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Short Communications

Mucosa cytopathology for research of Sinegaglia-Lentz corpuscles in canine distemper

Citopatologia de mucosa para pesquisa de corpúsculos de *Sinegaglia-Lentz* na cinomose canina

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A R T I C L E   I N F O

A B S T R A C T

Canine distemper (CD) is a multisystemic and infectious disease caused by a *Morbillivirus*. The search for viral inclusions by mucosal cytology is a low cost and high practical alternative, which establishes the definitive diagnosis of CD, but is little reported in the literature. The objective was to encourage the use of mucosal cytopathology to identify *Sinegaglia-Lentz* corpuscles in the veterinary routine as an alternative or complementary to blood screening, providing a selection of photographs of the viral inclusions. Selected were 16 dogs with classic systemic or neurological disorders and positive for the chromatographic immunoassay for CD. Samples of the conjunctival, nasal and genital epithelium were collected with the aid of a sterile swab for making slides. Whole blood was also collected to make a blood smear. The slides were stained with a fast panoptic and observed by optical microscopy to directly search for eosinophilic viral inclusions, at 40 and 100x magnification. *Sinegaglia-Lentz* corpuscles were detected in nine of the 16 dogs (56.25%), five in conjunctival mucosa (41.65%), three in nasal mucosa (25%), one in genital mucosa (8.33%) and three in blood smear (25%). It is concluded that mucosa cytopathology, especially conjunctival, for *Sinegaglia-Lentz* research is an auxiliary tool for the early and definitive diagnosis of canine distemper. However, the absence of viral inclusions in these samples does not rule out the possibility of the disease.

R E S U M O

Palavras-chave:
Cães
Inclusões virais
Morbillivirus

A cinomose canina (CC) é uma doença multissistêmica e infectocontagiosa causada por um *Morbillivirus*. A pesquisa de inclusões virais por citologia da mucosa é uma alternativa de baixo custo e alta praticidade, que estabelece o diagnóstico definitivo da CC, mas pouco relatada em literatura. Objetivou-se encorajar o uso da citopatologia da mucosa para identificar corpúsculos de *Sinegaglia-Lentz* na rotina veterinária como alternativa ou complementar à triagem no sangue, fornecendo uma seleção de fotografias das inclusões virais. Foram selecionados 16 cães com distúrbios sistêmicos ou neurológicos clássicos e positivos ao imunoensaio cromatográfico para CC. Amostras do epitélio conjuntival, nasal e genital foram coletadas com auxílio de swab estéril, para confecção de lâminas. O sangue total também foi colhido para confecção de esfregaço sanguíneo. As lâminas foram coradas com panóptico rápido e observadas por microscopia óptica para pesquisa direta de inclusões virais eosinofílicas, em ampliação de 40 e 100x. Os corpúsculos de *Sinegaglia-Lentz* foram detectados em nove dos 16 cães (56,25%), cinco em mucosa conjuntival (41,65%), três em mucosa nasal (25%), um em mucosa genital (8,33%) e três em esfregaço de sangue (25%). Conclui-se que a citopatologia de mucosa, sobretudo conjuntival, para pesquisa de *Sinegaglia-Lentz* é uma ferramenta auxiliar ao diagnóstico precoce e definitivo da cinomose canina. Contudo, a ausência de inclusões virais nestas amostras não descarta a possibilidade da doença.

INTRODUCTION

Canine distemper (CD) is a multisystemic and infectious disease caused by a *Morbillivirus* in the family *Paramyxoviridae* (UHL *et al.*, 2019). It has high morbidity and mortality and worldwide distribution. The disease has no seasonality or predilection for sex or race, but mainly affects young animals (HEADLEY *et al.*, 2012; COSTA *et al.*, 2019; DIGANGI *et al.*, 2019; GREEN *et al.*, 2020).

Although quite suggestive, the clinical picture presents variable symptoms, related to gastrointestinal, respiratory and neurological disorders. In addition, infected dogs develop immunosuppression, allowing secondary infections by opportunistic agents (RENDON-MARIN *et al.*, 2019). Thus, laboratory tests are necessary for differential diagnosis (BARBOSA *et al.*, 2011).

For diagnosis, sophisticated techniques such as immunoenzymatic assay, viral isolation, direct immunofluorescence, viral seroneutralization and PCR (polymerase chain reaction) can be used. However, these tests are expensive and require technical training and specific laboratory equipment (YI *et al.*, 2017; WANG *et al.*, 2018; LIU *et al.*, 2019; MICHELAZZO *et al.*, 2020).

The search for inclusion corpuscles by blood or mucosal cytology is a viable alternative due to its low cost, practicality and speed of performance. Observation of inclusion bodies establishes the definitive diagnosis of CD, given its specificity for viral species. These inclusions are nucleocapsid aggregates and cell residues resulting from replication of the distemper virus during the viremic phase and can be visualized in the cytoplasm of blood, endothelial or epithelial cells under eosinophilic staining (MARTINS; LOPES; FRANÇA, 2009; NOLETO *et al.*, 2011; GRANJEIRO *et al.*, 2020).

However, the methodology is little used for the mucosa, with blood smear being the predominant technique (VICENTE; ABREU; PASSOS, 2010). Although inclusions in the cells of the ocular or genital mucosa are characterized as pathognomonic, there are few studies in the literature on the subject, and no illustrations of corpuscles found in epithelial cells. These illustrations serve as a model for other veterinarians who wish to use the technique in their clinical routine.

In this context, the objective was to encourage the use of mucosal cytopathology to identify *Sinegaglia-Lentz* bodies in the veterinary routine as alternative or complement to blood screening, providing a selection of photographs of viral inclusions.

MATERIAL AND METHODS

The study was carried out according to the ethical principles of animal experimentation, approved by the Animal Use Ethics Committee of the Federal University of Acre, under license number 81/2015.

For non-probabilistic convenience (ROUQUAYROL; GURGEL, 2017), 16 unvaccinated dogs were selected, regardless of sex, age and breed, with classic systemic or neurological disorders (RENDON-MARIN *et al.*, 2019) and positive to the chromatographic immunoassay for qualitative detection of IgG antibody against canine distemper virus (Alere®, São Paulo, Brasil). Thus, the sample profile was made up of males (50% - 8/16) and females (50% - 8/16), with age group less than six months (43.75% - 7/16), between six months and one year (31.25% - 5/16) and greater than one year (25% - 4/16), of different racial patterns or without defined ration (43.75% - 7/16).

Samples of the conjunctival, nasal and genital epithelium were collected with the aid of a sterile swab, which was introduced with rotational movements in the respective cavities (Fig. 1). The biological material was transferred to glass slides with scrolling movements, and Diff Quick stain was used. An aliquot of the whole blood was also used to make a blood smear and search for viral inclusion (THRALL, 2015; ATHANASIOU *et al.*, 2018).

Figure 1. Canine sample collection with distemper. A) Nasal epithelium sample; B) Sample of conjunctival epithelium; C) Sample of genital epithelium.
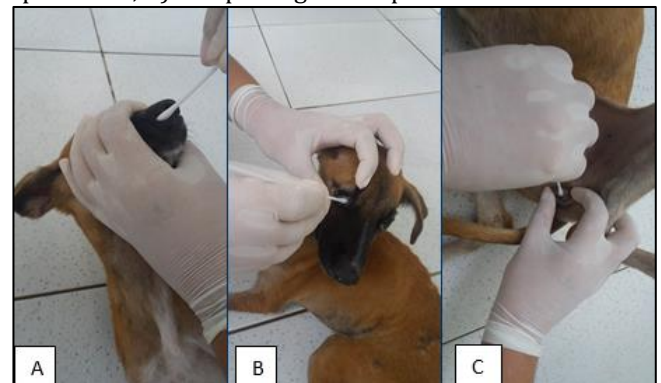
The search for *Sinegaglia-Lentz* corpuscles was performed by optical microscopy, with 40x and 100x magnification, considering as positive the eosinophilic staining inclusions in the leukocyte, erythrocyte or epithelial cell cytoplasm, with an average diameter between 600 and 1,000 nm (ATHANASIOU *et al.*, 2018). The results were analyzed using descriptive statistics (ROUQUAYROL; GURGEL, 2017).

RESULTS AND DISCUSSION

Sinegaglia-Lentz corpuscles were detected in nine of the 16 dogs (56.25%), five in conjunctival mucosa (41.65%) (Fig. 2), three in nasal mucosa (25%) (Fig. 3) and one in the genital mucosa (8.33%) (Fig. 4). Comparatively, in three blood smear samples (25%) (Fig. 5) viral inclusions were also identified.

Figure 2. *Sinegaglia-Lentz* corpuscles in conjunctival mucosa samples. Obj. 100x.
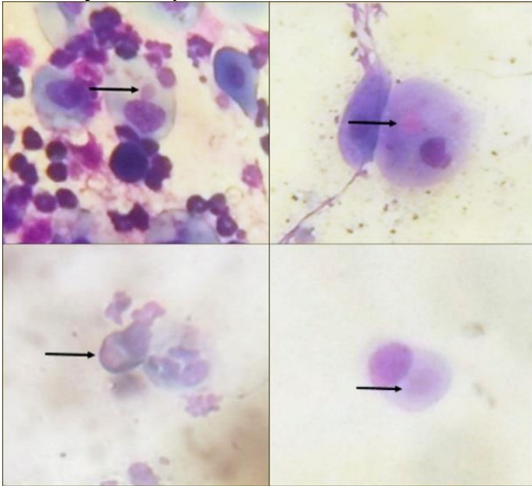

Figure 3. *Sinegaglia-Lentz* corpuscles in nasal mucosa samples. Obj. 100x.
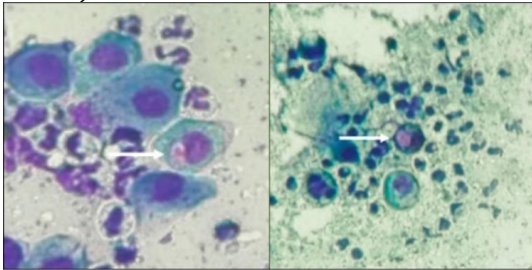

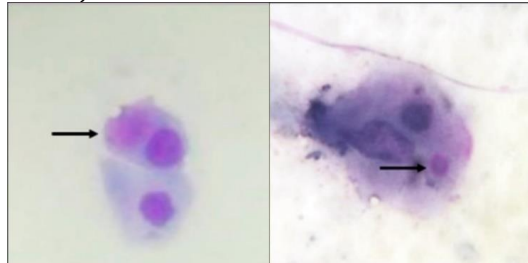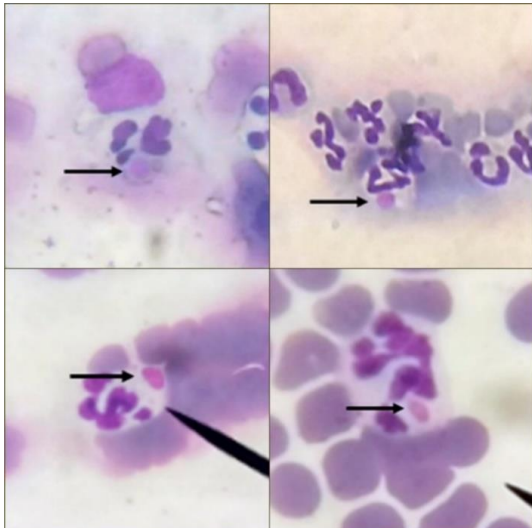Figure 4. *Sinegaglia-Lentz* corpuscles in genital mucosa samples. Obj. 100x.

Figure 5. *Sinegaglia-Lentz* corpuscles in blood samples. Obj. 100x.

Variations in relation to the morphology of the corpuscles were also evidenced. When evaluating blood smears, the inclusions are well delimited, oval and of equivalent dimensions. However, in mucosal cytopathology, the inclusions had variable and poorly defined dimensions. It was also observed that, depending on the type of sample, the presence of cellular debris and amorphous material can moderately hinder the visualization of the corpuscles.

Scheigert *et al*. (2008), when analyzing a small population of dogs with clinical suspicion of CD, identified inclusion bodies in 38.8% of the blood smear samples, but none in eye cytopathology. Similarly, Cowell *et al*. (2009) claim that these corpuscles are rarely found in epithelial cells. However, this study corroborated the results found by Gonçalves *et al*. (2012), who tested two dogs with clinical suspicion of CD and found corpuscles in the epithelial cells of both.

Although blood is classically selected as a sample to detect *Sinegaglia-Lentz* corpuscles (VICENTE; ABREU; PASSOS, 2010; SOUSA *et al*., 2015), in this study, the samples obtained from the conjunctival mucosa were more efficient. It is known that during the neurological phase of CD, low viremia occurs, however, viral persistence in ocular tissue is observed for a long period, resulting in nucleocapsid aggregates in the epithelial cell cytoplasm (MARTINS; LOPES; FRANÇA, 2009; HEADLEY *et al*., 2012; GRANJEIRO *et al*., 2020), which can justify the results found.

The rapid diagnosis of CD is essential to establish the prognosis and assist in the early therapeutic management of the disease, optimizing the chances of successful treatment (AMUDE *et al*., 2012). However, the clinical presentation is not always evident. Thus, although underused in the veterinary routine (ATHANASIOU *et al*., 2018), mucosa cytopathology for *Sinegaglia-Lentz* research is an alternative or complementary tool for the definitive diagnosis of CD. However, the non-visualization of viral inclusions in conjunctival samples does not rule out the possibility of the disease.

CONCLUSIONS

Mucosa cytopathology, especially conjunctival, for *Sinegaglia-Lentz* corpuscles research is an auxiliary tool for the early and definitive diagnosis of canine distemper. However, the absence of viral inclusions in these samples does not rule out the possibility of the disease.

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