), a 95% confidence level ($z = 1.96$), and a 5% margin of error. This resulted in a minimum required sample of 384 goats (Thrusfield, 2007). The locations and registrations of each property were obtained through local cooperatives. Seventeen farms located in eleven municipalities in the semi-arid region of Alagoas—Palmeira dos Índios, Estrela de Alagoas, Coité do Nóia, Arapiraca, Igaci, Limoeiro de Anadia, Major Isidoro, São José da Tapera, Piranhas, Pão de Açúcar, and Maravilha—were visited. The study included goats older than six months, regardless of sex or breed, raised on family farms where the owners consented to participate. Blood samples were collected exclusively from these animals. All goats present on each selected property were sampled. The collection occurred from September 2023 to August 2024. Blood was drawn from each goat, and after clotting, the resulting serum was transferred to microtubes and stored at −20 °C until serological testing.

## Serological Diagnosis

The diagnostic method used was Agarose Gel Immunodiffusion (AGID), employing an in-house kit. The viral antigen was produced from caprine corneal epithelial cells obtained from seronegative fetal donors and cultured in MEM supplemented with antibiotics and 10% fetal bovine serum. Cells were infected with the CAEV Cork strain and maintained in roller bottles. Supernatants were collected from the 10th day post-infection, concentrated by dialysis against PEG 8000, and purified using sucrose gradient centrifugation. The final antigen pellet was resuspended in 0.25% SDS. Protein concentration was determined using the Bradford method. A known CAEV-positive serum (confirmed by two AGID tests using a commercial kit) was used as the positive control. The AGID protocol involved diffusion in agar gel containing 1% agarose in PBS (pH 7.2), with the antigen placed in the central well and test sera in peripheral wells. Plates were incubated in a humid chamber at room temperature and examined for precipitation lines within 48 hours. The test has a sensitivity of 97.4%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 99.6% (data not shown). Although only goats were evaluated in this study, serological cross-reactivity between Caprine Arthritis-Encephalitis Virus (CAEV) and Maedi-Visna Virus (MVV) is well known. As both are classified within the small ruminant lentivirus (SRLV) group, AGID testing does not differentiate between them (Kalogianni et al., 2021). However, MVV remains rare in Brazil, with low prevalence and restricted geographic distribution (Cecco et al., 2022). In the state of Alagoas, no cases of MVV in sheep have been reported to date. Given the absence of significant sheep populations in the study area, the seropositive results are most likely attributable to CAEV.

## Statistical and Epidemiological Analysis

The prevalence of SRLV in the goat population was estimated as the ratio between the number of seroreactive animals and the total number of animals evaluated. The uncertainty associated with the point estimate was expressed through 95% confidence intervals (95% CI), calculated using either the Wald method or Wilson's correction. Data on animal management practices were collected for each property, including sex, breed, farming system, and production purpose. The analysis of risk factors was conducted in two stages. Initially, a univariate analysis was applied to assess the association between each independent variable and the outcome of interest (positive or negative serological result). Pearson’s chi-square test was used for categorical variables. When the assumptions of this test were not met (e.g., expected frequencies less than 5 in more than 20% of the cells), Fisher’s exact test was applied. In the second stage, a multivariate analysis was performed using binomial logistic regression, estimating adjusted odds ratios (ORs) and their respective 95% confidence intervals (95% CI). Variables with a p-value $< 0.20$ in the univariate analysis were included in the multivariate models, and the final variable selection was conducted using the backward stepwise method, with a removal criterion based on a p-value $< 0.05$. The presence of collinearity among variables was evaluated using the variance inflation factor (VIF), and the adequacy of model fit was assessed through the Akaike Information Criterion (AIC) (Zar, 1999; Thrusfield, 2007; Hosmer & Lemeshow, 2013). Statistical and epidemiological analyses were performed using R Studio (v4.1.2; R Core Team, 2021). The maps were generated using QGIS software (version 3.34 Prizren).



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## Results

This study investigated seventeen properties across eleven municipalities (Figure 1; Table I). A total of 388 goat serum samples were tested using the Agarose Gel Immunodiffusion (AGID) technique, revealing a seroprevalence of 9.3% (36/388; 95% CI: 6.8%–12.6%) (Figure 2). There was no statistically significant difference ($p > 0.05$) in the distribution of SRLV cases when comparing the two mesoregions (Agreste and Sertão) of the semi-arid region of Alagoas (Figure 3).

| Municipality | n | AGID positive (%) |
|---|---|---|
| Palmeira dos Índios | 22 | 0 |
| Limoeiro de Anadia | 40 | 2,5 (1/40) |
| Coité do Nóia | 31 | 3,2 (1/31) |
| Igaci | 40 | 2,5 (1/40) |
| Farm 01 | | |
| Farm 02 | | |
| Farm 03 | | |
| Farm 04 | | |
| Estrela de Alagoas | 17 | 23,5 (4/17) |
| Arapiraca | 6 | 0 |
| Pão de Açúcar | 41 | 2,4 (1/41) |
| Farm 01 | 23 | 4,3 (1/23) |
| Farm 02 | 18 | 0 |
| Maravilha | 38 | 0 |
| Piranhas | 54 | 5,5 (3/54) |
| Farm 01 | 23 | 0 |
| Farm 02 | 20 | 0 |
| Farm 03 | 11 | 27,2 (3/11) |
| São José da Tapera | 43 | 4,6 (2/43) |
| Major Isidoro | 56 | 41 (23/56) |

**Table** I. Number of lentivirus-seroreactive animals detected by Agarose Gel Immunodiffusion (AGID) in the semi-arid region of Alagoas, Brazil.



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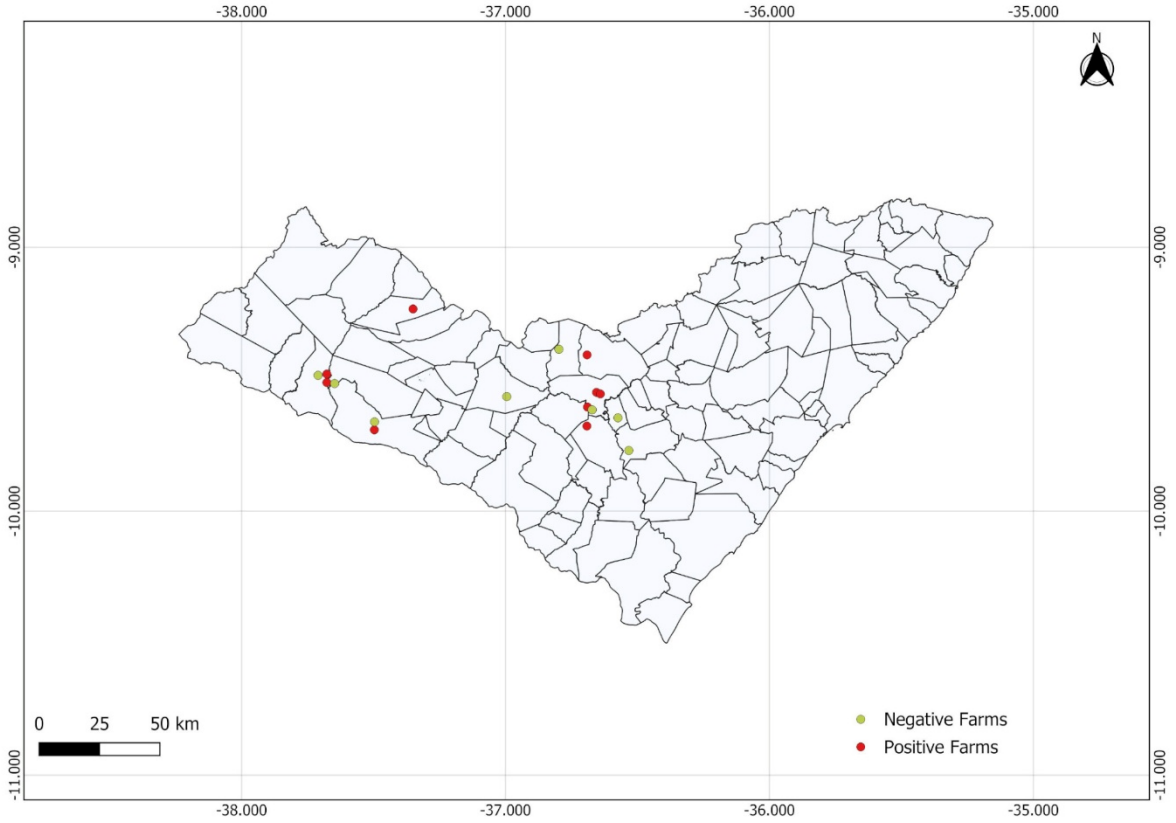


**Figure** 2. Distribution of Family Farming Properties with Positive or Negative Small Ruminant Lentivirus (SRLV) Cases in Caprines in the Semi-Arid Region of Alagoas, Brazil.

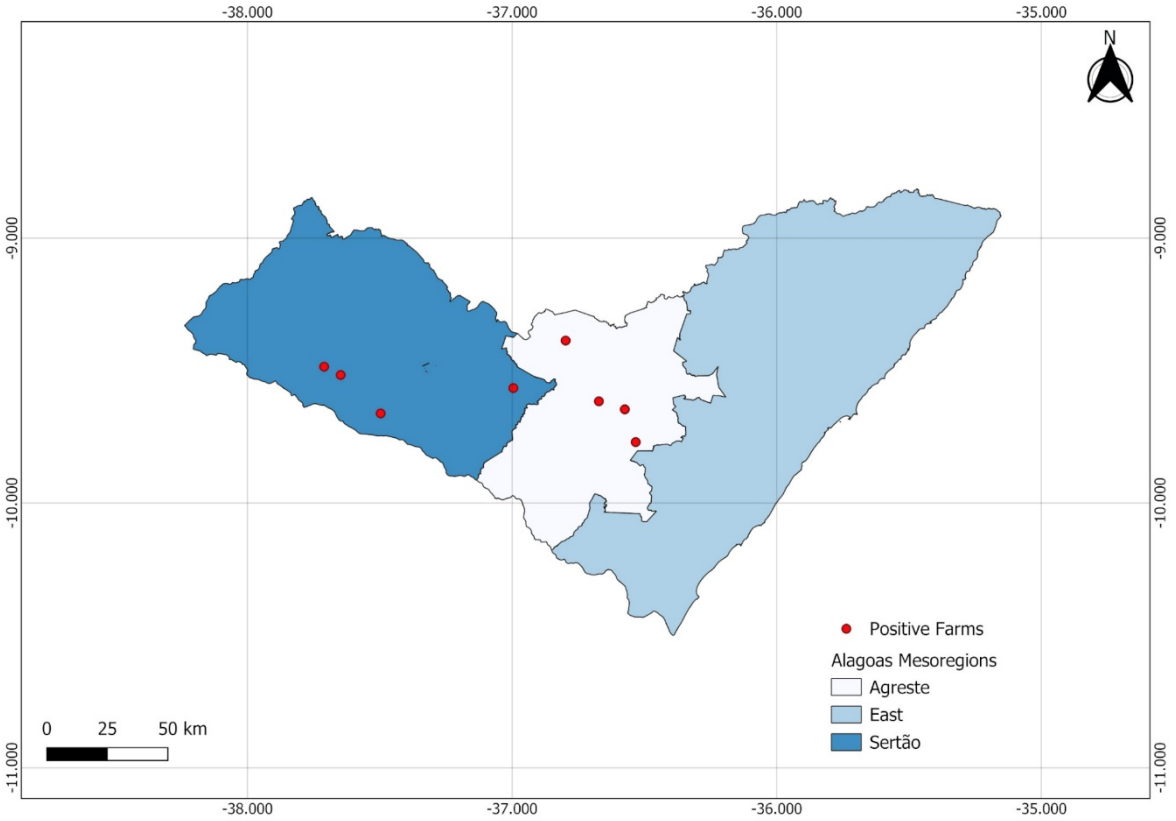


**Figure** 3. Distribution of family farming properties with positive or negative small ruminant lentivirus (SRLV) cases in caprine across the mesoregions of Alagoas, Brazil.

Among females, the seropositivity rate was 9.14% (33/361), whereas in males it was 11.11% (3/27). Regarding the



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farming system, seropositive animals were found in 16.1% (27/167) of confined goats and 4% (9/221) of semi-confined goats (Table II). Concerning the production purpose, the prevalence was 9.8% (34/345) among goats raised for milk production, and 4.6% (2/43) among those raised for mixed purposes (meat and milk).

| Variable | *N* | Positive (%) | p-value (univariate analysis) |
|---|---|---|---|
| | 388 | 36 (9,3%) | |
| **Sex** | | | |
| Male | 27 | 3 (11%) | 0,99 |
| Female | 361 | 33 (9,1%) | |
| **Farming System** | | | |
| Confined | 167 | 27 (16,1%) | < 0,001 |
| Semi-Confined | 221 | 9 (4%) | |
| **Production Type** | | | |
| Milk | 345 | 34 (9,8%) | 0,26 |
| Mixed | 43 | 2 (4,6%) | |

**Table** II. Occurrence and associated factors of lentivirus infection in goats from family farming properties in the semi-arid region of Alagoas, Brazil.

In the multivariate analysis, the only statistically significant independent variable was the type of farming ($p < 0.001$; OR = 5.13; 95% CI [2.26–11.62]) (Table III).

| Variable | Coefficient | Standard Error | p- value | *Odds Ratio* | Confidence Interval (95%) |
|---|---|---|---|---|---|
| Farming System | 1,64 | 0,417 | < 0,001 | 5,13 | (2,26 - 11,62) |
| Constant | -3,28 | 0,360 | 0,0000 | | |

**Table** III. Multivariate logistic regression analysis of factors associated with the risk of seropositivity for lentiviral infections in goats from family farming properties in the semi-arid region of Alagoas, Brazil.

## Discussion

The prevalence described in this study did not significantly differ ($p = 0.6443$; 95% CI [0.0632–0.1208]) from the average prevalence reported by other authors using the same diagnostic technique in similar contexts (Teixeira et al., 2016; Rizzo et al., 2018; Alves et al., 2022a; Teles et al., 2023). Small ruminant lentiviruses (SRLVs) can be transmitted through aerosols via the respiratory route (Costa et al., 2007; Rodrigues et al., 2012; Souza et al., 2013; Alves et al., 2022b). The higher prevalence observed in confined herds in the present study may be attributed to increased chances of horizontal transmission among animals housed in close proximity (Teixeira et al., 2016; Rizzo et al., 2018; Teles et al., 2023), as well as the stress-related immunosuppression associated with confinement (Rizzo et al., 2018). Conversely, the reduced contact between animals in extensive farming systems may limit horizontal transmission, reducing seropositivity rates (Souza et al., 2012). Although AGID is a classical method for SRLV detection and remains widely used in epidemiological studies due to its high specificity and low cost, it has inherent limitations. These include moderate sensitivity, which may affect detection in animals with low antibody titers or in the early stages of infection. However, given the use of a well-characterized antigen and appropriate controls, the results obtained in this study are considered reliable for estimating exposure levels in the population studied (Kalogianni et al., 2021). Teixeira et al. (2016) reported a 2.8% SRLV seroprevalence in Maranhão, Brazil, concluding that the farming system was not a risk factor, likely due to the low prevalence and the predominance of semi-extensive systems in the study area. In Sergipe, Brazil, Rizzo et al. (2018) found seroprevalence rates of 3.3% in intensively raised herds, 1.7%



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in semi-extensive systems, and 1.0% in extensive systems. Although these figures are lower than those found in the present study, they highlight the influence of farming systems on SRLV prevalence. In Paraíba, Brazil, Alves et al. (2022a) reported a prevalence of 10.5%, which is consistent with the results of this study. Their investigation was conducted among dairy herds managed under semi-intensive systems. Similarly, Teles et al. (2023) identified a 13.7% prevalence in a study using archived goat serum samples collected between 2011 and 2012 in Pernambuco, Brazil, primarily from dairy farms focused on genetic improvement. Notably, 73.5% of these properties had at least one AGID-positive animal. Teixeira et al. (2016) suggested that SRLV seroprevalence tends to increase as herds undergo genetic improvement, particularly with the introduction of specialized dairy goats. These findings highlight the need to implement control strategies to prevent the spread of SRLV in dairy operations. Although the ingestion of colostrum and milk from infected dams remains the primary transmission route, SRLV can also be spread via saliva, semen, and fomites—including towels, needles, tattooing instruments, and dehorning tools (Adams et al., 1983; Peretz et al., 1993; Callado et al., 2001; Rocha et al., 2022). In dairy systems, contaminated milking machine cups that are inadequately sanitized also pose a transmission risk (Callado et al., 2001; Alves et al., 2022a). This study did not evaluate these factors, as none of the farms assessed had established SRLV prevention or control protocols. Currently, no effective treatments or vaccines against SRLV are available (Oliveira et al., 2008). Prophylactic measures should prioritize regular testing, segregation of infected animals, and other practices such as early weaning to prevent colostrum ingestion from infected dams, generation isolation, structured milking routines, breeding control using seronegative sires, and strict sanitation of fomites and equipment (Callado et al., 2001; Megid et al., 2016; Azevedo et al., 2017). However, these actions can be logistically and financially challenging for small-scale family farmers, emphasizing the need for public policies that ensure consistent technical support and training for this group. Family farming is essential for ensuring food and nutritional security, preserving traditional diets and biodiversity, promoting responsible natural resource use, boosting local economies, and fostering social cohesion, cultural identity, and rural development. It also has considerable potential for job creation and for adapting to climate and environmental changes, while meeting growing demands for high-quality, nutritious food (Heberlê, 2017).

Nonetheless, smallholder farms often face significant limitations in implementing basic biosecurity practices. As such, the first step toward SRLV control in these systems is accurate diagnosis. This must be accompanied by public policies that offer structured technical assistance and capacity building for small producers (Vilela, 2017).

## Conclusion

The present study underscores the circulation of SRLV in family farming properties found in socially and economically vulnerable areas of Alagoas, emphasizing the immediate need for tailored public policies developed to assist these producers.

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## Ethical approval

This research was authorized by the Animal Use Ethics Committee of the Federal University of Alagoas (registration number: 04/2022).

## Conflict of interest

None.

## Author Contributions

Conceptualization: ACCZ, JCA; Methodology: ACCZ, SAN, AMC, JCA; Formal analysis: ACCZ, SAN, AMC, JCA; Investigation: ACCZ, EMLJ, ICLS, KEFS, MLCS; Writing original draft preparation: ACCZ, JCA; Writing, review and editing: ACCZ, JCA; Visualization: ACCZ, JCA; Supervision: ACCZ, SAN, JCA; Project administration: ACCZ, JCA; Funding acquisition: JCA.

All authors have read and agreed to the published version of the manuscript.

## Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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