

Canine nocardiosis due to *Nocardia caviae*

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SUMMARY

The literature on canine nocardiosis is briefly reviewed and critically discussed.

A full description is given of lesions in two cases. The histopathological changes are those of a purulo-granulomatous process in both naturally and experimentally infected animals.

Experiments have confirmed that the identity of the organism responsible for the disease in the two cases as *Nocardia caviae*. It occurs as comparatively long branched beaded filaments which are partially acid-alcohol-fast and unhomogeneously Gram-positive. Cultures were obtained aerobically on different media and their cultural and biochemical characteristics are described. The antibiotic sensitivity was determined in vitro.

The inoculation tests have proved that *Nocardia caviae* is pathogenic to dogs, monkeys, rabbits, guinea pigs and mice.

INTRODUCTION

In the literature, canine nocardiosis is largely subjected to confusion. Different species of the genera, *Actinomyces* & *Nocardia*, are thus blamed for the disease.

In their article, «A Sudanese actinomycosis» CHALMERS and CHRISTOPHERSON (1916/17) referred to the organism of canine nocardiosis as *Nocardia canis*. BAUDET (1934) described three cases and suggested the name *Cohnistrep-tothrix canis*.

BALAZET and PERNOT (1936) reported a case of canine meningitis and demonstrated an organism which they claimed to be the first record of *Actinomyces asteroides* from the dog.

EROMS (1939) described the disease in two dogs with a chronic purulent productive inflammation of the peritoneum and pleura; a histopathological picture typical of actinomycosis was revealed in both cases. Three other cases of canine actinomycosis were described by MARTIN (1942). The lesions were in the lungs, liver and abdominal cavity. They consisted of a fibrous

capsule surrounding areas of massive infiltration with polymorphonuclear leukocytes, lymphocytes, histiocytes and other mononuclear cells; rosettes were occasionally observed with peripheral radiating clubs and granular centres. It was only in 1948 that BREED, MURRAY and HITCHENS were able to list *Actinomyces canis* among the ill-defined species of *Nocardia*.

GINSBURG and LITTLE (1948) isolated a strict aerobe from two dogs. The organisms isolated differed in their morphological, cultural and pathogenic characteristics. The organism isolated from the first case was acid-fast, highly pathogenic to rabbits (mice were resistant) and resembled *Actinomyces asteroides* of MAC CALLUM (1902) in all features; the other one was non-acid-fast, markedly haemolytic and nonpathogenic to laboratory animals.

According to MANSI (1952), the disease in Egypt was common in an acute septicaemic form with swelling of all lymph nodes.

THORDAL-CHRISTENSEN and CLIFFORD (1953) reviewed the literature concerning canine

actinomycosis and described a case of canine distemper complicated by actinomycotic lesions in the lungs, kidneys and other abdominal organs. An acid-fast organism slightly different from *N. asteroides* was isolated. It produced no lesions in guinea pigs and rabbits which were injected intraperitoneally; similar results were obtained in the latter after intravenous inoculation.

The first case of canine nocardiosis in the American literature was recorded by BOHL, JONES, FARRELL, CHAMBERLAIN, COLE and FERGUSON (1953). They described the clinical, bacteriological and pathological picture of a dog spontaneously infected with *Nocardia asteroides*. The latter conformed to the one isolated by GINSBERG and LITTLE (1948) and BALOZET and PERNOT (1936). Two other cases which were associated with canine distemper as a primary disease were added by CROSS, NAGAO and MORRISON (1953).

More cases of canine nocardiosis were reported later — two by BLAKE (1954) and a fatal one by FROST (1959).

BROWN and OSBORNE (1962) recorded a case with omental and hepatic lesions and no purulent peritonitis.

In New Zealand, the disease was firstly recorded by MANKTELOW and RUSSELL (1965). They isolated *Nocardia species* from granulomatous pleuritis in two sheep dogs. The organism isolated was very obviously a low-grade pathogen as demonstrated by negative transmission tests in guinea pigs, mice, rabbits and pups.

In the Sudan, the disease was officially recorded in 1942. The Annual Report of the Veterinary Services, Sudan (1942), dealt with three cases; all characterized by very large swelling on the side of the neck which revealed, in a state of purity, an actinomyces morphologically indistinguishable from *A. farcinicus*. The only difference detectable by microscopic examination was that it was not in the least degree acid-fast. The condition was again recorded in the Annual Report (1956/57).

AWAD (1959) reported two cases; one showed discharging fistulae in neck and thorax while a cervical fistula and various lung abscesses were revealed in the other. The organism was a Gram-positive, acid-fast branching filamentous aerobe and was confirmed as *N. asteroides* var. *gypsoides*. It grew readily in normal media. The disease

was produced experimentally in rabbits, guinea pigs and a pup; mice were found to be refractory. Eight additional cases were also reported by AWAD and OBEID (1962) and susceptibility of rabbits and guinea pigs was confirmed.

In 1963, FAWI, OBEID and HASSANEIN reported the disease in four other dogs and *Nocardia asteroides* was said to be the cause.

In his article, «Complement fixing antitoxins in *Nocardia* with special reference to dogs», FAWI (1964) used a strain of *Nocardia asteroides* obtained from a dog which had died of the disease. Sera for the experiment were collected from nine other dogs suffering from nocardiosis.

MATERIAL AND METHODS

Two naturally infected dogs (94/66 and 105/66) were obtained from the Veterinary Hospital, Khartoum. Autopsy was carried out and material was made available for histopathology, bacteriology and experimental inoculation.

3 rabbits, 4 guinea pigs, 4 mice, 1 monkey and 1 dog were inoculated with cultures (94/66 and 105/66) originally isolated from the two cases. This was carried out through the intravenous, intramuscular and intraperitoneal routes.

Solid and fluid ordinary media were used for cultivation. These included Sugar media, Litmus milk and nutrient broth containing 37.5 units of penicillin per 1 ml. of medium. The sugars used were arabinose, glucose, glycerol, lactose, mannitol, rhamnose and xylose. Production of urease, indol and tryptophane desaminase were tested using the urea-indol medium (modified medium of Ferguson by ROLAND, BOURDON and SZTURM) as indicated in Milieux de Culture (1961), Tome II by H. CASSAGNE, Editions de la Tourelle, St-Mandé, France.

Antibiotic sensitivity test was carried out using Oxoid multodisk (code n° 11-140) on blood agar plates. Smears of pus and cultures were stained by Ziehl-Neelsen and Gram's method. Representatives of cultures were sent to London School of Hygiene and Tropical Medicine for confirmation.

Tissues from both naturally and experimentally infected animals were examined bacteriologically and histologically. Sections from various formalin-fixed organs were stained with haematoxylin and eosin, Ziehl-Neelsen and Gram's method.

RESULTS

Clinically-detectable lesions :

The clinical diagnosis was canine nocardiosis in both animals. The latter showed gradual weakness and general signs of emaciation.

The first case presented a deep perforated wound in close proximity to the root of the penis (Fig. 1). The axilla contained purulo-necrotic lesions and a few pea-sized nodules. These were dull red in colour, looked like oedematous haemal lymph nodes and extended downwards to involve a considerable part of the thorax. Their pus content was blood-stained, mealy in consistency and contained small granules.

The second case was characterised by two cervical fistulae extending from the seventh cervical vertebra to the upper part of the scapular spine. The lesions were associated with purulonecrotic involvement of the subcutis.

Morbid anatomy :

Post-mortem examination revealed internal and external lesions in both cases. These amounted to a deep purulent process with a fistula localised beside the root of the penis and closely related to the inguinal canal in the first case ; the subcutaneous tissue was involved and revealed blood-stained exudate. The process involved a bigger part of the abdominal subcutis and that of the hind limbs. The inguinal lymph nodes were swollen, moist and hyperaemic. The axillar region presented similar lesions.

In the second case, two cervical fistulae closely related to the scapular spine were found to have their ramifications beside the seventh cervical vertebra. The purulo-necrotic process was in no way different. The pleura was involved, the heart dilated and presented few foci with purulonecrotic centres.

Further examination of both cadavers revealed that lungs, kidneys, liver and spleen were affected.

The lungs were characteristically showered with foci which varied in size and appearance. Some were greyish and nodular while others were dull red and prominent resembling haemorrhagic infarcts in colour and shape (Fig. 2). Their diameter ranged from few millimeters to 2-3 cms. They projected above the surface and showed a thin cover of fibrinous exudate. The cut

surface was moist with a mostly yellowish purulent centre.

In the kidneys, liver and spleen, a varying number of similar yellowish foci was present. The lesions in the kidneys were of an embolic nature and showed clear indication of a haematogenous spread. The spleen was also swollen and hyperaemic.

Histopathology :

The characteristic histopathological changes were almost the same in both natural and experimental animals. The main features of all lesions were those of a purulo-granulomatous process with its usual arrangement and cellular content ; the purulent process being its dominant feature. This consisted of a thin fibrous capsule, lymphocytes, plasma cells, epithelioids and an intensive polymorphonuclear leukocytic infiltration in the central part of the nodules.

In sections stained by Gram's and Ziehl-Neelsen methods, Gram-positive, partially acid-fast beaded long branching filaments were easily seen under the microscope.

Isolation of the organism

Primary cultures from both cases (94/66 and 105/66) were aerobically obtained in ordinary media at an optimum temperature of 37°C. Representatives of these were confirmed as *Nocardia caviae* *.

Growth appeared in 24 hours in ordinary media. On nutrient, glycerin and serum agar slopes, it developed by age into a greyish nodular growth which was mostly formed of spreading flat colonies with a thin chalky whitish surface and lobate edges (Fig. 3). Some colonies projected from the surface and showed a mealy consistency.

Blood agar was in no way different apart from a light touch of a dirt like appearance which was conferred onto the colonies ; no haemolysis was noted after 30 days of incubation.

Dense growth was also obtained on Lowenstein-Jensen and Dorset's egg media ; the former being superior to others in yield. Colonies appeared as peach or creamy heaped-up

(*) We are indebted to Dr. I. G. MURRAY of the London School of Hygiene and Tropical Medicine, Gower Street, London W. C. 1., for confirming the identity of the organism.



Fig. 1. — A deep perforated wound in close proximity to the root of the penis (case n° 1).

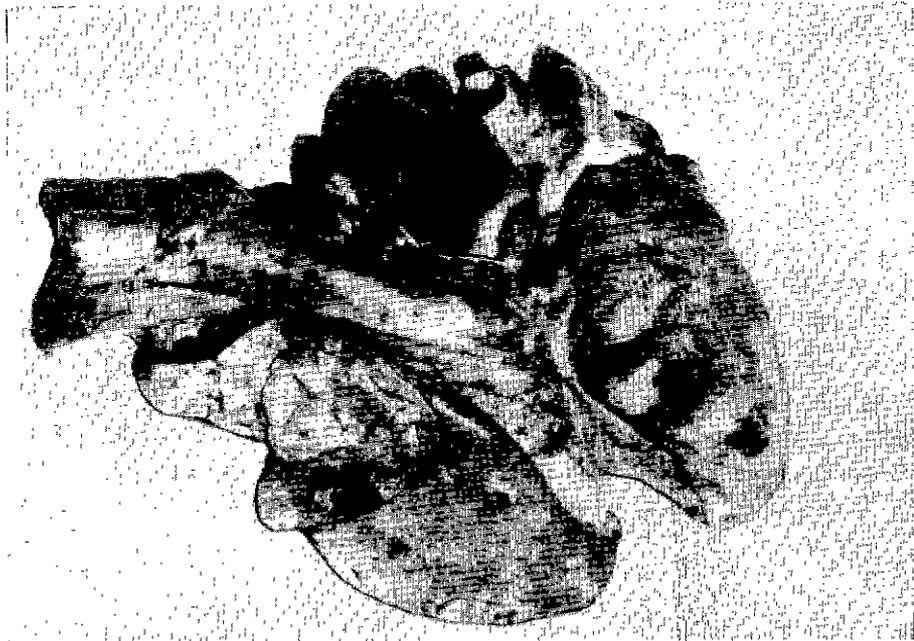


Fig. 2. — Lungs showered with variable greyish foci (case n° 2).

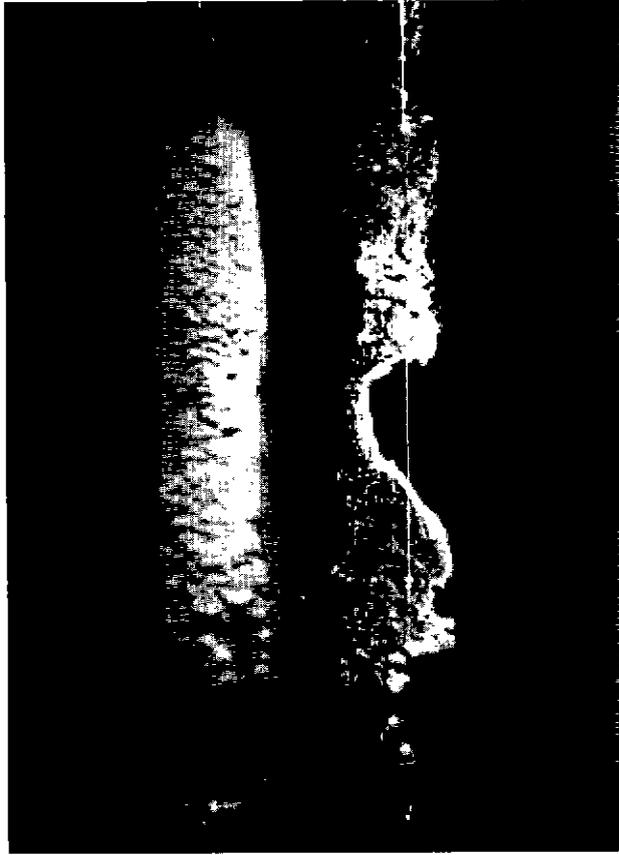


Fig. 3. — (Right) Two weeks'old culture on an agar slope
(Left) Two weeks'old culture on Lowenstein-Jensen medium.

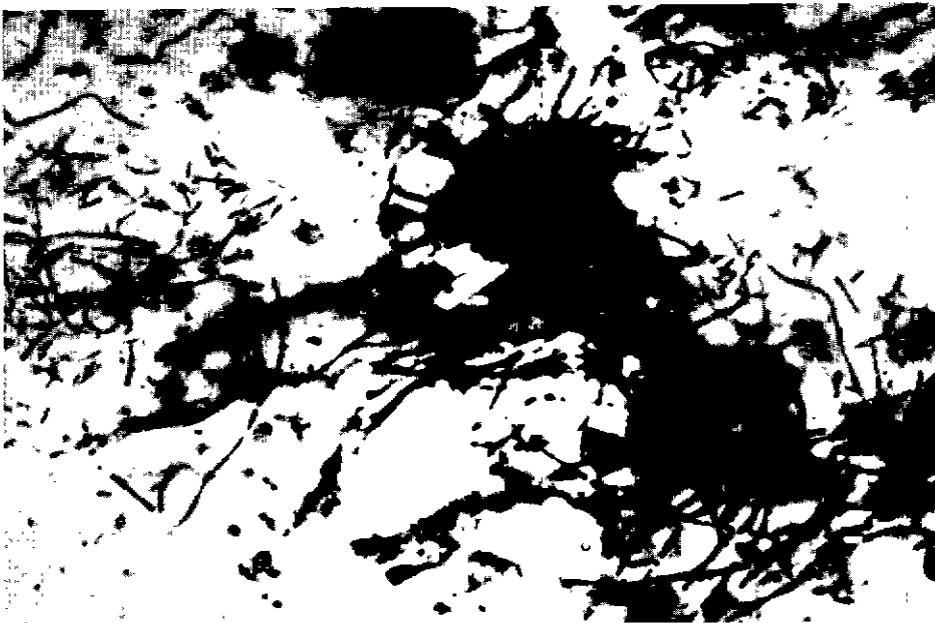


Fig. 4. — Film of pus stained by Ziehl-Neelsen.
Partially acid-alcohol-fast long beaded branched filaments of *N. caviae* are seen.

growth which increased after 5 days of incubation and coalesced in a mealy crumpled irregular yield covered with a thin chalky coating. Other colonies were single and centrally depressed or conical with radial furrows running down their chalky whitish surface. The edges were mostly irregular, lobate or crenated (Fig. 3). Lowenstein-Jensen's medium lost its greenish colour in the areas of growth and changed slowly to a fawn-yellowish one.

Penicillin, added at 37.5 units 1 ml. of nutrient broth, had no adverse effect on the growth. The initial colonies appeared in 16 hours of incubation at 37 °C ; these were greyish floating colonies surrounded by minute pin-pointed ones. In 24 to 48 hours, the surface of the broth was covered by a nodular growth of greyish white colonies which were connected by a fine web-like mesh. This increased rapidly and changed into a thick floating wrinkled pellicle in two weeks. Presumably, it sank to the bottom either spontaneously or after shaking the fluid ; the latter remained clear in spite of few web-like fragments of colonies that soon settled again to leave an unclouded broth. No adherent granular growth took place at the bottom of the tubes as in the case of *Nocardia farcinica* (Mostafa 1967).

Similar chalky whitish growth was obtained in all sugar media used. In two weeks, acid was only formed from glycerol, glucose and mannitol.

In litmus milk, at 37° C, the organism grew well as pale yellowish flakes ; the medium was not clotted but was slowly acidified in two weeks.

Urea test was positive in six to ten hours but both indol and tryptophane tests were negative. In 24 hours, nitrate was reduced to nitrite.

The antibiotic sensitivity test was carried out using sensitivity discs on blood and nutrient agar ; results were as follows :

Chloramphenicol	10 mcg	+ *
Erythromycin	10 mcg	++
Novobiocin	5 mcg	+++
Oleandomycin	5 mcg	—
Penicillin	1.5 unit	—
Streptomycin	10 mcg	++
Sulphafurazole	10 mcg	++
Tetracycline	10 mcg	+

(*) + : Slight positivity.
 ++ : Medium positivity.
 +++ : Strong positivity.

Morphology :

Microscopic examination of pus from both natural and experimental lesions revealed the presence of comparatively long breached beaded filaments (Fig. 4) ; these were partially acid-alcohol-fast and unhomogenously Gram-positive. Some of the beads were highly basophilic. Similar features were presented by cultures in spite of the excessive mechanical fragmentation due to smearing.

Pathogenicity :

The cultures proved to be highly infectious for dogs, monkeys, rabbits, guinea pigs and mice. The intravenous inoculation of culture emulsions in rabbit, monkey and dog induced generalisation and eventual death in 6 to 11 days. Numerous small greyish-yellow foci were scattered throughout all organs especially heart, lungs, brain, kidneys, viscera and serous membranes.

Guinea pigs succumbed in 7 to 30 days after intraperitoneal inoculation of culture emulsions ; mice survived for 3 to 6 months. The intramuscular inoculation provoked a local abscess formation in rabbits and guinea pigs ; adjacent lymph nodes were involved with eventual late generalisation. In one rabbit, fluid pus was discharged ; healing was apparently complete and only a scar was left 9 months after inoculation.

DISCUSSION

Canine nocardiosis appears to be world-wide in distribution ; the onset being mostly slow through a bite or a wound, with eventual generalisation and death. *Nocardia asteroides* was mostly blamed for the disease. In the available literature, *Nocardia caviae* was only mentioned in relation with otitis media in a guinea pig (SNIJDERS, 1924), and thus leaving our strains as the sole isolates from dogs.

Contrary to the findings of CROSS et al. (1953) and THORDAL-CHRISTENSEN et al. (1953), we have not found any association with canine distemper, not only in these two cases but also in other cases which were not included. The disease in dogs is spontaneous and fatal ; lesions being of a purulo-granulomatous nature. The intravenous and intraperitoneal inoculations of cultures were also fatal to dogs, monkeys, rabbits, guinea pigs and mice ; thus, no justification for

the statement of BLAKE (1954) that nocardiosis itself may rarely be fatal unless it is a complicating factor to some other diseases.

In their article « Identification of *Nocardia caviae* (ERIKSON) Nov. Camb », GORDON et al. (1962) reported that 5 of the 15 strains of *N. caviae* added to their collection were received as *N. asteroides* and were considered misnamed ; nine were specifically unidentified. The remaining strain that came to them bearing a specific name, *N. caviae*, was isolated from an infected middle ear of a guinea pig and deposited in the national collection of type cultures (N. C. T. C.) by SNIJDERS (1924).

The presence of the disease in Khartoum Province necessitates thorough methods of diagnosis and greater measures of observation in other parts of the Sudan where the disease cannot be excluded as a hidden spreading problem. There may be little agreement as to the species of *Nocardia* involved but we hope that this work will stimulate investigators in the Sudan to assert that although closely related, *N. caviae* could easily be distinguished from *N. asteroides* (GORDON et al. 1962).

Our strains differed from those of GINSBERG et al. (1968) and AWAD (1959) in being infective for mice, and, in contrast to the second strain of the former, they were nonhaemolytic, partially acid-fast and pathogenic to laboratory animals.

They showed sensitivity *in vitro* to Novobiocin, streptomycin, erythromycin, sulphafurazole, slightly to Chloramphenicol and tetracycline but not to penicillin or oleandomycin. AWAD (1959) reported on the sensitivity of his strains to chloramycin, terramycin and not to tetracycline. In a negative result of a simultaneous inoculation of culture and penicillin in a rabbit and a guinea pig, he concluded that the organism was sensitive to penicillin as well.

AWAD et al. (1962) reported on the sensitivity (*in vitro*) of *N. asteroides* isolated from Sudanese dogs to terramycin and chloromycetin. Two out of 5 cases responded to a combined treatment with penicillin and terramycin or streptomycin.

ACKNOWLEDGEMENT

The writers gratefully acknowledge the interest and help given by Professor C. A. Mc GAUCHY. Special thanks are due to Dr I. G. MURRAY of the London School of Hygiene and Tropical Medicine, to Dr P. PERREAU and Miss M. Th. BOTTO of the Institut d'Élevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, to the staff of the Veterinary Hospital, Khartoum and to Mr Mohamed Salih HASSAN of the department of Veterinary Pathology, University of Khartoum.

RÉSUMÉ

Nocardiose canine à *Nocardia caviae*

Les auteurs, après avoir passé en revue les diverses observations déjà publiées de nocardiose canine, montrent combien était imprécis à l'origine le genre de l'actinomycète en cause.

C'est l'espèce *Nocardia asteroides* qui a été rencontrée le plus fréquemment chez le chien, isolée de lésions diverses (méningite, péritonite, pleurésie purulente, pleurésie granulomateuse, abcès du foie, abcès du poumon, etc.).

Dans l'observation présente, il s'agit de deux cas naturels de nocardiose du chien, étudiés à la clinique vétérinaire de Khartoum (Soudan).

Chez le premier animal, les lésions visibles étaient constituées par une plaie fistuleuse de la région inguinale et des lésions nécrotiques et purulentes de la région axillaire.

Chez le second, deux fistules purulentes étaient situées au cou et en arrière, près du bord antérieur de l'épaule.

Les autopsies montrèrent que, dans les deux cas, la nocardiose était généralisée ; des nodules actinomycosiques, dont la taille variait de quelques millimètres à trois centimètres, parsemaient les poumons, les reins, le foie et la rate. Histologiquement ces lésions étaient absolument classiques.

Dans le pus prélevé au centre de ces nodules, l'examen microscopique montrait un actinomycète à longs filaments ramifiés, gram-positif, dont l'acidorésistance était variable.

Celui-ci fut isolé aisément, par culture aérobie sur les milieux ordinaires ; les colonies d'aspect crayeux, blanc grisâtre, apparurent en 24 heures. Des cultures luxuriantes furent obtenues sur les milieux de Lowenstein-Jensen et de Dorset. Non hémolytique, cet actinomycète, absolument insensible à la pénicilline mais sensible à la Novobiocine, l'erythromycine, la streptomycine et au sulphafurazole, fut identifié dans les deux cas à *Nocardia caviae*.

Ces deux souches étaient très pathogènes pour le chien, le singe, le lapin, le cobaye et la souris, comme le montrèrent les inoculations expérimentales (évolution fatale en 6 à 11 jours après l'inoculation intraveineuse, pour les trois premières espèces).

Dans les deux observations rapportées, cette infection spécifique évoluait seule et ne pouvait pas être considérée comme secondaire ou associée à une maladie classique du chien (du type Carré par exemple) ; d'autres cas sont venus depuis confirmer ce pouvoir pathogène essentiel.

Il y a donc lieu de considérer désormais qu'à côté de la nocardiose canine à *N. asteroides*, existe une nocardiose du chien, cliniquement identique et à évolution fatale, due à *N. caviae*.

RESUMEN

Nocardiosis del perro causada por *Nocardia caviae*

Se pasan en revista y se discuten los estudios ya publicados sobre la nocardiosis del perro.

Se describen las lesiones encontradas en dos casos.

Lesiones purulentas y fístulas son las modificaciones histopatológicas ocurridas en los animales naturalmente y experimentalmente infectados. Se identificó el germen causal de la enfermedad como *Nocardia caviae* en los dos casos. Aparece el dicho bajo forma de filamentos ramificados, relativamente largos, parcialmente ácido resistentes e irregularmente gram positivos.

Fue aislado por cultivo aerobio en varios medios. Se describen sus características bioquímicas y culturales. Se determinó su sensibilidad para con los antibióticos.

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