

MYCOBACTERIOSIS IN DOMESTIC DOGS. REPORT OF TWO CASES IN SÃO PAULO, BRAZIL *

MICOBACTERIOSE EM CÃES. RELATO DE CASOS EM SÃO PAULO - BRASIL

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SUMMARY

In veterinary dermatology, domestic carnivores sporadically are found with ulcerated lesions characterized by a prolonged course of evolution and by relative unresponsiveness to the usual drug therapy; this condition can be due to infections caused by microorganisms of the Genus *Mycobacterium*. Due to the scarcity of its description in Brazilian literature, and due to the inusitate lesions and response to preconized therapy, two cases of canine mycobacteriosis attended at the University Veterinary Teaching Hospital of the FMVZ/USP are then described. The adult bitches (German Shepherd and Doberman Pinscher) were found with ulcerated lesions at the posterior side of the pinna with granulomatous aspect and with exudation and covered by hemorrhagic crusts without showing any detectable satellite lymphadenomegalia or any symptoms in other systemic organs. Physical, hematologic and radiographic examinations were performed and lesions have been curetted and biopsied through punching technique, and specimens were examined histologically using Holst-Mitchinson and Radhakrishna and Ziehl-Neelsen staining, and through culture methods using Lowenstein-Jensen and Stonebrink & Leslie media at different temperature of incubation. Intradermal tuberculin test using PPD tuberculin of mammalian and avian origin were performed. Presence of alcohol-acid resistant bacilli were detected through histopathologic examination. The growth of bacilli, however, were not confirmed by the use of conventional media, even being incubated at different temperatures, although found with typical pyogranulomatous tissue reactions. After introducing the topical therapy, represented by the Domeboro's solution, rifamycin, and parenteral use of rifampicin, lesions showed marked remission. Etiology, pathogeny and other diagnostic and therapeutic maneuvers are also discussed.

UNITERMS: Mycobacterium; Dogs

INTRODUCTION

In dermatology, the clinician can sporadically observe ulcerated nodular skin lesions in domestic carnivores. Some of these lesions are characterized by their prolonged course and by the relative unresponsiveness to the usual drug therapy. For the establishment of an accurate diagnosis, it is necessary to exclude other possible causes, through complementary tests, like the fungal diseases (*Sporotrichosis*, *Cryptococcosis*, *Maduromycosis*, etc.); psychogenic (*neurodermatitis*, *acral like dermatitis*); neoplastic (*carcinomas*, *mastocytomas*, *lymphosarcomas*, etc.); immunopathic (*pemphigus*, *eosinophilic ulcers*); parasitic (*cutaneous dirofilariosis*); traumatic (*decumbecy ulcer*) and even the diseases secondary to self traumatic and allergic-inflammatory causes. By

eliminating the most frequent causes very often the owner and the clinician feel discouraged and forget to make connection to those skin exoulcérations or ulcerations of mycobacterial origin, because of their rarity and unawareness of the conditions.

There are reports of granulomatous lesions associated with ulcers due to the infection of microorganisms of the order *Actinomycetales* affecting the head and limbs (SNIDER¹⁰, 1971; FINNIE¹, 1978; GROSS; CONNELLY⁶, 1983).

These conditions seem to be more frequently seen in cats, especially in the Siameses, however, they have also been

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described in dogs, mainly in the Boxer, Bull Mastiff and even in the mongrel dog (FINNIE⁴, 1978; MULLER et al.⁷, 1989).

These alterations are usually found in the pinna, possibly being originated as the consequence of auto-traumatism due to the scratching act. Very often the affected animal do not manifest any other signs or symptoms in association, even the so common "satellite" lymph node enlargement is not encountered (FINNIE⁴, 1978).

In respect to their etiology, the ulcerated granulomas are consequent to the infections of skin wounds (animal bites, scratching, accidental or iatrogenic perforations) by the aerobic, non-sporogenic, non-motile baciliform bacteria, with the cell wall rich in lipid content, acid-alcohol resistant and capable of inducing cell-mediated hypersensitivity reaction and also presenting distinct pathogenic potential, often living freely in the environment as saprophytes. These morphophysiological characteristics are common to the microorganisms of the family Mycobacteriaceae, genus Mycobacterium. The complexities of the tests required for identification may outweigh the results received from the identification itself for this reason routine guides to a minimal test protocol which will permit identification of most slow growers and rapid growers should be followed the level of identification usually is considered to be sufficient for routine practical purposes (WAINE; KUBICA¹¹, 1986).

A typical mycobacteria, both rapid and the slow growers are classified, according to Runyon, into the types I, II, III and IV and can infect cats, dogs and human beings. These are mycobacteria of the species *M.kansasii*, *M.xenopi*, *M.chelonae*, *M.fortuitum*, *M.phlei* and *M.smegmatis* (GREENE⁵, 1984).

In nature, these mycobacteria are usually found in soil and preferably in small water collection or brooks (MULLER et al.⁷, 1989). After having invaded the organism of the animal host they cause pyogranulomatous lesions of chronic course, potentially suppurative and/or fistulating and sometimes remittent and controversially resistant to many antibiotics or antituberculosis drugs (WARD¹², 1975). In respect to their symptoms, painful manifestations are accompanied with pyrexia and anorexia, however, these manifestations are not usual.

The diagnosis can be made through information obtained from a careful anamnesis, dermatologic examination and by finding of the acid-fast bacteria in microscope slide smears, culture media or in histopathologic section of biopsed

materials.

The easiness in demonstrating and in identifying mycobacteria in impression smears obtained directly by making a gentle touch of the slides on the lesions and then stained by Ziehl-Neelsen, Fite-Faraco, or by the fluorescent technique using auramine (method of Holst, Mitchinson and Radhakrishna) quote by CENTRO PANAMERICANO DE ZOONOSES² (1988) is still a matter of controversy.

According to the species of the mycobacteria, the growth in culture media like the Lowenstein & Jensen or the Stonebrink & Leslie can be either fast or slow and these must be cultured at 22^o, 28^o, 33^o and 37^oC.

It is said that the histopathologic examination of the skin sections is of great diagnostic significance, the findings are characterized as nodular or diffuse dermatitis, paniculitis or both, because of the pyogranulomatous type of infection.

The Ziehl-Neelsen technique may reveal the presence of the numerous acid-fast microorganisms, being isolated or accumulated at the center of the vacuoli and circumscribed by the neutrophilic infiltration, inside the matured granulomas. They may be found free in necrotic areas or inside epithelioid cells.

These microorganisms often are found in rosary form and do not stain by the Gram stain, however, they are evidenced by the fluorescence using, auramine-rhodamine (FINNIE⁴, 1978; MULLER et al.⁷, 1989).

When a spontaneous remission does not occur, the condition can be controlled by topic therapy using antiseptics and antibiotics, (Lead or Aluminium acetat-Domeboro's solution) and rifamycin. Supporting systemic therapy is made by using kanamycin, gentamicin, amikacin or rifampicin. Usually the condition does not respond to erythromycin, chloramphenicol, tetracycline, polymyxin and other antituberculosis drugs (GREENE⁵, 1984; MULLER et al.⁷, 1989; ALMEIDA NETO, 1990**).

Some authors (MULLER et al.⁷, 1989) have stated that the influence made by these drugs upon the evolution of this type of infection has not clearly established yet.

In Brazil, the information on the subject is very scarce, there is only one report of mycobacteriosis in dog, made by CRUZ and LEAL³ (1987). Different therapeutic schemes are presently available for the treatment of the mycobacteriosis, though marked with controversies.

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The scope of this paper is to describe the occurrence of two autochthonous cases of atypical mycobacteriosis in dogs reared in the city of São Paulo and the clinical and therapeutic experiences acquired by these cases are also included.

Case Report nº 1

FMVZ/USP HOVET-File nº 166.064/90

Animal Species: Canine
Breed: Doberman
Sex: Female
Age: 6 years
Weight: 25,5 kg

The history of the animal refers to the manifestation of a skin lesion at the posterior site of the right pinna, approximately 30 days prior to the first examination and then progressed to an exulceration. Several weeks later, similar lesion was noted at contralateral pinna. The symptoms observed at the time were the shaking of the head, and a discrete pruritus of affected areas. No other symptoms were registered.

The animal was submitted to an adequate alimentation and sanitation schemes; and it was not recorded any trip to the coastal area of the state of São Paulo was recorded.

The dog had contact with tuberculous patients or with person having a chronic pulmonary manifestation. At the house the dog was reared without access to rats and there was another dog living together, apparently in good health condition. The immunization schedule was adequate and repeated annually.

At physical examination, the vital signs were all normal and no organic alterations were detected. The lymphonodes, including the peri-lesional ones, were not enlarged, with normal consistency, motility and sensibility. The general appearance was good, with disposition, mental alertness and good state of nutrition.

At dermatologic examination, an ulcerated lesion of approximately 1.5 cm in diameter was found in the right pinna, with granulomatous aspect showing discrete serosanguineous secretion with hemorrhagic scab. At the dorsal position of the contralateral pinna, a moist, exudative, nodular and ulcerative lesion of about 0.8 cm in diameter was also observed.

As a complementary examination, the thoracic radiography

was made, although lesions were not found at the level of the pulmonar parenchyma. The blood test revealed a normal erythrocyte counts. A discrete leucopenia (7.8×10^3 leucocytes/ mm^3) was detected by means of absolute neutropenia (4836 neutrophils/ mm^3). Coproparasitic examination did not reveal the presence of any protozoan, parasites or helminths.

In order to elucidate the etiologic process, the lesion was biopsied after a sedation with the use of dissociation anaesthetic (Tiletamina - Zoolazepan***). Right and left ear lesions were curetted and smears were taken directly on microscope slides, fixed at warm temperature and then stained by the Holst, Mitchinson & Radhakrishna technique and by the Ziehl-Neelsen technique, using the Malaquita green.

The biopsy was made through a punch technique of keys and fragments of the left pinna were removed for the histopathologic examination and after processing the material through Petroff technique (recommended by CENTRO PANAMERICANO DE ZOONOSES^{1,2}, 1985, 1988) the clinical specimens were inoculated into Lowenstein & Jensen and Stonebrink & Leslie culture media at the temperature of 22^o, 28^o, 33^o and 37^oC.

Bacterioscopic examination has revealed the presence of acid-alcohol resistant bacilli, both in materials stained by the Ziehl-Neelsen and by the Holst-Mitchinson & Radhakrishna techniques. No bacterial growth was observed with specimens harvested in culture media, even after 60 days.

The histopathologic examination of the biopsied fragments, after being fixed in 10% formalin solution and embed in paraffin and sections stained by Hematoxylin-Eosin and Ziehl-Neelsen stains allowed the observation of the traumatic epidermis and papillary epidermis covered with fibrinous-leucocytic scab. At the subjacent dermis, there was a dense and diffuse inflammatory infiltration consisted especially by macrophages and lymphocytes with numerous plasmocytes and neutrophilic leucocytes among them. Among macrophages, there were a number of epithelioid cells. Proliferation of capillary vessels and fibroblasts and collagenous fibrils circumscribing the area were also observed. In one of the fragments, the epithelioid cells formed a nodular agglomeration with the presence of the rare binucleated cells. A great number of acid-alcohol resistant bacilli were found inside the macrophages, with the cytoplasm slightly vacuolized. No signs of malignancy were detected. Based on histopathologic examinations, the diagnosis of mycobacteriosis was made.

*** Zooletil® - VIRBAC DO BRASIL, Ind. e Comércio Ltda.

The tuberculin test was applied at the medial face of the right and left pelvic limbs respectively with an avian type tuberculin (Avian type PPD - 25.10^3 IU/ml) and with a mammalian type (Mammalian type PPD-MA-01/90 - 50.10^3 IU/ml) both doses of 0.1 ml and the reactions were examined at 24, 48, 72 and 96 hours after the inoculation. However, no positive reactions to both tuberculins were found.

For the therapy, rifampin****, at a dose of 300 mg/day "per os" for 14 days was recommended. Topically, Burrow's solution (1:10) and application of rifamycin***** (Sodium Salt) were employed.

The evaluation made 15 days after the initial medication indicated the healing tendency of the lesion with reduction of 30% in the diameter.

After 33 days of follow-up drug treatment, there was complete remission of the lesion persisting until this day (120 days of observation).

Case Report nº 2 FMVZ/USP - File nº 169.020/90

Animal Species: Canine
Breed: German Shepherd
Sex: Female
Age: 5 years
Weight: 26,7 kg

The clinical history of the bitch shows the occurrence of pyometra, approximately 2 years ago, which was surgically solved by means of an ovariosalpingo hysterectomy.

The immunization procedure was adequate and up-to-date and annually repeat and the dog was also wormed semestrially.

Approximately 10 days before the consultation, a wound appeared at the dorsal side of the both pinna and another lesion at the dorsal side of the phalange of the left pelvic limb. The owner of the dog has medicated it with tiormeral and iodine solution, with lesions becoming aggravated. No other organic alterations were registered.

The alimentation and sanitation were adequate and another dog being reared together was apparently healthy. No history of ectoparasites and staying at coastal area was recorded. The owners were also in good health condition.

At physical examination, the vital signs and the general

appearance were normal, with good state of nutrition, alertness and no alterations were found through cardiopulmonary auscultation and by lymph nodes palpation.

The dermatologic examination of the left pad had revealed the presence of the small circular ulcerated lesion with defined margin at the dorsal side of the first digit. The lesion had a depression in its center, covered with "honey-like" materials with approximately 2.5 cm in diameter. At the dorsal side of both pinna there were ulcerated polycyclic lesions, flat and the size of the lesions at the left ear measured 5 x 3; 3 x 2.5 and the right one (2.5 x 2 cm) and they were covered with exuberant hemorrhagic scabs that bled easily when manipulated.

Complementary examinations were made on clinical specimens that were taken after sedating the dog with tiletamina-zoolazepam***, and then curetting the podal and auricular lesions. From the revived lesions the impression smears were taken directly on the microscope slides. The slides were submitted to heat fixation and subsequently stained by the Ziehl-Neelsen and the Holst-Mitchinson-Radhakrishna stains for the search of acid-fast bacteria. The biopsy was made by means of punch technique of keys, as mentioned previously and then the clinical specimens were harvested in Lowenstein & Jensen, and Stonebrink & Leslie culture media. The incubation of quadruplicated materials was made at varying temperatures of 22°, 28°, 33° and 37°C for two months. The examination of impression smears has revealed the presence of acid-alcohol bacilli. However, no bacterial growth was observed in culture media inoculated with the biopsied specimens.

After fixation in 10% formalin solution, tissue sections were prepared from one of the biopsied fragments and then stained by the Hematoxylin-Eosin, and Ziehl-Neelsen Technique.

At histopathologic examination, the biopsied fragment was found to have a dermal characteristic: surfaces covered by fibrinous-leucocytic scabs and presenting dense and diffuse inflammatory infiltrates, mainly constituted by macrophages and partly represented by epithelioid cells with vacuolized cytoplasm, with lymphoplasmocytic infiltration and also showing numerous neutrophilic leucocytes. A great number of acid-alcohol resistant bacilli usually of granular aspects were found, establishing the diagnosis of cutaneous mycobacteriosis.

Once established the diagnosis of cutaneous mycobacteriosis, a therapeutic scheme using rifampin**** at a dose of 300 mg/

*** Zooletil[®] - VIRBAC DO BRASIL. Indústria e Comércio Ltda.
**** Rifaldin[®] - MERREL LEPETIT FARM. LTDA. (Capsules of 300 mg)
***** Rifocina[®] - MERREL LEPETIT FARM. LTDA. (10 mg/ml)

day by oral administration for a period of two weeks was then initiated.

For topical treatment, it was recommended to clean the lesions daily with 0.5% Irgasan DP300***** and with 10% Burrow's solution, with daily administration of Rifamycin***** (Sodium Salt).

Ten weeks later the patient has returned to the hospital and the owner reported that the parenteral medication was continued for 14 days and topically, for the whole period.

The ulceration of the right pinna had totally remitted, only a small ulceration of 1.0 x 0.5 cm has persisted in the contralateral pinna with bright and moist appearance. Satellite lymphadenopathy was not found. The podal lesion has disappeared completely. It was recommended to keep continuing the same topical therapy and to resume the oral rifampicin therapy.

DISCUSSION

Despite the reports of specialized literature that cats, especially of the Seamese breed are more predisposed to acquire skin mycobacteriosis than dogs, until the present time, no case of feline mycobacteriosis was registered by the Dermatology Service of Department of Internal Medicine of the Veterinary Teaching Hospital of the FMVZ/USP. Only one report of canine mycobacteriosis affecting dogs of the Doberman and German Shepherd breeds was made by CRUZ and LEAL¹ (1987). The diagnosis was obtained from a necropsied German Shepherd with signs of cutaneous mycobacteriosis. By compiling the international literature concerning the subject, authors are in accordance that atypical cutaneous mycobacteriosis are more frequent in dogs of Boxer and Bull Mastiff breeds, and even in mongrel dogs (FINNIE², 1978; MULLER et al.⁷, 1989).

In both cases, patients were already matured, over than 5 years of age. Similar condition has been reported by FINNIE² (1978).

Although represented by a very minute sample, the fact that the condition, was found only in females would be a coincidence, occurred by chance.

Similarity to the finding of other authors, in both cases were not found any manifestation of general symptoms, associated to cutaneous illness.

The dogs have been brought to hospital for clinical examination merely due to esthetic concern of the owners.

Before and after the treatment of the skin lesions, both patients had maintained a general good health condition.

In both cases, the ulcerated lesions were situated at the posterior side of the auricular pinna, like the one describe by FINNE² (1978), but no major otical problem was detected, except the animal n°1 that showed discrete otopathic manifestations. In both cases the otoscopy did not reveal any meatal or membranaceous alterations and no deposits, secretions or exsudates were detected.

Despite these facts, during the scratching act to minimize the pruritus caused by flea or mosquitoes bites the inoculation of the mycobacteria should have occurred. In case of animal n° 2, a female German Sheperd, it presented an ulcerated lesion at the left dorsal pad that should have possibly been acquired by making contact with ipsilateral ear lesion.

The two bitches never have travelled or lived in the coastal area of the State where dirofilariosis is endemic. It is well known and, somewhat underestimated, that very frequently, many of the skin lesions are due to the erratic parasitism of **Dirofilaria immitis**, which are transmitted through bites of a number of culicid mosquitoes (SCOTT⁸, 1979; SCOTT; VAUGHN⁹, 1987). By analyzing the predisposing conditions or the determinants of the mycobacteriosis, no other morbid conditions could be associated, after a through anamnesis and subsidiary examinations of the two dogs.

Despite the report of the acute course of the illness in the case of n° 2, the lesional aspect in both cases was of the chronic course, as described in the literature (MULLER et al.⁷, 1989).

The lesions observed in both cases were quite similar, and despite the absence of general symptoms, the over all aspect of the lesions was bad, relatively large for the size of the ear and from the lesions there was draining and abundant serohemorrhagic material, causing repulsion for surrounding people. The lesional were comparable to those described in the literature.

The complementary tests, specially the radiographic and the blood tests were of no significance. After making the curettage and subsequent bacterioscopy of clinical specimens, the bacilli were evidentiaded in both cases.

***** Soapex - Creamy soap[®] - DARRROW LABORATÓRIOS LTDA.

***** Rifocina[®] Spray - MERREL LEPETIT FARM LTDA. (10 mg/ml)

The technique used for the isolations of the mycobacteria, although being traditionally employed for diagnostic purpose, was unsuccessful in both cases. The previous decontamination processing of the clinical specimens using the Petroff's Technique (CENTRO PANAMERICANO DE ZONÓSES^{1,2}, 1985, 1988) may have been too drastic, preventing the mycobacterial multiplication. Another possibility is that these mycobacteria might not grow in the culture media and/or temperatures used which may require other mediums or the inoculation in laboratory animals.

Because of relatively slow growth of pathogenic mycobacteria and because of the initial suspicion of the illness directed toward the pathogenic mycobacteria and due to the fact that the two dogs were guided to the Hospital with an interval of few weeks, any modification on the processing of freshly obtained clinical specimen was not possible.

As related by others (FINNIE⁴, 1978; MULLER et al.⁷, 1989), the histopathologic examination of the biopsied materials allowed the prompt establishment of the diagnosis. The histopathologic findings, in both cases were similar to those described in the literature.

The tuberculin test did not reveal a positive result when applied for animal n°1. According to GREENE⁵ (1984), however, the responses to tuberculin tests in carnivores are not always conclusive, and then accurate and useful.

Finally, the follow-up drug therapy prescribed in both cases was quite satisfactory and effective. The case of German Sheperd could not be accompanied more closely because of the owner's professional reasons and due to the distance of the place where they lived. Had the medication be continued for a more prolonged period, at the time of first prescription, the course and the cure be abbreviated.

RESUMO

Na clínica dermatológica veterinária por vezes atendem-se carnívoros domésticos com lesões ulceradas de evolução prolongada e, geralmente, rebeldes à terapia usual, que podem ser originárias de infecções por micobactérias do gênero **Mycobacterium**. Pela escassez de descrições na bibliografia brasileira, pelo inusitado das lesões e pela resposta à terapia preconizada, descrevem-se dois casos de micobacteriose em caninos atendidos na FMVZ/USP. As cadelas (Pastor Alemão, Doberman), adultas, apresentavam lesões ulceradas graves, nas faces posteriores dos pavilhões auriculares, de aspecto granulomatoso, exsudativas, recobertas por crostas hemorrágicas. Inexistiam linfadenomegalia satélite e tampouco sintomas nos demais sistemas orgânicos. Complementaram-se os exames físico, hematológico e radiológico por curetagem das lesões e biópsias de pele que foram então submetidas a exames histopatológico, bacteriológico (direto: técnicas de Holst-Mitchinson e Radhakrishna, Ziehl-Neelsen); cultivo (meios de Lowenstein-Jensen e de Stonebrink & Leslie), em diferentes temperaturas; teste de tuberculina (PPD mamífero e aviário). Demonstrou-se, na bacterioscopia e na histopatologia, a presença de bacilos ácido-álcool resistentes que não cresceram nos meios de cultivo convencionais, mesmo quando submetidos a várias temperaturas distintas, bem como quadro tecidual piogranulomatoso típico. Após a interposição da terapêutica tópica (solução de Burrow, sal sódico de rifamicina) e parenteral (rifampicina) obteve-se flagrante melhora com remissão do quadro lesional. Discutem-se a etiopatogenia do quadro e as condutas diagnóstica e terapêutica.

UNITERMOS: Micobacteriose; Cães

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