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An epidemiological study of caprine arthritis encephalitis virus (CAEV) in breeder goats from Northeastern Brazil

Estudo epidemiológico da Artrite Encefalite Caprina á vírus (CAEV) com ênfase em reprodutores de rebanhos do Nordeste do Brasil

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Abstract

Caprine arthritis encephalitis virus (CAEV) is a retrovirus that infects goats. This study evaluated the prevalence of CAEV in breeder goats from the states of Maranhão, Ceará, Piauí, Alagoas, Sergipe, Rio Grande do Norte, and Paraíba. We collected a total of 531 serum samples from 251 properties. On average, two male breeder goats were examined from each farm. Results from western blotting demonstrated that the prevalence of CAEV was 6.2% (32/513). In each state, the following prevalence values were found: Piauí, 5.9% (7/119); Maranhão, 2.0% (01/48); Sergipe, 7.1% (03/42); Alagoas, 17.6% (03/17); Rio Grande do Norte, 4.7% (05/105); Paraíba, 2.1% (02/94); and Ceará, 12.5% (11/34). We also conducted a univariate analysis to determine the risk factors that are associated with CAEV. This analysis revealed that breeding season, records of herd data, criteria adopted for the first mating of females, castration of male goats, origin of breeders, and identification of the animal were associated with CAEV. Adopting control measures to identify CAEV-positive animals and avoid virus transmission to females, especially during breeding seasons, is crucial since, males carrying CAEV can be sources of infection for the entire herd.

Key words: Goats. Lentivirus. Western Blotting.

Resumo

Objetivou-se com esse estudo avaliar a prevalência da Artrite Encefalite Caprina (CAE) em reprodutores dos estados do Maranhão, Ceará, Piauí, Alagoas, Sergipe, Rio Grande do Norte e Paraíba. Para tanto, foram examinadas em média dois reprodutores por criatório, totalizando 513 amostras de soros e 251

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propriedades. A prevalência encontrada através do *Western Blotting* foi de 6,2% (32/513). Em cada estado participante do estudo foram encontradas as prevalências descritas a seguir: Piauí 5,9% (7/119), Maranhão 2,0% (01/48), Sergipe 7,1% (03/42), Alagoas 17,6% (03/17), Rio Grande do Norte 4,7% (05/105), Paraíba 2,1% (02/94) e Ceará 12,5% (11/34). Na análise univariável para os fatores de risco, as variáveis associadas ($p \leq 0,20$) a frequência de positividade nos reprodutores foi: estação de monta, anotações em relação ao rebanho, critério adotado para a primeira cobertura das fêmeas, castração dos caprinos machos, origem dos reprodutores e identificação dos animais. Na análise de regressão logística múltipla, não foram encontrados fatores de risco para a infecção em estudo.
Palavras-chave: Caprinos. Lentivirus. Western blotting.

Introduction

Caprine arthritis encephalitis (CAE) is a chronic disease that progresses slowly in adult goats and is caused by the CAE virus (CAEV). CAEV is a lentivirus from the Orthoretrovirinae subfamily and Retroviridae family (FRANKE, 1998). The clinical signs of CAE include swollen joints (arthritis), especially in carpus, chronic interstitial pneumonia, and mastitis with progressive weight loss and a decrease in milk production (CALLADO et al., 2001). In young animals, encephalomyelitis may be observed; however, some infected animals do not present clinical signs.

Dairy goat breeding is an important economic activity in Brazil. Although there is a large population of goats throughout the country, Brazil only ranks 18th in goat milk production worldwide (FAO, 2004). This low ranking is associated with the low milk productivity in goats, which is the result of infectious diseases, such as CAE. The importance of CAE for breeder goats is directly related to the economic aspect. Several factors caused by CAE directly impact production, such as predisposition to bacterial infections (especially in the mammary glands), deficient growth or increased mortality of goat kids, shorter lactation periods, reduction of productive life and milk production, decreased reproductive efficiency, and reduction of fat and protein levels in milk (GREENWOOD, 1995; LILENBAUM et al., 2007; BRITO, 2009).

In addition, this disease predisposes of goats to gastrointestinal infection with the parasite, *Haemonchus* spp. (CARNEIRO, 2011). The indirect losses of CAE include the devaluation of herds; early replacement of animals; costs with control measures; and commercial barriers for breeder animals, semen, and embryos (CALLADO et al., 2001; ANDRIOLI et al., 2006).

Infected goats are considered the main reservoir and source of infection of CAEV (LARA et al., 2005). Transmission occurs more frequently by the ingestion of contaminated colostrum and milk by goat kids in their initial months of life. Other forms of transmission include direct contact and contact with fomites and semen (PINHEIRO et al., 2001; BLACKLAWS et al., 2004). Studies have demonstrated that the viral-free form of CAEV can be found in seminal liquid and in proviral DNA that is integrated into non-spermatic cells. This may explain how the virus is transmitted through artificial insemination and natural mating (ANDRIOLI et al., 2006; GREGORY et al., 2009; SOUZA et al., 2014; HASEGAWA et al., 2017).

Multiple studies have evaluated the presence of CAEV using the agarose gel immunodiffusion (AGID) technique in breeders that reside in regions that specialize in dairy production, including Ceará and Maranhão, and found that the prevalence of CAEV in these regions was 13.2% and 2.8%, respectively (PINHEIRO et al., 1999; TEIXEIRA et al., 2016). Additionally, Teixeira et al. (2016) used AGID to investigate the prevalence of CAEV in male and female animals over six years of age in Maranhão and reported that CAEV was higher in male goats (4.4%) than female goats (2.5%).

CAE control requires identification of CAEV-positive goats in flocks. Hence, some diagnostic tests are recommended by the World Organization

of Animal Health (OIE), such as AGID and enzyme-linked immunosorbent assay (ELISA). In Brazil, the Ministry of Agriculture, Livestock and Food Supply (MAPA) initiated the Sheep and Goat Health Program that includes the National Plan for Vigilance and Control of Small Ruminant Lentiviruses. In the guidelines, AGID was selected as the routine diagnostic measure for lentiviral infections in animals. In Addition, western blotting (WB) has been used as a confirmatory test to validate AGID results and for certification purposes (OIE, 2008).

Goat production in Northeastern Brazil is mostly characterized as a subsistence activity that has low technological levels and rare or absent technical assistance. Therefore, not all CAEV-positive male goats are identified. These animals are generally used for natural mating in uncontrolled breeding environments. Consequently, female goats are likely to get infected during this process. In addition, the practice of sharing a male breeder goat between several farms is common (SOUZA et al., 2010; RAMOS et al., 2014).

This study aimed to investigate the prevalence of CAE in male breeder goats from the states of Maranhão, Ceará, Piauí, Alagoas, Sergipe, Rio Grande do Norte, and Paraíba using WB.

Material and Methods

This study was performed according to the ethical principles of animal experimentation outlined by the National Council for Animal Experimentation Control, was approved by the Ethics Committee for the Use of Animals at the State University of Acaraú Valley (protocol number 012.12). The sera used in the study belong to mcroproject named "Zoosanitary study of tropical goat and sheep productions: epidemiology, risks and economic impact of diseases", of the Embrapa Goats and Sheep aproved in public notice 64/2008 of the National Council for Scientific and Technological Development (CNPq) Brazil/ Ministry of Agriculture Livestock and Supply (MAPA).

The minimum number of farms necessary for the study was calculated according to the guidelines established by Thrusfield (1995). Based on previous studies, we expected the prevalence of CAEV to be 4.6% (PINHEIRO et al., 2001) and assumed a 6% margin of error and a 95% confidence level. From these calculations, the minimum sample size (n) was 47 farms. However, 513 male breeder goats from 251 goat production units that were distributed among seven states in Northeastern Brazil were investigated in this study.

Antisepsis was performed at the site, and blood samples were collected from venipuncture of the jugular vein using vacuum tubes, which were adequately identified. Following complete retraction of the blood clot, samples were centrifuged at 1000 ×g for 5 min. Then, serum samples were transferred to 1.5 mL polypropylene microtubes and stored at -20°C until serological analysis was performed.

The detection of anti-CAEV antibodies was performed using a WB technique that has been previously described by Pinheiro et al. (2011).

Frequency of caprine arthritis encephalitis virus-positive male breeder goats in Northeastern Brazil (Table 1). The association of risk factors with the frequency of CAEV-positive goats was assessed using data collected from epidemiological questionnaires, which were distributed during farm visits (Table 2). The risk factors for CAEV were analyzed using univariate and multivariate analyses. Univariate analysis was conducted using the Chi-square test or Fisher's exact test, and each independent variable was crossed with the dependent variable (serological status of the individual). Significant results ($p \leq 0.20$) from the univariate tests were subsequently used in a multiple logistic regression (HOSMER; LEMESHOW, 2000). All analyses were performed with the SPSS software version 21.0 for Windows; $p \leq 0.05$ was considered as statistically significant.

Table 1. Frequency of caprine arthritis encephalitis virus-positive male breeder goats in Northeastern Brazil.

	Animals		Farms	
State	**n/N**	**%**	**n/N**	**%**
Piauí	07/119	5.9	5/60	8.3
Maranhão	01/48	2.0	1/24	4.2
Sergipe	03/42	7.1	3/23	13.0
Alagoas	03/17	17.6	2/9	22.2
Rio Grande do Norte	05/105	4.7	4/53	7.5
Paraíba	02/94	2.1	2/48	4.2
Ceará	11/88	12.5	11/34	32.4
Total	**32/513**	**6.2**	**28/251**	**11.2**

n = positive samples; N = tested samples.

Table 2. Frequency of caprine arthritis encephalitis positive samples and associated characteristics of flocks and farms.

Variables	Categories	Investigated samples	Positive samples (%)	p
Breed				
	Pure	139	18(3.5)	0.001*
	Half-breed	250	10(1.9)	
	Undefined breed	124	04(0.8)	
Production type				
	Meat	248	10(1.9)	0.002
	Dairy	143	11(2.1)	
	Mixed	108	07(1.4)	
	Trading of breeders	14	04(0.8)	
Goats graze in other farms				
	No	437	31(6.0)	0.035
	Yes	76	01(0.2)	
Flock is maintained indoors at night				
	No	29	00(0.0)	0.146
	Yes	484	32(6.2)	
Separation of females before birth				
	No	201	15(2.9)	0.357
	Yes	312	17(3.3)	
Separation of animals per sex				
	No	408	21(4.1)	0.044
	Yes	105	11(2.1)	
Separation of animals per age				
	No	451	21(4.1)	0.001
	Yes	62	11(2.1)	
Newborn mortality				
	No	108	11(2.1)	0.056
	Yes	405	21(4.1)	

continue

continuation

Goatling mortality at weaning			
No	52	03	1.000
Yes	461	29	
Reproductive practices/breeding season			
No	418	23(4.5)	0.159*
Yes	95	9(1.8)	
Records of herd data			
No	332	17(3.3)	0.156*
Yes	181	15(2.9)	
Criteria adopted for the first mating of females			
None	361	18(3.5)	0.071*
At least one (Age, weight...)	152	14(2.7)	
Castration of male goats			
No	201	20(3.9)	0.006*
Yes	309	12(2.4)	
Origin of breeders/bought in animal fair			
No	416	20(3.9)	0.006*
Yes	97	12(2.3)	
Identification of animals			
No	321	13(2.5)	0.008*
Yes	192	19(3.7)	

* Variables used in the multivariate analysis ($p \leq 0.20$).

Results and Discussion

Overall, CAEV was detected in 6.2% (32/513) of goat sand 11.2% (28/251) of farms in the seven states in Northeastern Brazil (Table 1). Alagoas had the highest prevalence of CAEV in goats (17.6%). Costa et al. (2011) investigated sera of 70 goats (males and females) in Alagoas for the occurrence of CAE using AGID, and only one male goat was positive. In 1999 (PINHEIRO et al.) the prevalence of CAEV in breeders from dairy herds in Ceará was 13.2% (9/68), which was similar to the prevalence of CAEV in breeders from Ceará in the current study (12.5%). In (PINHEIRO et al. 2001) 4019 serum samples of goats from 30 cities in Ceará were investigated for the presence of CAEV with AGID and a prevalence of 1% (40/4019) was reported. Additionally, Pinheiro et al. (2001) reported that males (2.3%) and male breeders (3.8%) were significantly more affected ($p < 0.05$) than female breeders (0.9%) and young females (6 to 12 months of age) (0.3%). In our study, only male breeders were investigated.

In Minas Gerais, Nascimento-Penido et al. (2017) investigated the occurrence of CAE in dairy goats with AGID and reported that 14.5% (7/48) of males were CAEV-positive. In two separate studies, the prevalence of CAEV was determined to be 5.8% (7/119) and 3.6% respectively in male breeder goats from Piaui (SAMPAIO JUNIOR et al., 2011).

In the current study the lowest frequency of CAEV was identified in Maranhão (2%). However, another study performed in Maranhão reported that 4.4% (10/225) of goats were infected with CAEV following analysis with AGID (TEIXEIRA et al., 2016).

Here, we reported that CAEV was present in 7.1% (3/42) of goats and 13% (3/23) of farms in Sergipe. Rizzo et al. (2016) used AGID to investigate the occurrence of CAE in 15 goat flocks from the city of Poço Verde (Agreste region of Sergipe). These authors identified 3.63% (10/276) of infected goats distributed among 26.7% (4/15) of farms, which were named focus farms, and did not observe differences between sexes.

Our results demonstrated that 4.5% (5/105) of goats were infected with CAEV, and the virus was present in 7.5% (4/53) of farms in Rio Grande de Norte. Silva et al. (2005) investigated dairy goats using AGID in Central, West, and Agreste Potiguar regions of Rio Grande do Norte; these authors reported a prevalence of 11% of CAEV in flocks and in all properties.

In the current study, 2.1% (2/94) of breeder goats were positive for CAEV in Paraíba. Similarly, Bandeira et al. (2009) reported that 8.2% of goats from the Cariri region in Paraíba were CAEV-positive, and the prevalence of CAEV in males was higher (28.3%) than in females. These authors suggested that the reported findings could be associated with the habit of trading breeder male goats, which are often acquired from other areas in Brazil that have elevated CAE prevalence (PINHEIRO et al., 2004).

Some practices, such as the habit of borrowing, exchanging, or buying goats with unknown health status and the lack of quarantine, may lead to the introduction of diseases, including CAE, in flocks. To reduce the presence of CAEV (especially during reproduction), control measures, such as the identification of CAEV-positive animals through serological testing, separation of infected and healthy animals, and elimination of positive individuals from breeding programs, should be established (SARDI et al., 2012; NASCIMENTO-PENIDO et al., 2017).

The univariate analysis for risk factors revealed that some variables ($p \leq 0.20$), such as breeding season, records of herd data, criteria adopted for the first mating of females, castration of male goats, origin of breeders, and identification of animals, were associated with the frequency of CAEV-positive males. However, no risk factors were associated with CAE in the multiple logistic regression analysis (Table 2).

Previous studies have investigated goat production habits in Northeastern Brazil and revealed that most of the farms have low quality conditions, including poor nutrition and reproductive and health status (SAMPAIO JUNIOR et al., 2011; SARDI et al., 2012). In addition, the genetic potential is unfavorable, which renders inferior zootechnical levels. Further, uncontrolled natural mating is the most commonly used form of mating, and the breed of goats is frequently undefined (SANTOS et al., 2011; RAMOS et al., 2014).

In this study, results demonstrated that goats from farms that did not establish breeding seasons had a higher prevalence of CAEV, suggesting that the absence of organized reproductive practices on farms predisposes animals to infection. This may be related to the breeding system; most goats are reared extensively without separation by categories. Hence, males are gathered among females, and mating cannot be controlled.

Sardi et al. (2012) studied sheep and goat production in Bahia and identified that natural mating was practiced in 88.4% of breeders, there was no reproductive control. Additional studies describing breeding practices in Northeastern Brazil confirm that there are no methods of selection or reproductive practice that aim at improving production and standardizing of flocks (COSTA et al., 2008; RIET-CORREA et al., 2013).

Most of the CAEV-positive goats (3.5%) were found in farms that did not adopt any criteria to determine the adequate moment for the first mating of females as compared to farms that used age or weight to decide the moment for the first mating (2.7%). Other studies have described that farmers

do not consider age or weight when deciding on an appropriate mating time (SOUZA et al., 2007; SARDI et al., 2012). In addition, the sex separation variable presented a higher percentage of CAEV-positive animals (4.1%) on farms that did not perform such practice. Hence, this suggests that young goats, which demand more energy to grow, and pregnant females may be more susceptible to infection by CAEV.

In this study, the highest frequency of CAEV-positive animals was identified in farms that did not recording flock related events. These results reinforce the importance of zootechnical annotations, which have fundamental value for identifying and controlling problems that may interfere with successful production (ALENCAR et al., 2010). Studies that have demonstrated practices of goat and sheep production in Northeastern Brazil reveal that most farmers do not maintain zootechnical records (COSTA et al., 2008; SANTOS et al., 2011. Hence, events in these farms could not be controlled, which impairs management and decision-making and contributes to the low efficiency of production (ALENCAR et al., 2010; LOPES, 2008).

The origin of breeders is an important variable in CAEV epidemiology, considering that they may actively participate in the transmission and dissemination of the pathogen in flocks. Studies have demonstrated the capacity for CAEV transmission to females and offspring via semen of infected breeder goats (CAVALCANTE et al., 2013; HASEGAWA et al., 2017). The results obtained in this study demonstrated that a higher frequency of CAEV-positive animals lived on farms where breeders were not purchased at animal fairs. Farmers often negotiate or lend these animals, which are not subjected to any diagnostic tests that can determine if they are positive for CAEV. Therefore, these individuals are potential disseminators of the disease in flocks (BANDEIRA et al., 2009; MOURÃO et al., 2016).

Farm goats are identified to control and follow up the flock. In this study, the highest prevalence of CAEV was observed in farms that performed identification in an isolated manner. During the questionnaire application, we observed that most farmers performed identification through signaling or with given names, which is a common practice. Therefore, farmers have personal control of the animals and often consider the use of other forms of monitoring to be unnecessary. This is an unreliable method because only the owner can identify the individual animals, and previous studies have demonstrated that a reduced number of farmers are using earrings for identification (PORTO et al., 2013).

Conclusion

Overall, our findings demonstrate that CAEV was identified in male breeder goats from flocks located in Maranhão, Ceará, Piauí, Alagoas, Sergipe, Rio Grande do Norte, and Paraíba. Therefore, it is important to adopt control measures, such as periodical serological testing, for identifying CAEV-positive animals and avoid the transmission of the virus to females, especially during breeding seasons.

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