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**Case Report**

# Cardiac hypertrophy associated with *Corynebacterium* spp. in *Didelphis albiventris* cubs

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**Abstract**
The purpose of this manuscript is to describe the clinical-pathological aspects of cardiac hypertrophy related to the presence of *Corynebacterium* spp. in three *Didelphis albiventris* cubs. In necropsy, macroscopically, in the heart, cardiomegaly, concentric hypertrophy of the ventricles and the ventricular septum were observed, with consequent reduction of the chamber. Microscopically, the primary lesions found in the heart were cardiomyocyte hypertrophy and necrosis, myocytolysis, and the presence of myriad basophilic bacteria. Liver fragments and endocardial swabs were sent for bacterial culture, in which pleomorphic Gram-positive rods grew, forming small and hemolytic colonies. Chemical tests demonstrated characteristics compatible with *Corynebacterium* spp. Thus, this report represents the first description of cardiac hypertrophy associated with *Corynebacterium* spp. in white-eared opossums cubs, representing an essential contribution to studying diseases in wild animals.

**Keywords:** cardiac remodeling, opossum, corynebacteria, wildlife.

## Introduction

Cardiomyopathies are a group of diseases that affect the heart muscle, compromising its function and structure. They are currently classified into five categories - hypertrophic, dilated, restrictive, right ventricular arrhythmogenic, and indeterminate cardiomyopathy when the disease does not fit any previous pattern (5). In a body condition with systemic organ damage and dysfunction due to abnormal host response to an infection, the heart is one of the most sensitive organs to sepsis and sepsis-induced cardiomyopathy (2).

Newborn opossums can get passive immunity through their mother's milk; however, most newborns admitted to animal recovery centers are orphans, and they may not have been able to obtain their mother's milk with antibodies, which may increase susceptibility to disease (17). Various *Corynebacterium* species have been associated with severe infections such as sepsis, surgical infections, and endocarditis. *C. diphtheriae* is a producer of diphtheria toxin (DT), responsible for the classic forms of diphtheria, and characterized by grayish pseudo membranes at the infection sites caused by the multiplication of the agent and the host's immune response. The toxin has a tropism for organs such as the kidneys, adrenals, and heart (4). Pericarditis and pyopericardium due to *Corynebacterium diphtheriae* have been reported *in a human being.* Echocardiography discovered an ejection fraction of the left ventricle of 20% and mild pericardial effusion, but the electrocardiogram and the cardiac enzymes were normal (9). Also, in a child case report, *C. diphtheriae* was identified from a throat swab. The patient developed multiple organ dysfunctions, such as

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impairment of myocardial function, and died of respiratory and heart failure (14). This study aims to report the occurrence of cardiac hypertrophy related to the presence of *Corynebacterium* spp. and its clinical-pathological repercussions in three white-eared opossum cubs (*Didelphis albiventris*).

## Case description

Three orphaned *D. albiventris* cubs, approximately 50g and 60 days old, were under the care of the Wild Fauna Rehabilitation Center of the Federal University of Pelotas, and demonstrated neonatal management, inappetence, dyspnea, diaphragmatic breathing, hematochezia, and mild diarrhea. Animals 1 and 2 shared the same enclosure and had an acute condition that evolved to death in three days, while animal 3 presented a subacute condition plus ascites and intensification of clinical signs, with subsequent death in 12 days.

After general clinical examination, all cubs were treated with Gentamox® (Gentamox®, Gentamicin and Amoxicilin, Hipra, Brazil) diluted 1:10 in Mercepton® (Mercepton®, Vitamin complexes, and antitoxic amino acids, Bravet, Brazil) at a dose of 1mL/kg, every 12 hours (BID), by deep intramuscular route. In animal 3, Furosemide (Diurax®, Furosemide, Agener União Saúde Animal, Brazil) 4mg/kg BID was additionally administered subcutaneously, and fluid therapy with Ringer Lactato® (Lactated Ringer's solution, Fresenius Kabi Brasil Ltda, Brazil) 50mL/kg associated with Sorovita® (Fluid and vitamin complexes, UCBVet, Brazil) 2.5 mL/kg subcutaneously. In addition to nutritional taurine supplementation, it added 10% feline feed to the milk substitute. Concomitant to treatment, an echocardiogram and electrocardiogram were performed.

The corpses of animals 1, 2, and 3 were sent to the Regional Diagnostic Laboratory of the Federal University of Pelotas for necroscopic examination, and tissue fragments were collected and subjected to routine histological technique for further analysis by optical microscopy. Fragments of liver and pericardial swabs were collected and sent for bacteriological culture to be sown on 5% blood agar and Mac Conkey agar, incubated at 37°C for up to 72 hours. The isolated colonies were stained with Gram, and a biochemical battery was used to identify the agent.

Clinically, the opossums presented grayish stools with a slight presence of live blood, dyspnea associated with diaphragmatic breathing, loss of appetite, and progressive weight loss. One of the animals, with a subacute condition, showed ascites and, because it survived longer than the other cubs, he underwent electrocardiography (InCardio Duo - InPulse Animal Health) and echodopplercardiography (LOGIQ *e* – GE HealthCare, microconvex and linear probe). The electrocardiography showed a heart rate of 168 bpm, a ventricular axis shifted to the left (0°; reference value 43°±18), and a T wave with a larger dimension than expected (-0.13mV; max -0.1mV) (16, 18). EchoDopplercardiography, in the objective evaluation, showed the left ventricle (LV) with a thick wall (3mm) and decreased internal chamber (4mm), but with normal movement and uniform contraction. LV systolic function was shown to be increased (ejection fraction - 90%; left ventricular fractional shortening - 56%). In the subjective evaluation, the right ventricle showed increased wall thickness, regular movement, and consistent contraction (Fig. 1).

All cubs underwent treatment, but the clinical signs evolved to death, and thus were referred for necropsy and histopathological evaluation. At the opening of the abdominal cavity of all animals, there was moderate generalized splenomegaly, and in cadaver one the surface of the liver presented pale to whitish multifocal areas that also extended through the hepatic parenchyma.

In the thoracic cavity, there was evident cardiomegaly, characterized by the globose-shaped heart, which occupied a large part of the chest. When cutting the heart, concentric hypertrophy of the ventricles and left interventricular septum walls was observed, with a consequent reduction in the ventricular chamber (Fig. 2).

Histologically, in the myocardium of animal 1, a focal area of extensive necrosis was observed with myriads of basophilic bacterial staining, fibrin, and cellular debris in the subendocardium, interventricular septum, and left ventricle. In the liver of the same animal, a multifocal presence of myriad bacteria with basophilic color was observed, located mainly in the hepatic portal spaces. The lung had diffused pulmonary edema.

In the microscopy of animal 2, multifocal and coalescent areas of necrosis of cardiomyocytes with fibrin, cellular debris, and myriad bacteria were observed in the myocardium, located mainly in the interventricular septum, atria, and left and right ventricles. The lung had interstitial pneumonia with myriad bacterial basophilic stains and an inflammatory infiltrate of moderate multifocal polymorphonuclear cells. In the kidney, interstitial nephritis was observed, characterized by inflammatory polymorphonuclear infiltrate in the

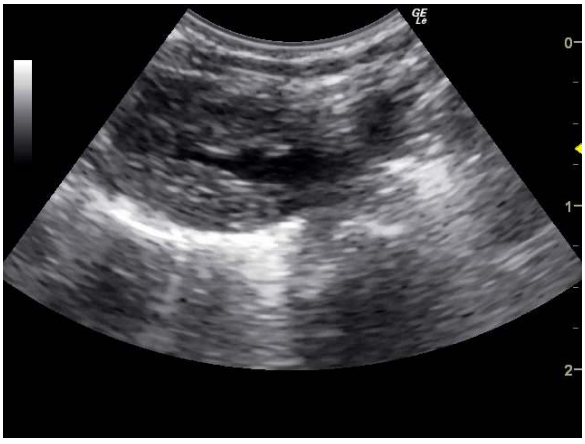


**Figure 1**. Echodopplercardiogram in a *D. albiventris* cub with cardiac hypertrophy. The image shows the systole in the right longitudinal parasternal section of the heart (animal 3).

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renal interstitium. Small and large intestines demonstrated multifocal areas containing inflammatory polymorphonuclear infiltrates and myriad bacterial basophilic stains in the muscular and mucous layers.

In the myocardium of animal 3, myocytolysis was observed, characterized by vacuolar degeneration in cardiomyocytes, with a consequent increase in the volume of muscle fibers (Fig. 3). Multifocal fatty degeneration was also present. In the auricles, myriads of basophilic-colored bacteria adhered to the endocardium and were associated with a marked inflammatory infiltrate of polymorphonuclear cells. The kidney had many proteinaceous cylinders in the lumen of the renal tubules. In the lung, there was interstitial pneumonia.

After 48 hours, in the samples of pericardium and liver, the growth of small and hemolytic colonies was observed in blood agar. Colonies were stained, and Gram-positive pleomorphic rods were observed. The catalase test was positive. In biochemical tests, nitrate, urea, lactose, trehalose, and arginine tests were negative, and glucose, sucrose, maltose, xylose, and mannitol tests were positive, characterizing the genus *Corynebacterium* spp.

## Discussion

The neonatal casuistry of opossums presents itself as one of the most expressive screening centers for wild animals due to the high reproductive efficiency of the species and its synanthropic character (8). Opossums are wild marsupial mammals, often associated with a reservoir of pathogens, which justifies studies on the dynamics of diseases that affect humans and animals (3, 12). In the present report, cubs demonstrated a rapid drop in body condition due to a lack of appetite and marked dyspnea, evidenced by respiratory effort. This finding suggested that progressive weight loss was related to malnutrition, which translated into difficulty in swallowing food and maintaining breathing physiologically and synchronously, which led to the decompensation of animals such as prostration, cyanosis, and severe dyspnea, commonly observed in insufficiency Congestive Heart Failure (CHF). The feces had a grayish color and a soft consistency with discreet traces of live blood. It is proposed that such clinical signs are related to the infection caused by *Corynebacterium* spp., found in pericardial samples of the cubs, which represents one of the sites of action of this bacterium in humans (4).

Corynebacteria, although commensal, can manifest varied pathogenicity according to the species, infection, and carrier immunity conditions (11). Research carried out in Mexico pointed out the presence of *Corynebacterium* spp. in evaluating the antibiotic susceptibility of bacterial isolates from the oral cavity of adult specimens of free-living opossums (*Dildelphis virginiana*) (1). A similar study was carried out in the state of Texas (USA) to isolate aerobic bacteria associated with infected bites of *D. virginiana*, where *Corynebacterium* spp. was again found (7). While in Kansas (USA), a study with 17 healthy possums found the presence of *Corynebacterium* spp. in the microbiota of the ocular conjunctiva of 12% of the samples (13). In possums kept

**Figure 2**. Cross section of the heart of a *D. albiventris* cub with cardiac hypertrophy. Normal heart. Animal with same age (left). In the center, a globose heart. Sliced heart showing thickening of the walls and ventricular septum - concentric hypertrophy with decreased cardiac chambers (animal 3).

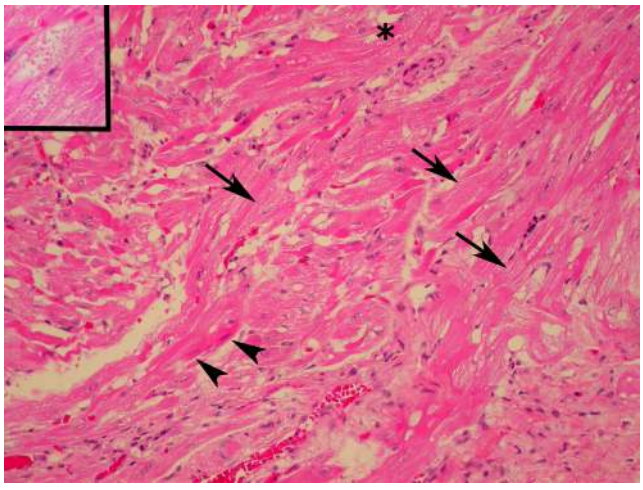

**Figure 3**. Left ventricle of a *D. albiventris* cub with cardiac hypertrophy. Swollen cardiac muscle fibers (arrow), with intracytoplasmic vacuoles and bundles of fibers showing myocytolysis (*). (Inset) Small eosinophilic globules in myocytolysis area. Normal cardiomyocytes (Arrowhead). H.E. 100X (animal 3).

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in captivity (15), the study showed macroscopic and microscopic alterations of bacterial endocarditis with evolution to myocarditis in some specimens, however, the etiological agent has not been elucidated. Thus, few references address the presence of coryneform bacteria in *Didelphis* spp., especially causing disease in these animals and even less related to cardiac hypertrophy.

Cardiac hypertrophy is an early compensatory change of the heart in response to many stresses (19), but cardiac hypertrophy with an infectious origin is rarely documented in wild animals. However, it suggests a cardiac dysfunction characterized by changes in cardiac pump function in terms of left ventricular ejection fraction (EF%), fractional shortening (FS%), and cardiac output (19). Such changes lead to heart failure, as observed in the animals in this study. The electrocardiographic examination complemented the cardiac evaluation by showing a left ventricular axis deviation and an increase in the T wave. These results occur in cardiac enlargements and cases of myocardial hypoxia, which may be secondary to myocytolysis and cardiomyocyte necrosis, evidenced in the anatomopathological evaluation of this report (18). It is suggested that the presence of myocytolysis and cardiomyocyte necrosis correspond to the host's response to the injury of *Corynebacterium* spp. to the myocardium, promoting structural hypertrophic adaptations with a consequent increase in the thickness of the ventricular walls, as demonstrated in echoDopplercardiography in animal three and the necroscopic examination in all the corpses.

Reactive oxygen species (ROS) involvement has been indicated in the pathogenesis of cardiac hypertrophy and heart failure. Furthermore, ROS have associated with sepsis-induced cardiac injury. Ferroptosis is a form of cell death marked by the oxidative modification of phospholipid membranes via an iron-dependent mechanism, and it participates in the development of cardiomyopathy, including cardiac hypertrophy. In sepsis, ferritinophagy-mediated ferroptosis is one of the critical mechanisms contributing to cardiac injury, triggering lipid peroxidation, malondialdehyde (MDA), and ferroptosis of cardiomyocytes (10, 2). According to the findings, this is probably the mechanism for developing cardiac hypertrophy in our study.

It can be inferred that the presence of myriad bacteria in several organs associated with the lesions found results from the sepsis caused by *Corynebacterium* spp. In a study with ferrets affected by lethal sepsis, a new strain of corynebacteria (*C. mustelinae*), with phylogenetically similar characteristics to *C. pseudotuberculosis*, was isolated from fragments of lung and liver. Such discovery points to the virulence potential of certain coryneform bacteria and the possibility of newly isolated species in wild animals (6).

In summary, this report represents the first description of cardiac hypertrophy associated with *Corynebacterium* spp. in white-eared opossums cubs, constituting an essential contribution to the study of diseases in wild animals, especially of the species in question, given the proximity to humans and the high casuistry in wild animal screening centers.

## Conflict of Interest

The authors declare no competing interests.

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