Brazilian Journal of Development

Molecular detection of *Mycoplasma haemofelis* in domestic cats from municipality of mineiros, Goiás

Detecção molecular de *Mycoplasma haemofelis* em gatos domésticos do município de mineiros, Goiás

DOI:10.34117/bjdv6n5-021

Recebimento dos originais: 15/04/2020
Aceitação para publicação: 04/05/2020

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ABSTRACT

Feline mycoplasmosis is caused by *Mycoplasma haemofelis* agent and your transmission is through the sanguine meal of ectoparasitas, due the lack of studies on the disease is considered serious and of difficult diagnnosis being more necessary studies in order to explain the transmission, patogenia and control. This work had as objective analyzed animals of Mineiros-GO and to verify possible the existence of this microorganism, where all the tested felines obtained negative result through the technique of Polimerase chain reaction (PCR).

Keywords: Felines. Feline mycoplasmosis. PCR.

RESUMO

A micoplasmose felina é causada pelo agente *Mycoplasma haemofelis* e sua transmissão é através do repasto sanguíneo de ectoparasitas, mas por falta de estudos sobre a doença é considerada grave e de difícil diagnóstico sendo necessário mais estudos a fim de esclarecer melhor sua transmissão, patogenia e controle. Este trabalho teve como objetivo analisar animais de Mineiros-GO e verificar possível a existência deste microrganismo onde todos os felinos testados obtiveram resultado negativo através da técnica de Reação em cadeia de polimerase (PCR).

Palavras-chave: Felinos. Micoplasmose felina. PCR.

1 INTRODUCTION

Mycoplasma haemofelis is the etiological agent of feline mycoplasmosis, which is considered a small bacterium with a size between 0.3 and 0.8 μm. The mycoplasmas are hemotropic, Gram-negative extracellular bacteria with pleomorphic ability, without cell wall and have the ability to aggregate to erythrocyte walls (Santos *et al*., 2011) And can be present in a coccoid, annular or rod-shaped format, and may occasionally compose currents of 3 to 6 organisms (Haefner *et al*.,2003). *M. haemofelis* is the most virulent and clinically significant described so far.

Feline mycoplasmosis known as feline hemotropic mycoplasmosis or feline infectious anemia is caused by the etiological agent designated *M. haemofelis*, which was formerly called *Haemobartonella felis* (Urquhart *et al.*, 1998). Hemotropic mycoplasmas are Gram negative bacteria that use the surface of the erythrocytes of several mammalian species for their survival (Messick, 2004; Biondo *et al*. 2009) presenting a pleomorphic shape and being predisposed to be in the form of cocci, coccobacilli or small stems and usually constitute small currents (Almosny e Souza, 2012). They are microorganisms with little extension of the genome that reproduce through binary fission (Messick, 2004). In electron microscopy, they are found in shallow depressions on the surface of erythrocytes (Almosny e Souza, 2012).

Species such as *Candidatus M. haemominutum* and *Candidatus M. turicensis* are also apt to infect felines inducing the appearance of clinical signs and even mild laboratory modifications (Messick e Harvey, 2011). The species of hemoplasmas were not described in Antarctica alone, revealing a global distribution of organisms (Macieira, 2011). The first feline *M. haemofelis* was found in South Africa in 1942 (Martinez et al. 2016) being named *Eperythrozoon felis* (Sykes, 2010; Barker *et al*. 2011) in the United States, a similar organism was found that caused infectious anemia in felines and after research, the organism was assigned to the *Anaplasmataceae* Family, Rickettsiales Order and *Haemobartonella* genus (Sykes, 2010). In Brazil it was identified in 1976 (Martinez *et al*. 2016).

There were two types of *Haemobartonella felis*: one larger and isolated from Ohio, and one isolated from California (Santos, 2012). In order to evaluate the effect of the 16S rRNA gene on the 16S rRNA gene, the genotypes *Haemobartonella* and *Eperythrozoon* were renamed and began to participate in the Mollicutes class and the genus *Mycoplasma* (Neimark *et al.*,2001; Messick e Harvey, 2011; Biondo *et al.* 2009), as a consequence the larger and pathogenic form of *H. felis* was named *M. haemofelis* while the smaller and less pathogenic form of *Candidatus M. haemominutum* (Santos, 2009; Neimark *et al*.,2013). The third species of hemoplasm in cats was found by Willi et al. In 2005 and designated *Candidatus M. turicensis* (Sykes, 2010) and these mycoplasmas that have '*Candidatus*' to their nomenclature are recent and with unfinished characterization (Messick, 2004).

Hemotropic mycoplasmas have four distinct species detected in cats by PCR tests: *M. haemofelis, Candidatus M. haemominutum, Candidatus M. haematoparvum* and *Candidatus M. turicensis* (Javinky *et al.* 2012). The most pathogenic is *M. haemofelis*, which is mainly related to hemolytic anemia (Stockham e Scott, 2012).The mycoplasmas present the

peculiarity of not being able to be cultured in vitro, the infecting dose can not be established with experimental accuracy interfering in the results (Carneiro, 2015).

This study had as a deciding factor the amount of cats that arrive at the veterinary clinics presenting ectoparasites, which can cause damages in the health and well being of the animal. Since few studies indicate the prevalence of this species of *M. haemofelis* and it is not yet known how this disease is transmitted, making treatment difficult, it was observed the need to develop this study in order to bring new data and information about this disease using blood samples of felines from the municipality of Mineiros-GO from November 2017 to June 2018.

2 MATERIAL AND METHODS

This study was carried out in the municipality of Mineiros-GO from November 2017 to June 2018, where 110 blood samples were randomly selected, as they arrived at the Bio Veterinary Laboratory, and most of these samples came from the Veterinary Office of the UNIFIMES and the NeoVet Veterinary Office. These whole blood samples were stored under refrigeration at -20ºC and later sent to the University Hospital of the Federal University of Mato Grosso (UFMT) for the laboratory of Molecular Biology. This research was approved by the Committee on Ethics in the Use of Animals (CEUA), of the University Center of Mineiros, under protocol number 15/2017.

The DNA extraction technique was carried out using phenol-chloroform with addition of alcohol according to Del Peta (1999). (A Isis vai procurer, tbm não achei). After adding the sample amount and centrifuging it, glass beads (Fig. 1A) were added, guaranteeing better extraction of DNA. It was agitated in the Vortex apparatus and washed the sample with alcohol removing the excess and sending the eppendorf tubes to the oven (Fig. 1B), drying and the DNA remaining in the wall of the tubes.

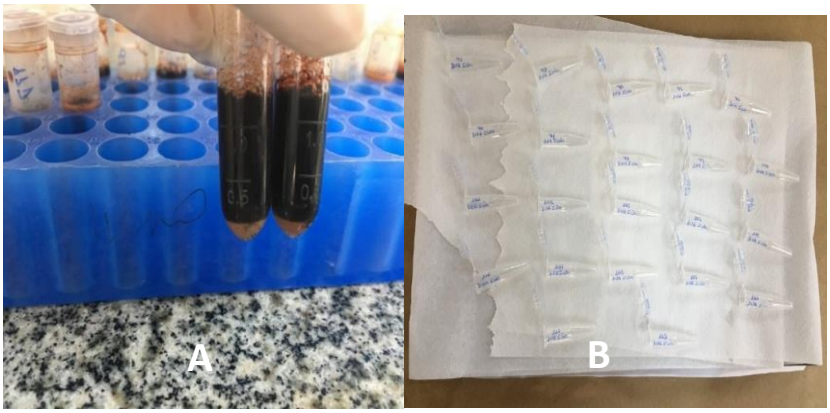

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FIGURE 1 - DNA extraction technique, (A) Addition of glass beads (white part at the bottom of eppendorf tube) guaranteeing better extraction of DNA. (B) Eppendorf tube after removal of excess alcohol and ready to be placed in an oven.

The polymerase chain reaction (PCR) technique was used according to Watanabe *et al.*, (2003), using specific primers for M. haemofelis: OH-OK (5'-ATGCCCCTCTGTGGGGGATAGCCG-3') and CA-B2 5'-CTGGGAAACTAGAGCTTCGCGAGC-3'), aiming to amplify a 100pb fragment of the 16S rRNA gene in the Applied Biosystems™ Vetiri™ 96-Well Fast Thermal Cycler (Fig. 2A). The samples were taken from the thermocycler and placed to run through the 1.5% agarose gel, after which electrophoresis was performed (Fig. 2B) and placed in the transilluminator to visualize the result of the samples (Fig. 3).

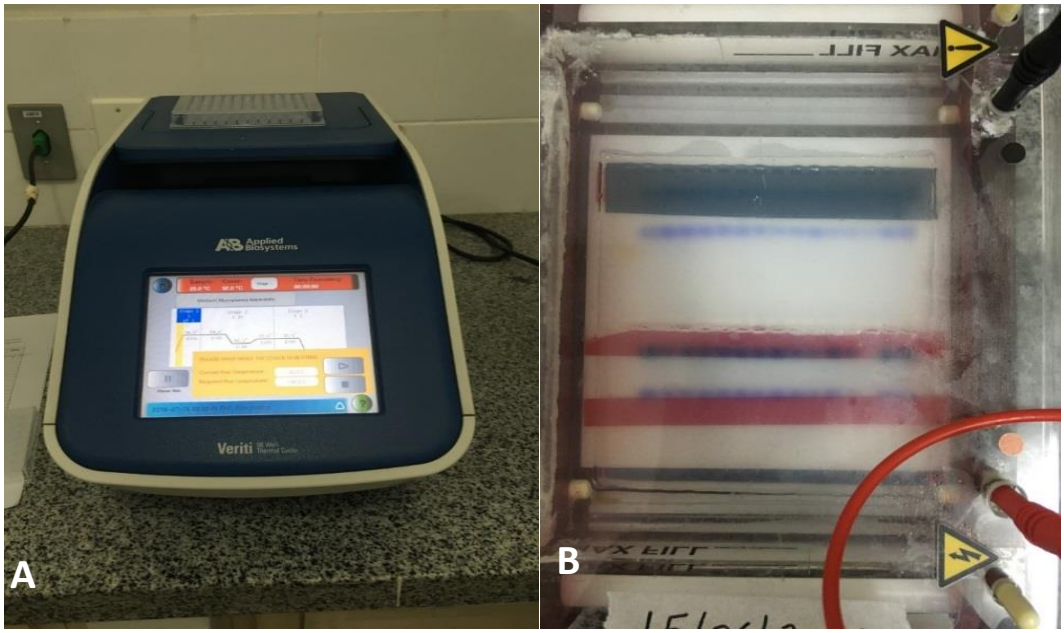

FIGURE 2 - Polymerase Chain Reaction (PCR) technique, (A) Applied Biosystems™ Vetiri™ 96-Well Fast Thermal Cycler, (B) 1.5% agarose gel samples for electrophoresis.

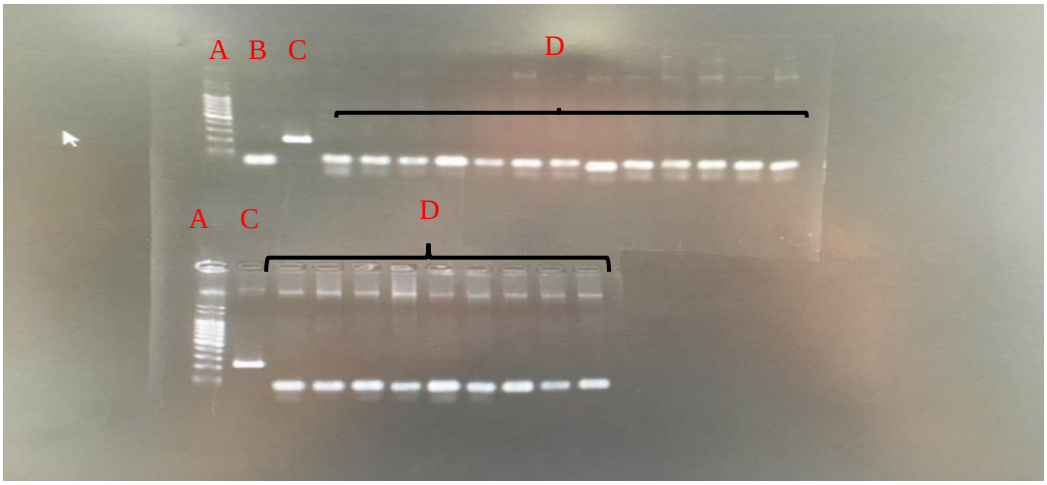

FIGURE 3 - Negative result of the PCR test of *M. haemofelis* after electrophoresis where (A) shows the molecular marker of 100bp. (B) negative control, (C) positive control and (D) samples.

3 RESULTS AND DISCUSSION

In this work the tested animals were negative in the PCR test for *M. haemofelis*, it is necessary to repeat the PCR to confirm the negative result and to conclude that these animals are really negative without occurring the chance of being carriers and provided they do not present clinical signs, that is, to exclude the chance that in the first test the animal did not present bacteremia when the material was collected and excluded the option of being an asymptomatic carrier.

Feline mycoplasmosis is a disease caused by epicellular microorganisms (Willi, 2005). They aggregate to the surface of erythrocytes causing hemolysis (Messick, 2004), and consequently acute or chronic hemolytic anemia (Page, 2003) and evolving to death. According to Biondo *et al.* (2009), the most predisposed felines are males with free access to the street for being involved in fights by females in estrus and territory dispute.

In the study conducted by Martinez *et al.(*2016) a presence considered feline hemoplasmosis in Brazil was reported between 5 and 23%. According to Harrus et al. (2002) and Paula *et al.(*2012),in their studies, the most threatened animals were cats up to 6 years of age and the expectation of infection falls on cats older than six years of age confronted with a reported in the city of Jataí-GO, where a 10-year-old female and with access to the street was for dermatological consultation and in the result of the hemogram the *M. Haemofelis* (Terra, 2015) and also contradicts Da Silveira's *et al.*(2014), where a Persian cat, domiciled, aged 2 years with signs of anorexia and when requested PCR for *M. haemofelis*, was attended at a veterinary clinic in the city of Nova Friburgo, RJ. result was positive.

These animals are vulnerable to relapses but are not considered a contagious feline (Norsworthy, 2004), complicating the diagnosis as cited by Almosny (2012) who considers it difficult to obtain the diagnosis due to the lack of identifiable parasitemia in chronic infections or by the slight decrease of the after the establishment of the symptomatology in the acute cases and still considers the PCR, the most recommended test for the diagnosis considering the greater sensitivity when compared to the research with the blood smear. The efficacy in the treatment is dependent on a correct diagnosis, which becomes complicated by being an underdiagnosed disease in Brazil and directly affected in the treatment, in the preventive and therapeutic measures and also in the epidemiological studies (Martinez *et al*. 2016).

A study conducted by Martinez *et al.(*2016) in Osasco, São Paulo, in the year 2013 to 2014 diagnosed 15 cats with feline mycoplasmosis where it was described that the low frequency may be related to the non-appearance of clinical signs or lack of authorization for the accomplishment of hemograms and blood smear by the owners, still differing from this study carried out in Mineiros where it did not obtain any percentage of positive cats with *M. haemofelis*. Also according to Martinez *et al.* (2016) when harvesting is performed in tubes containing EDTA as anticoagulant, the result may be influenced because feline erythrocytes are more susceptible to EDTA, releasing the microorganism from the surface of the erythrocytes

being then suggested to collect this blood in tubes that use heparin, as anticoagulante (Alleman *et al*.1999).

4 CONCLUSIONS

With this work we can conclude that the sampled cats of the city of Mineiros-GO did not present positive results as in other studies described above where the prevalence was present in considerable values, but even with this result the animals should always be taken care of, and the clinical suspicions should not be ruled out because of the difficulty of finding the microorganism in the blood and because it is not an easily diagnosable disease besides not yet having a totally clear means of transmission.

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