

## *Nocardia niwae* Infection in Dogs

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### ABSTRACT

**Background:** Bacterial dermatopathies are common causes of skin disorders in dogs. The genus *Nocardia* belongs to the group of aerobic actinomycetes and is responsible for causing disorders in immunosuppressed animals, with cutaneous, subcutaneous, pulmonary or septicemic manifestations. The present report describes 2 cases of cutaneous nocardiosis, caused by *Nocardia niwae* in 2 semi-domiciled dogs in the municipality of Barra, BA, Brazil. The emphasis is given to its laboratory, clinical, therapeutic and prognostic analysis.

**Cases:** A 10-month-old male mixed breed (MB) dog (*Case 1*), was seen at the Veterinary University Hospital of the Federal University of Western Bahia (HVU-UFOB), with a history of bloody diarrhoea, vomiting, lack of appetite and swelling in the submandibular region lasting for 15 days. On physical examination, the animal presented myoclonus in the posterior limbs, motor incoordination and hyperkeratosis in the foot pads. A few days later (*Case 2*), a 5-month-old male MB dog, from the same domicile, was admitted to the (HVU-UFOB). The main complaint reported was the presence of a nodule on the left side of the thoracic region that had appeared 2 weeks earlier, during the physical examination the presence of a firm nodule was noted, it was adherent and limited to the middle portion of the 6<sup>th</sup> left rib. In both cases, blood samples for a complete blood count (CBC) and samples of the content of the fistula (*Case 1*) and nodule (*Case 2*) were collected by the capillary method and fine needle aspiration puncture (FNAP), for cytological and microbiological examination (respectively). After performing the Matrix Associated Laser Desorption-Ionization - Time MALDI-TOF technique the pathogen causing the disease in both cases was classified as *Nocardia niwae*. By evaluating the resistance profile of this bacterium by the disc diffusion method to several classes of antimicrobials, sensitivity was found only to amoxicillin + clavulanate, amikacin, ceftazidime, cefotaxime and meropenem. The drug therapy instituted for animal 1 was based on thymomodulin [Leucogen - 1.8 mg/kg, p.o, SID, 4 mL, during 15 days], Disodium cytidine phosphate, Trisodium uridine triphosphate, Hydroxocobalamin acetate [ETNA - 1 capsule, p.o, BID, during 20 days] and phenobarbital [Gardenal - 2.2 mg/kg, p.o, SID, 10 drops, during 10 days]. This animal died before the results of the cytological and microbiological analyses were available. For animal 2, Cefadroxil [Cefadroxil - 35 mg/kg, dose, p.o, SID, 1 tablet, during 14 days] was used, and also Prednisone [Prednisone - 0.7 mg/kg, p.o, SID, half- tablet, during 6 days], after this application a clinical improvement was noted.

**Discussion:** The macroscopic, microscopic and biochemical characteristics of the bacterium, *Nocardia niwae*, are in agreement with that cited in the literature. This bacterial species was first identified in a 60-year-old human in 2011, and there are no reports in the literature of infections involving animals. High mortality rates may be associated in part with concomitant diseases with nocardiosis in the case of dog 1, the death may have correlated with canine distemper and the absence of specific treatment for nocardiosis. The early diagnosis of cutaneous nocardiosis and the absence of comorbidities in dog 2, positively influenced the choice of therapy and its clinical cure. These are the first reports of canines naturally infected with *Nocardia niwae*. Their importance should be emphasised since it is a zoonotic disease. Future studies should be conducted in order to determine the prevalence of the agent in the region of Barra, Western Bahia.

**Keywords:** bacterial diseases, cytology, *Nocardia niwae*, nocardiosis.

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## INTRODUCTION

In small animal medical practice, bacterial dermatopathies are common causes of skin disorders in dogs [21]. Nocardiosis is an infectious bacterial disease of zoonotic character, caused by actinomycetes of the genus *Nocardia*, microorganisms of bacillary to filamentous character, Gram-positive, aerobic, and partially alcohol-acid resistant [1,11]. *N. asteroides*, *N. brasiliensis*, *N. otitidiscaviarum* and *N. nova* [4] stand out as causative agents of affections in companion animals, can commonly be found in water, soil or decomposing organic matter [6], and their transmission occurs mainly through mechanical skin lesions, wound contamination, or inhalation, since they are considered opportunistic bacteria [5].

Cutaneous and respiratory manifestations are demonstrated in cases of nocardiosis [13]. In canines, the cutaneous form with the appearance of suppurative abscesses is more common and immunosuppression of the host is an important factor in establishing infection [13].

Presumptive diagnosis is based on the clinic and epidemiology of the disease, laboratory confirmation is done by cytology, histopathology, serological techniques, PCR, culture and bacterial identification [12,20].

Treatment is prolonged and made more difficult by the pathogenic potential of the bacteria, due to its maintenance inside phagocytes, the constitution of its bacterial wall that hinders the passage of the antibiotics and naturally acquired resistance to some enzymatic mechanisms [11,12].

This paper describes 2 cases of cutaneous nocardiosis in dogs undergoing treatment at the Veterinary Hospital of the Federal University of Western Bahia (HVU-UFOB), Barra, BA, and their laboratory, clinical, therapeutic and prognostic analysis are emphasised.

## CASES

**Case 1.** A 10-month-old male mixed breed (MB) dog was seen at the Small Animal Clinic (CMPA), of the University Veterinary Hospital (HVU) of the Federal University of Western Bahia (UFOB), Barra Multidisciplinary Campus. With a history of bloody diarrhoea, vomiting, lack of appetite and swelling in the submandibular region lasting for 15 days. His guardian also reported previous treatment with dipyrone, enrofloxacin, mercepton, probiotic, floratil, injectable and oral metoclopramide, and human sulfa+trimethoprine for 5 days. On physical examination the animal was

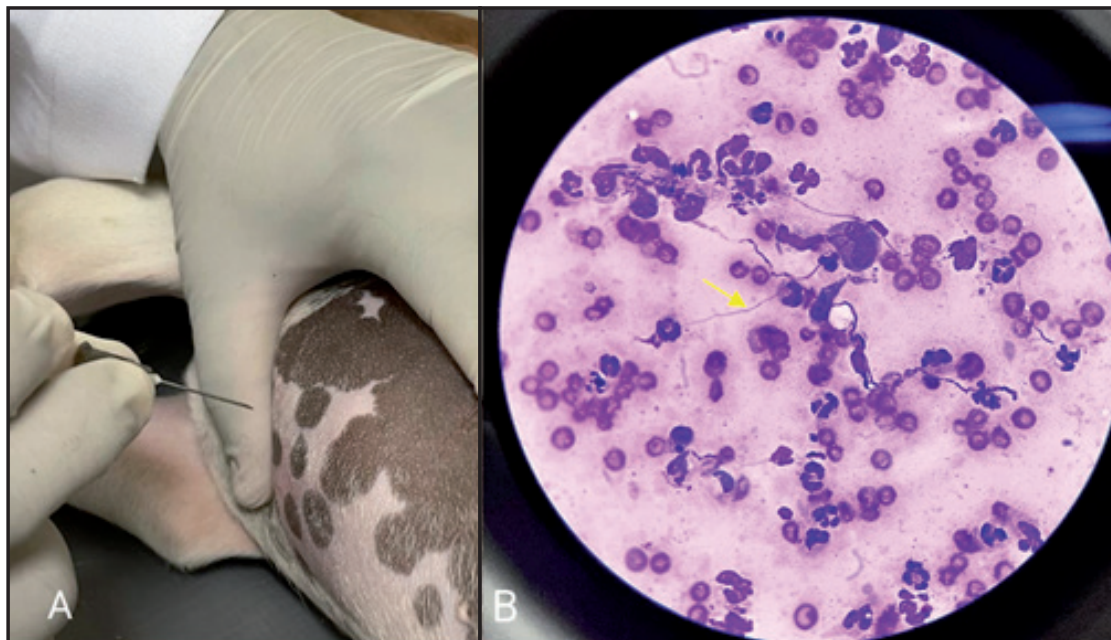
apathetic and the left submandibular lymph node was reactive and, myoclonus was evidenced in the posterior limbs, motor incoordination and hyperkeratosis in the foot pads. Given the clinical signs, canine distemper was suspected. It was adopted as a therapy the use of Timomodulin<sup>1</sup> [Leucogen - 1.8 mg/kg, p.o. SID, 4 mL during 15 days], Disodium cytidine phosphate, Trisodium uridine triphosphate, Hydroxocobalamin acetate<sup>2</sup> [ETNA - 1 tablet, p.o, BID, during 20 days] and phenobarbital<sup>3</sup> [Gardenal - 2.2 mg/kg, p.o, SID, 10 drops, during 10 days]. Three days later, it was verified enlargement and the presence of a fistula in the right prescapular lymph node, draining purulent secretion.

**Case 2.** An apparently 5-month-old male MB dog, from the same domicile, was seen at the Small Animal Clinic (CMPA) sector of the HVU-UFOB, Barra Multidisciplinary Campus. In the anamnesis, the main complaint was the presence of an increase in volume situated on the left side of the thoracic region, visualised 2 weeks before the consultation. During the physical examination, it was possible to verify the presence of a firm, adherent nodule limited to the middle portion of the sixth left rib. The left bilateral submandibular, popliteal and prescapular lymph nodes were reactive, congested ocular mucosa and pale penile mucosa. Furthermore, all the physiological parameters of the animal were unchanged. At first, a bacterial pyogranulomatous affection or neoplasm was suspected.

In both cases to confirm clinical suspicion, blood samples for a complete blood count (CBC) and samples of the content of the fistula (animal 1) and nodule (animal 2) were collected by the capillary method (Figure 1A) and fine needle aspiration puncture (FNAP), intended for cytological and microbiological examination (respectively), sent to the Veterinary Clinical Analyses and Veterinary Microbiology Laboratories of the same institution. Animal 1 died before the results of these tests were made available.

The haematological examination of animal 1 showed macrocytic normochromic anaemia, leukopenia with neutropenia and lymphocytosis. Animal 2 showed neutrophilic leukocytosis with a left deviation of regenerative character.

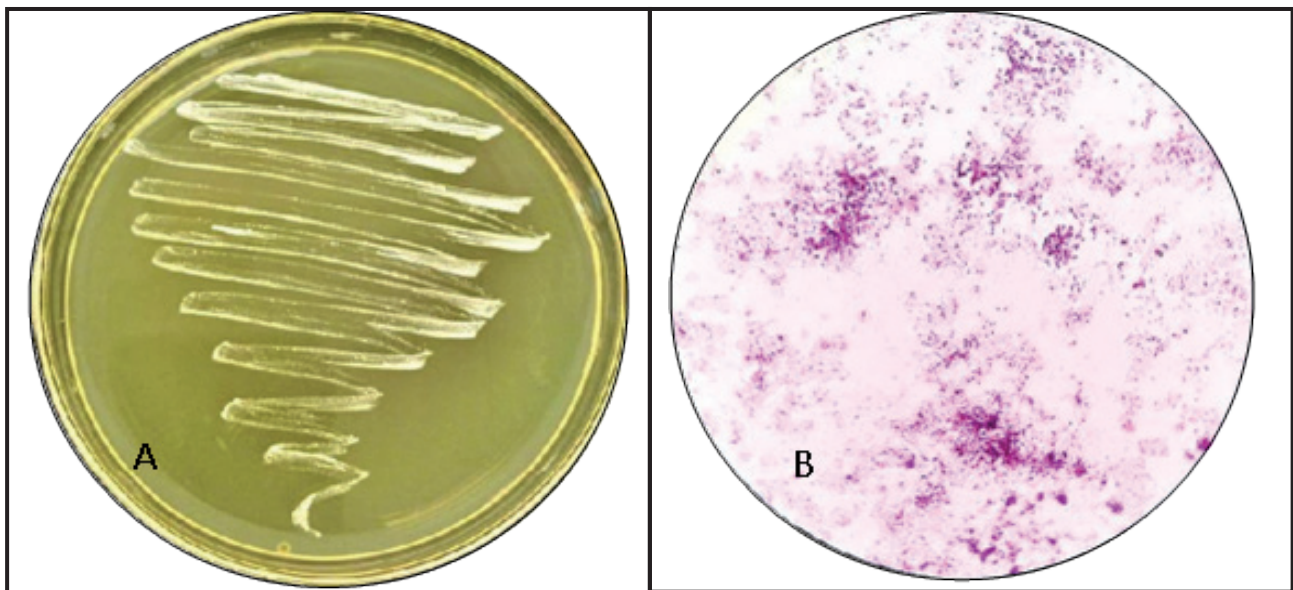
Cytological examination of the 2 cases showed thin branched coccoid bacterial filaments, in the shape of rosary beads, surrounded by many neutrophils showing degenerative karyorrhexis changes, suggestive of a case of nocardiosis (Figure 1B).



**Figure 1.** Cytological diagnosis of nocardiosis. A- Use of the capillary cytological technique to collect samples from a firm nodule, adhered and limited to the middle portion of the sixth left rib of a canine. B- Microscopic aspect. Fine-branched coccoid bacterial filaments are observed, rosary bead-shaped, surrounded by many neutrophils showing degenerative changes of karyorrhexis [Panoptic fast staining; Obj.100x].

For bacteriological culture, biological samples were collected and seeded in Brain Heart Infusion (BHI)<sup>4</sup> broth, Blood Agar plus 5% sheep blood<sup>4</sup>, MacConkey<sup>4</sup> Agar and Sabouraud Dextrose<sup>4</sup> Agar incubated at 37°C, in aerobiosis, for 24-48 h. During the readings, turbidity of the liquid medium and morphological characteristics of the colonies in solid medium - rounded colonies - were observed, with irregular, white and friable edges, strongly adhered to the culture medium (Figure 2A). Subsequently, morphotyping analysis of

the pathogens was performed using the Gram staining method, in both cases, Gram-positive rod-shaped micro-organisms with filamentous characteristics were found. Bacteria were identified using biochemical tests based on Bergey's Manual of Microbiology [15]. To perform the species identification, the Matrix Associated Laser Desorption-Ionization - Time<sup>5</sup> (MALDI-TOF) technique was used for confirmation [4] and classified as *Nocardia niwae* (Figure 2B).



**Figure 2.** Microbiological diagnosis of *Nocardia niwae*. A- Macroscopic appearance. Colony seeded on Sabouraud Dextrose Agar medium: rounded colonies, with irregular, white, friable edges, strongly adherent to the culture medium, isolated firm nodule adhered and limited to the middle portion of the 6<sup>th</sup> left rib of a canine. B- Microscopic aspect. Pleomorphic rod-shaped to filamentous Gram-positive bacilli are observed, alcohol-acid-resistant [Gram staining; Obj.100x].

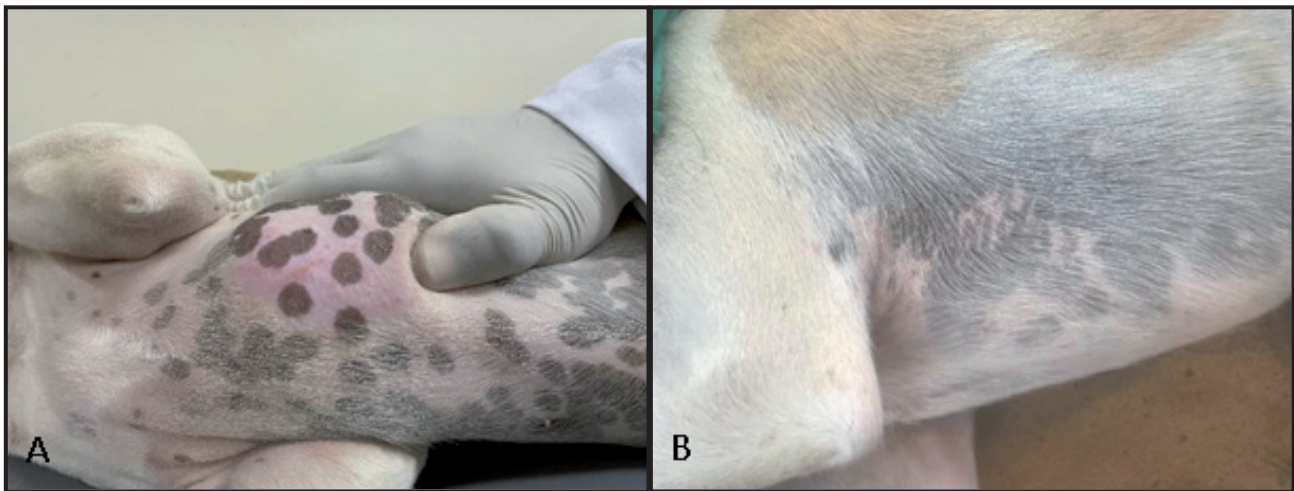


After the identification of the species, to assess the antimicrobial resistance profile, the disk diffusion method proposed by the Clinical and Laboratory Standards Institute - CLSI [2] was used. The antibiotics tested were: Amoxicillin + Clavulanate (20-10 µg), Ampicillin (10 µg), Penicillin (10 µg), Amikacin (30 µg), Cephalexin (30 µg), Gentamicin (10 µg), Tetracycline (30 µg), Cephalothin (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30µg), Azithromycin, (15 µg), Meropenem (10 µg), Cefaclor (30 µg), Tobramycin (10 µg), Cefoxitin (30 µg) (Laborclin)<sup>6</sup>. The American

Type Culture Collection (ATCC) standard strain was used as quality control: *Escherichia coli* n. 25922 and (ATCC): *Staphylococcus aureus* n. 25923.

Susceptibility was found only to amoxicillin + clavulanate, amikacin, ceftazidime, cefotaxime and meropenem.

Adopting as drug therapy Cefadroxil<sup>7</sup> [Cefadroxil - 35 mg/kg, p.o, SID, 1 tablet, during 14 days] and Prednisone<sup>8</sup> [Prednisone - 0.7mg/kg, p.o, SID, half tablet, during 6 days]. After this period, the animal 2 presented a clinical cure (Figure 3A & 3B).



**Figure 3.** Nocardiosis of the Animal 2. A- First day of clinical consultation exhibiting a firm nodule, adhered and limited to the middle portion of the 6<sup>th</sup> left rib. B- Clinical cure after oral therapy based on cefadroxil (14 days) and prednisone (6 days).

## DISCUSSION

The clinical study of the cases, associated with cytological, microbiological and MALDI-TOF mass spectrometry diagnosis, confirmed the clinical suspicion of bacterial infection, specifying in both animals nocardiosis, caused by *Nocardia niwae*. Disease emerging among domestic animals and humans worldwide, which may be correlated to the opportunistic potential of this pathogen [8], corroborating what has been demonstrated in the present work, where both animals presented primary immunosuppression, which possibly contributed to the installation and secondary manifestation of the infection [12].

During observation of the cytological slide, branched bacterial filaments in the shape of a rosary bead were visualised, characteristic structures in cases of infection by *Nocardia* spp. [17]. It is emphasised, however, that finding this bacteriological profile justifies treatment for nocardiosis, but does not exempt the realization of bacterial cultures for confirmatory diagnosis [9].

The macroscopic, microscopic and biochemical characteristics of the *N. bacterium*, are in agreement with that cited in the literature [1]. *N. niwae* was first identified in a 60-year-old human in 2011, There are no reports in the literature of infections involving animals [13].

The dogs of cases 1 and 2 presented fistulated and non-fistulated abscesses respectively, with clinical signs resembling those found in cutaneous-type nocardiosis [17]. A total of 14 dogs with nocardiosis were analysed, among the animals with lymphatic involvement, the reactive lymph nodes were close to the areas of the lesions, going in agreement with what was evidenced in the animals reported here [6].

Primarily the animal of case 1 presented characteristic signs of canine distemper, a disease responsible for generating immunosuppression and increasing the susceptibility of the carrier to other infections [16]. This fact may have predisposed to the infection by *Nocardia* [3], given its opportunistic potential. This pathogen is known to be present in soils, water and decaying organic matter, presenting

transcutaneous lesions as a gateway to the organism [6], it should be noted that inhalation of aerosols should also be considered in the epidemiological chain of the disease, particularly in dry climate environments [12]. Moreover, the fact that the animals have free access to the street (semi-domiciled), may have made it possible for dogs to be exposed to factors that determine the transmissibility of the disease. Animal 2, on the other hand, considered to be a puppy, presented a developing immune system [10], possibly the direct contact with animal 1, naturally left it vulnerable to the affection.

In bacterial infections, the first leukocytes to be mobilised are the neutrophils [9,19], which could explain the neutrophilia evidenced in the blood count of dog 2. Dog 1 showed anaemia and lymphopenia, similar results were observed by Souza [18] who evaluated 18 cases of dogs diagnosed with canine distemper, observed in the haematological findings that 72.2% presented anaemia, while 38.8% presented lymphopenia [18]. The canine distemper virus acts by causing damage directly to lymphocytes, which generates lymphopenia early in the infection [9]. Previous studies of nocardiosis cases in dogs show that the erythrocytic series of infected animals in general presents themselves unaltered, while the leukogram is characterised by leukocytosis and neutrophilia with deviation to the left [8,15]. These indexes may be altered according to the severity of the clinical picture and presence of sepsis.

The treatment of nocardiosis in dogs presents an uncertain prognosis and becomes severe when the primary focus spreads and causes systemic disease, as few antimicrobials reach therapeutic concentrations within pyogranulomatous foci and intracellular localisation of the bacteria also hinders the therapeutic effi-

cacy of conventional drugs [12]. The use of cefadroxil and prednisone proved effective in the treatment of nocardiosis in animal 2, this is corroborated by studies conducted in which demonstrates *in vitro* the effects of drugs against *Nocardia* spp. highlighting cephalosporins and amikacin as the most effective drugs [3].

In this work, the high resistance shown by *N. niwae* may be related to the inherent characteristics of the species, as well as their enzymatic and virulence factors.

The establishment of the diagnosis based on cytology, microbiology and MALDI-TOF, was essential for establishing the causative agent of infection, important for therapeutic instauration and prognosis.

The association of canine distemper and nocardiosis, amplify the severity of the disease [9], while weakening the patient's immune system, hindering therapeutic success, and establishing prognoses ranging from reserved to bad, which happened in case 1.

These are the first reports of canines naturally infected with *Nocardia niwae*. Their importance is emphasised, as it is a zoonotic disease. Future studies should be carried out in order to determine the prevalence of the agent in the region of Barra, Western Bahia.

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