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Case report

Multisystem infection by *Dipetalonema* spp. in a capuchin monkey (*Sapajus nigritus*)

Infecção multissistêmica por *Dipetalonema* spp. em um macaco-prego (*Sapajus nigritus*)

Bibiana da Rosa Pereira[1], Ezequiel Davi dos Santos[1], Aline Bielak dos Santos[1], Paula Michelson Vanzin[1], Bruno Webber Klaser[1], Marina Gatto[1], Sabrina Pavan Flores[1], Tanise Policarpo Machado[1], Michelli Westphal Ataíde[1], Maria Isabel Botelho Vieira[1], Adriana Costa da Motta[1*]

[1]Universidade de Passo Fundo (UPF), Passo Fundo, Rio Grande do Sul, Brazil
[*]Corrsponding author: acmotta@upf.br

Abstract
The capuchin monkey (*Sapajus nigritus*) is one of the primates that make up the wild fauna of Rio Grande do Sul. Like other wild animals, primates suffer from infectious diseases transmitted by insects, including filariasis. This report presents the anatomopathological aspects of a multisystemic infection by *Dipetalonema* spp. in a capuchin monkey, female, adult, of wild origin, rescued along a highway in the north of the state of Rio Grande do Sul by the road policing service and taken for assistance. In the clinical evaluation, nystagmus and involuntary head movements, semi-comatose state, hyperthermia (38.7ºC), hypovolemia, multiple abrasions and deep skin lacerations covering muscle layers were observed. The patient was hospitalized and medicated, but his clinical picture worsened, which resulted in death. At necropsy, the main findings were in the peritoneal cavity, where there was an accumulation of yellowish serous fluid, marked formation of fibrinous adhesions and numerous free nematode parasites in the cavity. Histologically, microfilariae have been observed in the lumen of blood vessels in the lung, liver, heart, kidneys, spleen, small and large intestines, and brain. Peritonitis, pneumonia and bronchiolitis associated with nematodes, liver damage and cerebral microgliosis with satellitosis, neuronophagia and hemorrhagic malacia were also observed. Adult microfilariae and filarids were submitted to morphometric identification and characterized as belonging to the genus *Dipetalonema*. Thus, it was concluded that this was a case of multisystemic infection by *Dipetalonema* spp..
Keywords: wildlife animal; dipetalonemosis; microfilaria; parasite; monkey

Resumo
O macaco-prego *(Sapajus nigritus)* é um dos primatas que compõem a fauna silvestre do Rio Grande do Sul. Assim como outros animais silvestres, os primatas padecem de doenças infecciosas veiculadas por insetos, entre as quais, as filarioses. Este relato apresenta os aspectos anatomopatológicos de uma infecção multissistêmica por *Dipetalonema* spp. em um macaco-prego, fêmea, adulta, de origem silvestre, resgatado junto a uma rodovia do Norte do Estado do Rio Grande do Sul pelo serviço de policiamento rodoviário e conduzida para atendimento. Na avaliação clínica observaram-se nistagmo e movimentos involuntários da cabeça, estado semi-comatoso, hipertermia (38,7ºC), hipovolemia, múltiplas escoriações e lacerações cutâneas profundas abrangendo camadas musculares. O paciente foi hospitalizado e medicado, mas apresentou piora do quadro clínico, que evoluiu a óbito. Na necropsia, os principais achados foram na cavidade peritoneal, onde observaram-se acúmulo de líquido seroso amarelado, marcada formação de aderências fibrinosas e numerosos parasitas nematódeos livres na cavidade. Histologicamente foram observadas microfilárias no lúmen de vasos sanguíneos do pulmão, fígado, coração, rins, baço, intestinos delgado e grosso, e cérebro. Também foram constatadas peritonite, pneumonia e bronquiolite associadas à nematódeos, danos hepáticos e microgliose cerebral com satelitose, neuronofagia e malácia hemorrágica. Microfilarias e filarídeos adultos foram submetidos à identificação morfométrica e caracterizados como pertencentes ao gênero *Dipetalonema*. Assim, foi concluído tratar-se de um caso de infecção multissistêmica por *Dipetalonema* spp.
Palavras-chave: animal silvestre; dipetalonemose; microfilária; parasita; macaco

1. Introduction

The capuchin monkey (*Sapajus nigritus*) is a primate characteristic of the native fauna of southern Brazil, particularly the state of Rio Grande do Sul, and is considered a species of wide geographic distribution among neotropical primates[1,2]. This is because they are animals with great adaptive capacity due to their omnivorous diet and social structure that allows cohesion between males and females[2].

Like other wild animals, capuchin monkeys suffer from infectious diseases transmitted by insects, including those of parasitic origin[3]. The filarial nematodes of the

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genus *Dipetalonema*, transmitted by insects of the genus *Culicoides*, constitute an important cause of illness and death among neotropical primates[4]. Currently, six species of the genus *Dipetalonema* have been reported, namely, *D. caudispina*, *D. graciliformis*, *D. yatesi*, *D. gracile*, *D. freitasi*, and *D. robini*, where adult filarial nematodes are exclusively found in the chest and/or peritoneal cavities of neotropical primates[5,6].

In the biological cycle, adult filarial nematodes of *Dipetalonema* spp. are located mainly in the peritoneal cavity of primates. The parasite's microfilariae remain in the host's bloodstream, and in insects of the genus *Culicoides*, which act as vectors in enzootic areas, they develop to the infectious stage (L3)[7,6,8,9]. The clinical presentation of dipetalonemiasis and the worsening of the clinical condition of infected primates depend on the animal's immunological status and the presence of comorbidities and/or stressors[6,10,11]. This report aims to present a case of infection by *Dipetalonema* spp. with multisystem evolution in a capuchin monkey, with emphasis on anatomopathological aspects.

2. Case report

A female adult capuchin monkey (*Sapajus nigritus*) of wild origin was cared for at a Veterinary Hospital after being rescued from a highway in the north of the state of Rio Grande do Sul, Brazil, by the road policing service. Clinical evaluation revealed the presence of nystagmus and involuntary head movements, semi-comatose state, hyperthermia (38.7 ºC), hypovolemia, multiple abrasions, and deep skin lacerations covering muscle layers, in addition to wasting. Blood was collected for hemogram and serum biochemistry. No significant alterations were observed in the hemogram, except for the microfilariae seen in the peripheral blood evaluation. With the purpose of taxonomic identification of microfilariae, a sample of peripheral blood was subjected to the Knott test for the preparation of slides and morphometric study. Serum biochemistry analysis indicated a marked increase in the creatine kinase enzyme (41,620.0 U/L, reference: 137-1337 U/L). The established treatment included antibiotic therapy, analgesia, anti-inflammatories, and fluid therapy. However, the clinical picture evolved to death, and an anatomopathological examination was performed to establish the diagnosis.

In the macroscopic evaluation, the peritoneal cavity showed a slight accumulation of yellowish serous fluid suggestive of exudate, as well as abundant presence of fibrin with marked formation of adhesions between viscera, mainly involving the omentum, liver, right kidney, intestinal and gastric serosa, and ventral abdominal wall. In the peritoneal cavity, numerous free nematode parasites measuring between 10-15 cm in length were also found, which were also collected for morphometric study. In the liver and kidneys, there was marked pallor, fibrin deposition and fibrosis on the capsular surface. Upon sectioning, they exhibited pale yellowish parenchyma. The liver also showed an increase in volume and a slight accentuation of the lobular pattern. In the thoracic cavity, there was an accumulation of serohemorrhagic fluid, in addition to an accumulation of serobullous content in the tracheobronchial lumen. On the lung surface, hyperemic and hemorrhagic areas were found, in addition to marked foci of subpleural emphysema. Upon sectioning, the parenchyma was markedly edematous and, at times, hemorrhagic, with areas of consolidation. Figure 1 illustrates the main macroscopic findings at necropsy.

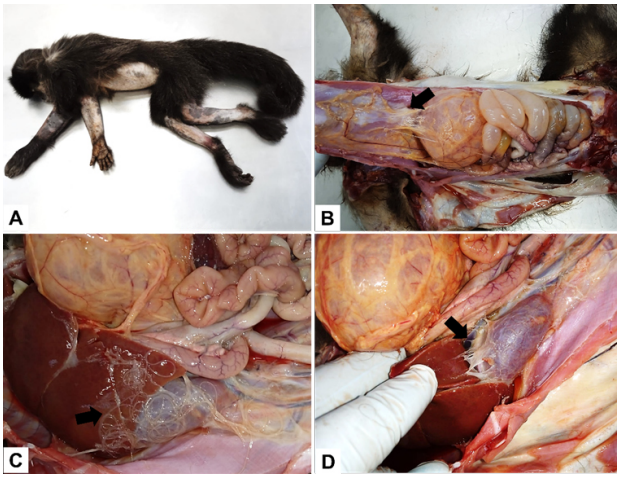

Figure 1. Multisystem infection by *Dipetalonema* spp. in a female capuchin monkey (*Sapajus nigritus*) rescued on a highway in the north of the state of Rio Grande do Sul. **A)** External view of the cadaver. Areas devoid of hair are the result of trichotomy. **B)** Topographic view of the abdominal cavity, highlighting the adhesions (arrow). **C)** Numerous adult filarial nematodes free in the abdominal cavity. **D)** Hepatorenal synechiae, right laterality.

In the heart, the epicardial and endocardial surfaces were pale. The skeletal musculature of the large muscle groups of the pelvic limbs and cervical region was brownish. Upon opening the cranial cavity, the bilateral frontal and occipital lobes showed areas of leptomeningeal hemorrhage, in addition to marked congestion. On cross sections, the cerebellum exhibited focal hemorrhage. Organ samples were collected, fixed in 10% formalin, processed by conventional histological methods, and stained with hematoxylin and eosin (HE) for histopathological analysis.

The microfilariae obtained by the Knott test and the adult filarial nematodes collected from the peritoneal cavity were submitted for morphometric identification. The microfilariae were between 262 and 269 µm in length and had a blunt anterior end, which characterized them as belonging to the genus *Dipetalonema*. On the other hand,

filarial nematodes from the peritoneal cavity were between 10 and 15 cm in length and, due to their location, were also characterized as belonging to the genus *Dipetalonema*.

Histopathological examination revealed numerous microfilariae in the lumen of blood vessels in the lung, liver, heart, kidneys, spleen, small and large intestines, and brain. There was also mononuclear peritonitis of moderate intensity, sometimes fibrin-eosinophilic, severe pneumonia, and mononuclear bronchiolitis, with nematodes visible and, sometimes, neutrophilic and eosinophilic polymorphonuclear infiltrate. The liver evaluation showed lipidosis, areas of centrilobular necrosis, atrophy of hepatocyte cords, and pericolangitis, in addition to cholestasis and congestion. In the brain, there was diffuse microgliosis, satellitosis, neuronophagia, multifocal spongiosis, as well as focal malacia, hemorrhage, and gitter cells. In the cerebellum, focal hemorrhagic necrosis was observed. Figure 2 illustrates the main microscopic findings in different organs and tissues.

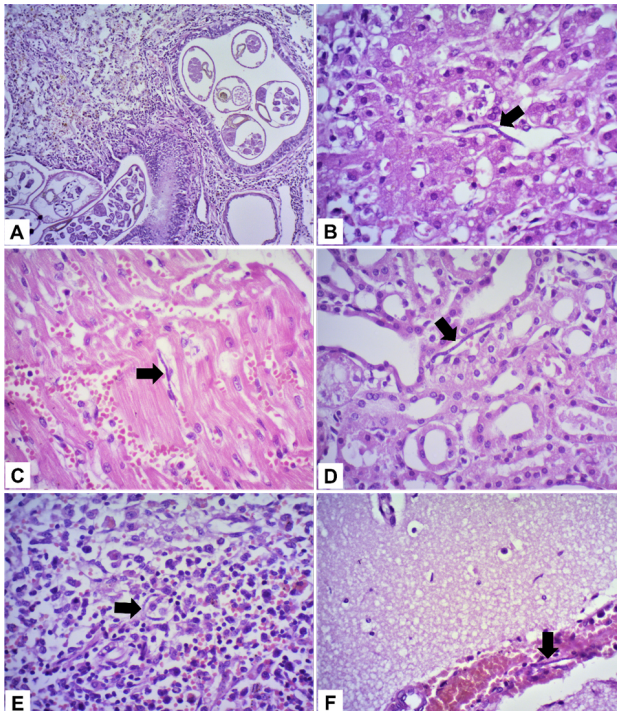

Figure 2. Photomicrographs of multisystem infection by *Dipetalonema* spp. in a female capuchin monkey (*Sapajus nigritus*) rescued on a highway in the north of the state of Rio Grande do Sul. **A)** Lung. Bronchopneumonia associated with adult filarids inside bronchioles (HE, 100x) **B)** Liver. Degeneration and necrosis of hepatocytes and microfilaria in the sinusoid (arrow) (HE, 400x). **C)** Heart. Intravascular microfilaria (arrow) (HE, 400x). **D)** Kidney. Microfilaria in intravascular location (arrow) between the renal tubules (HE, 400x). **E)** Spleen. Microfilaria (arrow) and predominantly polymorphonuclear inflammatory infiltrate (HE, 400x). **F)** Brain. Microfilaria in the vascular lumen (HE, 400x).

3. Discussion

Adult nematodes of the genus *Dipetalonema* parasitize the peritoneal and pleural cavities of their definitive hosts, while their microfilariae are found in the bloodstream. Infections described in neotropical primates are characterized by mild inflammatory reactions such as chronic peritonitis or pleuritis with areas of fibrosis and sometimes fibrinous adhesions associated with the parasites. However, clinical signs have not been presented by infected animals that develop these lesions[12]. Nonetheless, in this case study, the pathological findings were relevant because there were more severe lesions involving several organs, associated with clinical signs and the death of the primate.

In the present case, the diagnosis of dipetalonemiasis was made through the correlation between the clinical aspects, anatomopathological findings, and morphometric characterization of the microfilariae present in the peripheral blood and of the adult filarids found in the abdominal cavity. Cases of primates presenting with anatomopathological findings associated with filariasis have been reported in different regions of Brazil[10,11,13,14]. In the present case, microfilariae were also visualized in the blood count examination, as well as in the lumen of tissue blood vessels during the histopathological examination, as reported in the literature[14].

For microfilariae in the bloodstream, the Knott test was applied for morphometric characterization, as recommended for cases of filariasis[15,16,17,18,19,20,21]). Among the main characteristics established for the identification of the genus *Dipetalonema* are the length of the microfilariae, which can range from 250 to 288 µm, and the obtuse anterior end[18,20]. Another aspect that reinforces the diagnosis of dipetalonemiasis is the presence of adult filarial nematodes in the peritoneal cavity of the capuchin monkey in this case, which is common in *Dipetalonema* species that affect primates, in addition to the morphology and length also consistent with the genus[4, 13,14,22].

The genus *Dipetalonema* comprises six species, and of these, five were diagnosed as parasitizing primates[6]. In southern Brazil, this genus was diagnosed in primates such as *D. gracile* in red howler monkeys in the state of Santa Catarina[13]. When there is a high degree of infection, microfilariae can be constantly viewed in peripheral blood analysis, as well as in the lumen of blood vessels during histopathological analysis[4,6,14,23]. In the present case, numerous microfilariae were observed in the lumen of blood vessels in the lung, liver, heart, kidneys, spleen, small and large intestines, and brain. The observation of adult filarids is considered an incidental finding during necropsy[24,25]. The clinical presentation

and anatomopathological findings of the primate in the present case agree that the cause of death was caused by the intense and disseminated parasitic infection associated with its complications, especially the inflammatory ones. Currently, the precise identification of species belonging to the genus *Dipetalonema* is achieved through molecular tests[4,17,23]. However, in the present study, morphometric characteristics of the filaria found in the blood smear associated with the affected primate species and the location of the adult parasites were used, which only allowed the identification of the genus.

During necropsy examination, the presence of yellowish serous fluid and fibrin in the abdominal cavity already indicated peritonitis and possible liver disease. Macroscopic and microscopic examination revealed notable findings in the liver. Macroscopically, the liver was enlarged and covered by fibrin. Microscopically, histological analysis showed lipidosis, areas of centrilobular necrosis, and pericolangitis, along with evidence of cholestasis and congestion. These findings were also related to dipetalonemiasis[26]. Upon inspection of the peritoneal cavity, abundant formation of adhesions between viscera was observed, mainly involving the omentum, liver, right kidney, intestinal and gastric serosa, and ventral abdominal wall, all resulting from peritonitis triggered by the presence of parasites. Furthermore, there were numerous free nematode parasites in the cavity, adjacent to the fibrin areas, causing mononuclear and/or fibrino-eosinophilic peritonitis. These systemic inflammatory alterations are closely related to the filaremia caused by the nematode species *Dipetalonema gracile*[5,13,14,24].

In the present case, there were multiple abrasions and deep skin lacerations covering layers of skeletal muscles, which explain the increase in the creatine kinase enzyme. However, the creatine kinase enzyme is also present in high concentrations in cardiac and smooth muscles, as well as in lower concentrations in the brain, intestines, liver, and spleen[27]. Thus, as the primate exhibited multisystem damage, especially in tissues with the presence of the enzyme, its sharp increase is justified. It should be noted that deep cutaneous-muscular lesions are not related to dipetalonemiasis. In addition, the capuchin monkey was free-living and was found in a semi-comatose state with multiple abrasions and deep skin-muscle lacerations, which suggests that the cause is an animal interaction with other primates in the group.

The capuchin monkey in this report also had a severe pulmonary impairment, characterized by severe bronchopneumonia associated with the presence of adult filarial nematodes, in addition to diffuse edema. This miscellany of lung lesions impaired the process of adequate gas exchange, culminating in respiratory failure and death of the primate due to dipetalonemiasis[4,26,28]. Remarkably, the primate also exhibited lesions in the central nervous system, a characteristic not described in reported cases of dipetalonemiasis. The lesions found in the central nervous system of this primate, including leptomeningeal hemorrhage, diffuse cerebellar microgliosis, satellitosis, and neuronophagia, in addition to spongiosis and hemorrhagic malacia, explain the neurological clinical picture of nystagmus and involuntary head movements that the patient displayed when being treated. In addition, these lesions may be related to the high degree of filaremia shown by the capuchin monkey, to the damage that the presence of microfilariae causes in vessels and tissues, as well as to the inflammatory and hemodynamic process triggered.

Given the above, this study is of great relevance as it presents a case of multisystemic infection by *Dipetalonema* spp. in a capuchin monkey, which died due to severe systemic complications of filariasis.

4. Conclusion

The definitive diagnosis of dipetalonemiasis was established through meticulous morphological characterization of both microfilariae and adult filariae from the blood, along with detailed anatomopathological examinations. Moreover, the anatomopathological examination confirmed the presence of a multisystemic infection by *Dipetalonema* spp. The report also highlights the concerning occurrence of this infection in primates in the northern region of Rio Grande do Sul.

Declaration of conflict of interest

The authors declare no conflicts of interest.

Author contributions

Methodology: B. da R. Pereira and E. D.dos Santos. *Investigation*: B. da R. Pereira, E. D. dos Santos, A. B. dos Santos, P. M. Vanzin, B. W. Klaser, M. Gatto, S. P. Flores, T. P. Machado, M. W. Ataide, M. I. B. Vieira and A. C. da Motta. *Visualization*: B. da R. Pereira, E. D. dos Santos, B. W. Klaser, M. I. B. Vieira and A. C. da Motta. *Writing (original draft)*: B. da R. Pereira and E. D. dos Santos. *Writing (review & editing)*: B. da R. Pereira, E. D. dos Santos, A. B. dos Santos, P. M. Vanzin, B. W. Klaser, M. Gatto, S. P. Flores, T. P. Machado, M. W. Ataide, M. I. B. Vieira and A. C. da Motta.

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