Positive dogs for ehrlichiosis in the immunochromatographic test and negatives in the polymerase chain reaction: report of six cases with therapeutic success

Cães positivos para ehrliquiose no teste imunocromatográfico e negativos na reação em cadeia da polimerase: relato de seis casos com sucesso terapêutico

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ABSTRACT
Canine Monocytic Ehrlichiosis is a hemoparasitosis whose etiologic agent is Ehrlichia canis, a gram-negative and obligate intracellular bacteria. The clinical signs of this disease are considered non-specific, therefore, laboratory tests are needed to confirm the diagnosis. The present study aims to report six cases of ehrlichiosis in dogs positive in the immunochromatographic test and negative in the polymerase chain reaction (PCR). These animals showed complete recovery after clinical treatment, suggesting that carrier animals may present negative results even in laboratory tests of high sensitivity and specificity, such as PCR. The findings of this report allowed us to conclude that the treatment, in some cases, can be based only on diagnostic suspicion, in association with clinical and hematological signs.

Keywords: dog, *Ehrlichia canis*, PCR, rapid test.

RESUMO
A Erliquiose Monocítica Canina é uma hemoparasitose cujo agente etiológico é a *Ehrlichia canis*, uma bactéria gram-negativa e intracelular obrigatória. Os sinais clínicos desta doença são considerados inespecíficos, portanto, exames laboratoriais são necessários para confirmar o diagnóstico. O presente estudo tem como objetivo relatar seis casos de erliquiose em cães positivos no teste imunocromatográfico e negativos na reação em cadeia da polimerase (PCR). Esses animais apresentaram recuperação completa após tratamento clínico, sugerindo que animais portadores podem apresentar resultados negativos mesmo em exames laboratoriais de alta sensibilidade e especificidade, como a PCR. Os achados deste relato permitiram concluir que o tratamento, em alguns casos, pode ser baseado apenas na suspeita diagnóstica, associada a sinais clínicos e hematológicos.

Palavras-chave: cão, *Ehrlichia canis*, PCR, teste rápido.

1 INTRODUCTION

Canine tick-borne diseases are caused by bacteria or protozoa that parasitize blood cells. Among the etiologic agents of hemoparasitic infections, *Ehrlichia canis*, *Anaplasma platys* and *Babesia vogeli* are noteworthy due to their wide geographic distribution and high prevalence (ARAÚJO et al. 2015). These diseases include canine

monocytic ehrlichiosis (CME) which is caused by the bacterium Ehrlichia canis (PAULINO et al., 2018).

Canine monocytic ehrlichiosis is a worldwide distribution hemoparasitosis, being more frequent in countries with tropical and temperate climates, which occurs due to the high prevalence of the biological vector of the disease (*Rhipicephalus sanguineus*) in these places (VIEIRA et al., 2011). CME mainly affects dogs and its etiologic agent (*Ehrlichia canis*) is an obligate intracellular gram-negative bacterium (TAYLOR; COOP; WALL, 2017).

The severity of CME depends on several factors, including the pathogen strain, the dog's immune response and the presence of concomitant infections (SAINZ et al., 2015). However, the clinical course of the infection can be divided into acute, subclinical and chronic phases (NEER et al., 2002). Among the most commonly presented signs are fever, weight loss, apathy, emesis, epistaxis, petechiae and ecchymosis on skin and mucosal surfaces, lymphadenopathy, vasculitis, liver disease, nephropathy and ophthalmopathies such as uveitis, in addition to neurological signs such as seizures and ataxia (PARMAR et al., 2013; SILVA et al., 2021). These signs are considered non-specific and may present in different degrees of severity, depending on the virulence of the strain involved in the infection, the resistance or susceptibility of the host or the stage of the disease (acute, subclinical or chronic) (LIMA et al., 2021; SILVA et al., 2021).

The diagnosis of CME can be made based on clinical signs in association with hematological alterations (PARMAR et al., 2013; SILVA et al., 2021). A history of tick infestation is relevant information and contributes to clinical suspicion, especially in association with laboratory diagnosis. The blood count is considered a screening test and is of great importance for diagnosis, in which the most commonly observed changes are thrombocytopenia, leukopenia and normochromic normocytic anemia (PARMAR et al., 2013; SILVA et al., 2021). In the blood smear, morulae can be seen in infected cells (PARMAR et al., 2013). However, such morulae are difficult to visualize, being observed only in the acute phase of the disease and in a low percentage of laminas (PARMAR et al., 2013). A method often used to diagnose the disease is the serological enzyme-linked immunosorbent test (ELISA), which detects anti-Ehrlichia canis antibodies, also called immunochromatographic tests or rapid tests. The 4Dx plus test (IDEXX Laboratories®, USA) is one of the most used in Brazil for this purpose, with a percentage of 97.8 and 92.3 sensitivity and specificity, respectively (STILLMAN et al., 2014). Due to their high sensitivity, the use of immunochromatographic tests is recommended in clinical routine

to identify exposure to the causative agent of CME (PARMAR et al., 2013; STILLMAN et al., 2014). Molecular biology is also used in the diagnosis of canine ehrlichiosis such as the polymerase chain reaction (PCR). The PCR technique has more specificity when compared to other methods previously described and can be used in different clinical stages of the disease (NAKAGHI et al., 2008; HARRUS; WANER, 2011; PARMAR et al., 2013). The gold standard for diagnosis of CME is Indirect Immunofluorescence (IFAT) serology, revealing exposure to the agent in both acute and chronic cases (NAKAGHI et al., 2008; HARRUS; WANER, 2011).

The antibiotic of choice for the treatment of canine ehrlichiosis is doxycycline (MYLONAKIS; HARRUS; BREITSCHWERDT, 2019), in addition to antiemetic drugs, gastric protectors, appetite stimulants, antipyretics, multivitamins, among other drugs used to provide support during the recovery of the animal (CHETHAN et al., 2016).

The aim of this study is to report six cases of canine ehrlichiosis whose patients were on immunochromatographic test positive and negative PCR, but responded to treatment with complete recovery.

2 CASE REPORTS

Six dogs with a history of anorexia or decreased appetite, apathy and presence of ticks were attended at a Teaching Veterinary Hospital (TVH) (Table 1).

After analyzing the history and clinical examination, ehrlichiosis was suspected, and hematological tests were then requested, which revealed thrombocytopenia in almost all animals suspected of the disease (Table 2). The immunochromatographic tests of these patients were positive in all cases, however, the PCR tests for ehrlichiosis were negative for the presence of E. canis in the analyzed material.

Although negatives in the PCR test, the suspicion remained that the dogs were carriers of the disease due to tick infestation, clinical signs and laboratory findings (Table 1, Table 2). Thus, treatment based on Doxycycline was instituted at a dose of 5mg/kg every 12 hours for 28 days and Prednisolone at a dose of 0.5mg/kg every 24 hours for seven days, which was reduced by 50% every five days, in a total of 21 days. After treatment, all dogs recovered completely and did not show any clinical signs of illness.

Table 1 – Clinical data of dogs with suspected ehrlichiosis (n=6).

Patient	Presence of ticks	clinical signs				
		Fever	Changes in appetite	pale mucous membranes	weakness	Others
1	yes	yes	hyporexia	no	no	apathy
2	yes	no	hyporexia	yes	yes	apathy
3	yes	no	hyporexia	no	no	apathy
4	yes	yes	anorexia	yes	no	apathy and emaciation
5	yes	no	anorexia	yes	yes	apathy and emaciation
6	yes	no	anorexia	no	no	apathy

Source: the auhors

Table 2 – Laboratory data of dogs with suspected ehrlichiosis (n=6).

Patient	platelet count (10^3/µl)*	Anemia	immunochromatographic test	Chain reaction Polymerase
1	183	no	positive	negative
2	8	no	positive	negative
3	126	no	positive	negative
4	83	Yes	positive	negative
5	26	Yes	positive	negative
6	38	no	positive	negative

*Reference parameters for the canine species:175.000 a 500.000 (FELDMAN; ZINKL; JAIN, 2000).
Source: the auhors

3 DISCUSSION

Among the six dogs treated with suspected ehrlichiosis, two clinical conditions occurred in all animals. One of them was the presence of ticks (Table 1), and this information is important in the composition of the diagnosis, since ticks are vectors of ehrlichiosis (OLIVEIRA et al., 2019). Thus, dogs affected by the disease are, in most cases, infested by ticks (CIRINO et al., 2021), as observed in the dogs in this study. Another clinical condition observed in all animals was apathy (Table 1). This clinical sign is frequently mentioned in studies on ehrlichiosis in dogs (PARMAR et al., 2013; LIMA et al., 2021; MACIEL; SILVA; ESPÍRITO SANTO, 2021). Apathy is probably due to fever. On clinical examination, only a few animals presented fever, however, in affected animals the temperature fluctuates, and the dog may present fever peaks according to the clinical stage of the disease (NELSON; COUTO, 2015; MYLONAKIS; HARRUS; BREITSCHWERDT, 2019).

Thrombocytopenia was detected in five of the six dogs in the present study (Table 2), as reported by several authors (PARMAR et al., 2013; LIMA et al., 2021; MACIEL; SILVA; ESPÍRITO SANTO, 2021). Causes for thrombocytopenia include increased consumption of platelets as a result of inflammatory changes in the endothelium of blood

vessels, increased splenic platelet sequestration and immunological destruction of platelets (RAMAKANT; VERMA; DIWAKAR, 2020).

Infection by Ehrlichia canis often causes a diagnostic dilemma, since clinical signs are nonspecific (PARMAR et al., 2013). That said, it is essential to carry out laboratory tests, especially the blood count, which may show changes suggestive of the infection. It is understood that although immunochromatographic tests indicate the dog's exposure to the antigen, this does not necessarily imply the occurrence of the disease (VIEIRA et al., 2011). In the present study, all animals were positive in the immunochromatographic test (Table 2), however, the PCR tests were negative in all animals evaluated (Table 2). In the present report, all patients had clinical signs of the disease (Table 1). Thus, it was inferred that, although PCR negative, the dogs had ehrlichiosis. Several studies demonstrate that animals with clinical signs of ehrlichiosis may present PCR negativity (PARMAR et al., 2013; HARRUS; WANER, 2011; LARA et al., 2020), which can occur in cases of low bacteremia (LARA et al., 2020). It is noteworthy that among the available laboratory tests, false positives and false negatives can occur, since the effectiveness of such methods depends on the clinical stage of the patient. Animals in the acute phase of the disease can respond positively to the tests, however, animals in the chronic phase of the disease usually show negative results (NAKAGHI et al., 2008). Thus, it is suggested that the diagnosis of ehrlichiosis in dogs can be based on clinical history, physical examination findings and additional laboratory tests (PARMAR et al., 2013), as used in the dogs in the present study.

Regarding treatment, doxycycline is considered the antibiotic of choice in the treatment of dogs with ehrlichiosis and, for this reason, it was used in the treatment of reported patients (SILVA et al., 2021). In the present study, the animals responded positively to the instituted therapy, which reinforces the diagnostic suspicion.

4 CONCLUSION

The present report highlights the fact that, even tests with high specificity and sensitivity, such as PCR, can be negative in E. canis infections, thus suggesting that, if the occurrence of the infection is suspected, the diagnosis can be based, with reasonable safety in the association between patient history, clinical and hematological findings.

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