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First Seroepidemiological Study about Caprine Arthritis Encephalitis Virus in Paraná: Association Between Technification and Seropositivity

Primeiro Estudo Soroepidemiológico Sobre o Vírus da Artrite Encefalite Caprina no Paraná: Associação Entre Tecnificação e Soropositividade

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Abstract
Caprine Arthritis Encephalitis (CAE) is a disease globally notifiable to the World Organization for Animal Health (OIE). The causative agent is a Lentivirus, so the viral replication implies in their integration into the host cell genome. The present study aimed to verify the seroprevalence of CAE in caprine herds from Paraná, evaluating risk factors associated with the disease. Serum samples of 666 goats from farms located in the most relevant goat-producing cities of the state were tested using the agar gel micro-immunodiffusion (micro-AGID) test. Every farm owner answered an epidemiological survey containing questions about the infrastructure technological profile of the property, sanitary management, presence of other species, and productive and commercial aspects. About the animals, breed, sex and age group were considered. Data were analyzed by EpiInfo and R environment. Of the 666 goats, 19,2% were seropositive for CAE and 68.0% of the farms had at least one seropositive animal. In the multiple logistic regression analysis, the following factors were associated with seropositivity: semi-confined production system (p=0.0001; OR 4.41), property with slaughterhouses (p=0.0045; OR 2.21) and domestic dogs fed with remaining placental tissue (p=0.0276, OR 1.91) This is the first description of the occurrence of anti-CAEV antibodies in the state. Considering that CAE is an incurable disease, we can conclude that the virus is circulating in the herds where the study was carried out. This should be considered an alarming finding.

Keywords: Small Ruminantes. Micro-AGID. Lentivirus.

Resumo
A artrite encefalite caprina é uma doença viral de notificação obrigatória segundo a Organização Mundial de Saúde Animal. Seu agente é um lentivírus (CAEV) cuja replicação implica na incorporação do vírus ao genoma do hospedeiro, determinando uma infecção crônica. O objetivo do presente estudo foi verificar a soropositividade para o CAEV em rebanhos paranaenses e avaliar os fatores de risco associados à enfermidade. Para isso, foram submetidos ao teste de micro-IDGA 666 caprinos provenientes de propriedades localizadas nos municípios com maior relevância para a caprinocultura. A cada propriedade foi aplicado um questionário epidemiológico para avaliação da infraestrutura, perfil tecnológico, manejo sanitário, presença de outras espécies, e aspectos produtivos e comerciais. Quanto aos animais, considerou-se raça, sexo e faixa etária. Os dados foram tratados pelo programa EpiInfo e ambiente R. Do total de animais, 19,2% foram soropositivos em 68,0% das propriedades. Na análise de regressão logística múltipla foram observados associados a soropositividade para CAEV os seguintes fatores: sistema de produção semi-confinado (p<0,0001; OR 4.41), propriedade com local para abate de animais (p=0,0045; OR 2.21) e cães domésticos alimentados com restos placentários (p=0.0276, OR 1,91). Essa é a primeira descrição de ocorrência de anticorpos anti-CAEV no estado do Paraná. Considerando que a CAE é uma doença incurável, podemos concluir que o vírus está circulando nos rebanhos onde foi realizado o estudo, e por ser um rebanho que está em crescimento, tal dado deve ser visto como alarmante.

Palavras-chave: Pequenos Ruminantes. Micro-IGDA. Lentivirus.

1 Introduction

Small Ruminant Lentiviruses (SRLV) belong to the Lentivirinae subfamily and Retroviridae family. They are classified into two phylogenetic groups: the Maedi-Visna virus (MVV), which infects sheep, and the Caprine Arthritis Encephalitis Virus (CAEV), which infects goats. The diseases caused by these viruses are classified as notifiable by the World Organization for Animal Health (WOAH). SRLVs is enveloped, single-stranded RNA viruses with tropism for cells of the mononuclear phagocyte system. Their proviral DNA, resulting from the action of the reverse transcriptase enzyme, is incorporated into the host genome, characterizing a chronic and degenerative infection (WOAH, 2008).

Caprine Arthritis Encephalitis is a silent disease that is present in herds in several countries (Yang *et al.*, 2003). In Brazil, the prevalence rate of the disease oscillates between 8.2% and 14.0% (Bandeira *et al.*, 2009: Lilenbaum *et al*., 2007). However, animals may show symptoms after a drop in immunity,

which leaves them vulnerable to viral replication. Clinical signs include arthritis, pneumonia, mastitis, progressive weight loss, encephalitis and paralytic syndromes, drop in production, and in some cases may cause the animal's death (Martínez-Navalón *et al.,* 2013). Viral dissemination occurs mainly through secretions, with colostrum and milk being the most important route of transmission. In addition, Gregory *et al.* (2011) identified positive seminal samples using the RT-Nested-PCR technique and Araújo *et al.* (2020) reported vertical transmission. The Ministry of Agriculture and Livestock (MAPA) - the Brazilian government agency responsible for developing policies and regulations for agriculture, livestock and food safety - and the OIE recommend the Agar Gel Immunodiffusion (IDGA) test for the diagnosis of SRLV (World Organization for Animal Health, 2008). There is no treatment or vaccine (Tu *et al.,* 2020), the control is based on performing periodic herd examinations, discarding positive animals, artificial breastfeeding with pasteurized milk or colostrum (Michiels *et al.,* 2018).

Based on the Municipal Livestock Research, in 2020, the state of Paraná presented a herd of 85.845 goats. In comparison to 2019, there was a decrease of 16.1%. Even so, the state has the ninth largest herd in the country, second only to states in the Northeast (Instituto Brasileiro de Geografia e Estatística, 2023). No studies have accurately reported the direct economic impact of herd infection by SRLV; however, the losses are related to the restriction of international trade, death of animals, early discard, expenses with treatments and diagnosis, decrease in productivity, delay in growth, and devaluation of the herd (Michiels *et al.,* 2018).

The objective of the present study was to verify the seropositivity for the caprine lentivirus in Paraná herds and evaluate the risk factors associated with the disease.

2 Material and Methods

The study was approved by the Ethics Committee on the Use of Animals of the Brazilian Agricultural Research Company (EMBRAPA) - Goats and Sheep, Sobral/CE under number 010/2014. These activities were part of the project Animal Health Characterization of goat and sheep in Brazil: epidemiology, associated factors and economic impact of diseases of EMBRAPA, number 02.12.01.032.00, of which the authors participated in collaboration. Animals from properties in the state of Paraná were evaluated, located at latitudes 23ºS to 26ºS, with CFA (Subtropical) and CFB (Temperate) climates. To calculate the sample size (n), a population of 86000 was used as reference.

The estimated prevalence used was 50%, based on the absence of studies in the state of Paraná. The admitted error was 4%, resulting in a sample size higher or equal to 597 animals with a confidence level of 95%. Due to the absence of a list of all goat producers in the state, a non-probabilistic sampling of the properties was performed, considering the municipalities with greater representation in goat farming in Paraná. In each property, 20 animals were sampled, which were stratified according to the

following classification: 60% (Feitosa *et al.,* 2011) were reproductive females, 35% (Araújo *et al.,* 2020) were young animals with six to 12 months of age and 5% (World Organization For Animal Health, 2008) were male reproducers. A questionnaire developed by EMBRAPA was applied to each property with information such as: infrastructure, production and commercialization characteristics, technological profile, sanitation and presence of other species on the property, both domestic and wild. From each sampled animal, information on sex, age, and breed were obtained. The epidemiological questionnaire used presents 23 pages, was comprehensively designed to contemplate variables associated with a large number of sheep and goat diseases, because it is a broad study.

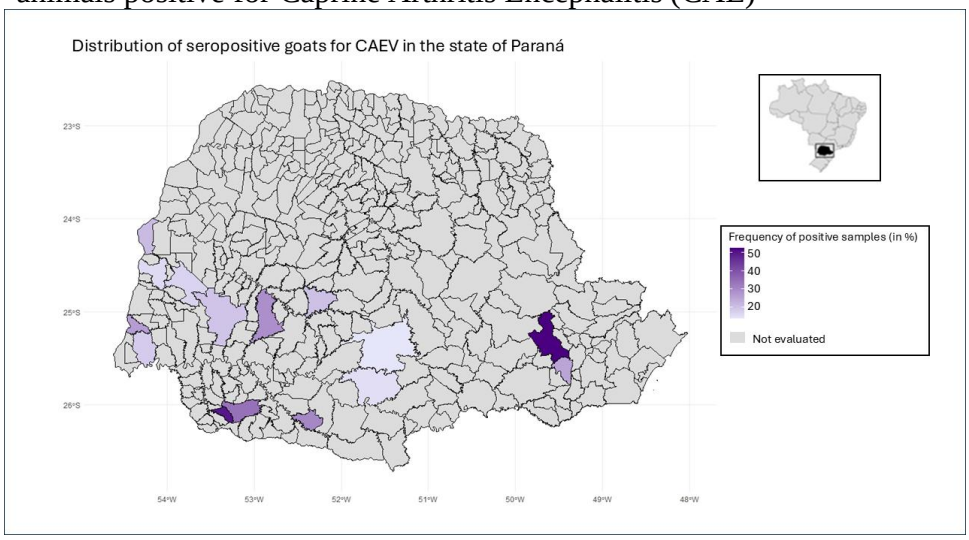
Blood was collected by jugular puncture in a tube without anticoagulant and kept refrigerated until centrifugation to obtain serum. The diagnosis was performed using the BIOVETECH® micro-IDGA kit (Arruda *et al.,* 2001), the examination, reading and interpretation were performed according to the manufacturer's instructions.

EpiInfo 7.1.5.2 software was used to tabulate the epidemiological questionnaire variables together with the serological results. The variables of the epidemiological questionnaire were screened using the chi-square test corrected by Yates and Fisher's exact test; those with $p < 0.20$ were selected for inclusion in the multiple logistic regression analysis. The strength of association was estimated by prevalence ratio (PR) and its respective 95% confidence intervals using the R environment version 3.6.2. It is worth noting that only variables whose percentage of completed questionnaires was greater than 85% were used.

3 Results and Discussion

A total of 666 animals were analyzed, of which 19.2% (128/666) were reagent to the micro-IDGA, in 68.0% (34/50) of the evaluated properties. The municipalities that present positive animals belong to the Metropolitan Curitiba, Northwest, South Central, West and Southwest regions, with a total of 15 positive municipalities (Figure 1). Campo Largo had the highest rate of seropositive animals, with 52.6%. The results according to the municipality are described in Table 1. Bivariate analysis was performed and the following variables from the epidemiological questionnaire: place of slaughter of animals on the property, ingestion of placental remains by domestic dogs, herd gathered in the sheepfold, free access to water on the property, production system adopted, and breed showed $p<0.20$. Table 2 presents the results of the bivariate analysis and Table 3 the multiple logistic regression.

Figure 1 - Map of the state of Paraná representing municipalities with animals positive for Caprine Arthritis Encephalitis (CAE)



Source: research data.

Table 1 - Distribution of CAEV-reactive goats using the micro-IDGA method, by municipality in the state of Paraná, with at least one seropositive animal

Municipality	Total	Positives	Positives (%)
Campo Largo	19	10	52.6
Manfrinópolis	18	9	50.0
Francisco Beltrão	79	27	34.2
Honório Serpa	43	13	30.2
Guaraniaçu	21	6	28.6
Itaipulândia	19	5	26.3
Araucária	20	5	25.0
Guaíra	19	4	21.1
Palmital	30	6	20.0
Cascavel	41	8	19.5
São Miguel do Iguaçu	44	8	18.2
Toledo	60	10	16.7
Marechal Cândido Rondon	39	6	15.4
Pinhão	20	3	15.0
Guarapuava	59	8	13.6

Source: research data.

Table 2 - Frequencies and bivariate analysis (P<0.20) of variables statistically associated with positivity for anti-CAEV antibodies in goats in the state of Paraná, Brazil

Variables	Positive/Total (%)	P	PR (CI 95%)
Breed			
Boer	41/307 (13.4)	0.0000	
Saanen	10/61 (16.4)		1.46 (0.69-3.11)
Mixed	77/257 (30.0)		3.20 (2.10-4.88)
Presence of a place for slaughtering animals on the property,			
Yes	70/283 (24.7)	0.0032	1.82 (1.22-2.72)
No	52/341 (15.2)		
Ingestion of placental remains by domestic dogs			
Yes	50/158 (31.6)	0.0000	2.63 (1.73-4.00)
No	73/489 (14.9)		
Production system adopted on the farm			
Intensive	12/168 (7.1)	0.0000	
Semi-intensive	87/339 (25.7)		4.48 (2.37-8.46)
On pasture	12/80 (15.0)		2.29 (0.98-5.35)
Herd gathered in the sheepfold			
Yes	119/648 (18.4)	0.0027	0.22 (0.08-0.57)
No	9/18 (50.0)		
Herd has free access to the property's water sources			
Yes	74/300 (24.7)	0.0007	2.00 (1.33-3.00)
No	46/327 (14.1)		

Source: research data.

The seropositivity of 19.2% for CAE observed in this study is the first data reported for the state of Paraná and is considered low (10-39%). According to Reina *et al.* (2009), herds with moderate (40-69%) to high (>70%) prevalence typically exhibit clinical symptoms, which were not reported by the farmers in this study. Feitosa *et al.* (2011) isolated CAEV from an animal in Rio Grande do Norte, Brazil, that showed clinical signs and tested positive in both IDGA and Western Blotting. Tu *et al.* (2020) stated that seropositivity detected by IDGA confirms viral circulation within herds. Globally, seropositive animals for CAEV have been documented. In Kosovo, Cana *et al.* (2020) reported a 15% seropositivity, while in Italy, Cirone *et al.* (2019) found a 51.42% positivity rate. In Brazil, Nascimento-Penido *et al.* (2017) reported 49.5% seropositivity in confined animals in Minas Gerais, while Teixeira *et al.* (2016) noted a 2.8% prevalence in a semi-extensive production system in Maranhão.

Table 3 - Final model of multiple logistic regression analysis of variables statistically associated with positivity of anti-CAE antibodies in goats from the state of Paraná, Brazil (P<0.05).

Variables	Coefficient	Standard error	Degrees of freedom	P-value	Adjusted PR (95% CI)
Production system adopted on the farm is semi-intensive	1.4853	0.3429	2	0.0000	4.41 (2.25-8.64)
Presence of a place for slaughtering animals on the property	0.7518	0.2645	1	0.0045	2.21 (1.26-3.56)
Ingestion of placental remains by domestic dogs	0.6515	0.2957	1	0.0276	1.91 (1.07-3.42)
Constant	-3.5547	0.3987		0.0000	

Source: research data.

In the present study, the semi-intensive production system, compared to confinement, was identified as a significant risk factor for seropositivity against CAEV, with an Adjusted Prevalence Ratio (PR) of 4.41. Although more intensive systems increase animal density per area, potentially elevating the chance of contact with infectious agents (Ramalho, 2010), they also facilitate sanitary control, allowing for regular diagnostic testing. These practices are crucial in managing CAEV, given the persistent nature of the disease and the absence of an effective commercially available vaccine.

Other factors associated with increased seropositivity risk, indicating a lower degree of technical management, included the presence of slaughter areas on the property, feeding dogs with placental remains, and unrestricted access to water sources on the property, likely due to more frequent exposure of animals to environments contaminated with biological waste. On the other hand, practices indicative of a higher degree of technical management, such as taking animals to the corral, were associated with a reduced risk (PR:0.22).

Regarding genetics, the statistical relevance of the Saanen breed and crossbred animals was noted. Indeed, the predisposition of dairy goat breeds is described in the literature (Thomann *et al.*, 2017), while the breeding of non-pedigree animals is also linked to a lower degree of technical management. In this sense, genetic selection of animals resistant to CAE exists and can contribute to controlling the disease in dairy herds, particularly in those where serological diagnosis is not performed regularly (Schultz *et al.*, 2020).

Seropositivity for CAEV has considerable economic impacts by reducing animal productivity both quantitatively and qualitatively. These factors not only decrease the direct economic yield of properties but also increase costs related to the management and treatment of infected animals. Furthermore, the lack of an effective control program can lead to the exclusion of producers from more competitive markets that require sanitary certification (Tavella *et al.*, 2018; Rodrigues *et al*., 2018). In

2023, the Brazilian goat herd expanded by 4%, reaching 12.9 million animals, while the sheep population grew by 1.3%, totaling 21.8 million. These represent the highest levels recorded for both livestock types in the history of the survey. Despite this, the goat herd in Paraná declined by 10.56% compared to the previous year, reaching a total of 67.438 animals in 2023 (Instituto Brasileiro de Geografia e Estatística, 2024). Although the South region of Brazil has a smaller herd than the Northeast, the South surpassed it in more than R$ 1.61 million of production value (Instituto Brasileiro de Geografia e Estatística, 2023). According to Monteiro *et al.* (2021), the economic competitiveness of the region is related to technological advances employed, highlighting the innovation in management techniques and the introduction of genetically improved breeds.

4 Conclusion

CAEV is circulating in herds from Paraná state, as well as the technification of the production system reduces the chance of seropositivity for this virus. The variables that indicate a low degree of technification on the property (raising animals without a defined breed, presence of a place for slaughtering animals on the property, feeding dogs with placental remains, having free access to water on the property, and confined production system) are considered factors associated with the risk of seropositivity for CAEV. On the other hand, taking the animals to the fold is considered a protective factor.

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