

Mastitis Due to *Nocardia Braziliensis*

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In a mastitis laboratory where approximately 55,000 milk samples are processed each year, the finding of a causative organism other than *Streptococcus* or *Staphylococcus* arouses considerable interest. Further interest is aroused when the organism proves to be one which has not been observed as a cause of bovine mastitis. Such a finding was the isolation of an organism resembling *Nocardia braziliensis*.

Nocardia is a free-living, aerobic genus of the higher fungi *Actinomycetes*. This broad group is placed taxonomically in a position intermediate between the true bacteria and the true fungi, since it appears to have some characteristics of both groups. All of the *Actinomycetaceae* are common saprophytes of the soil.

In man several *Nocardia* species may be introduced directly into wounds producing the typical "actinomycotic mycetoma" — a firm mass of fibrous tissue riddled with discharging sinuses. In addition one of the species, *N. asteroides*, is able to produce a systemic disease. This often follows infection by inhalation and appears to have a special predilection for the central nervous system and subcutaneous tissue.

In recent years numerous cases of actinomycotic mycetoma in dogs have been reported. Barnum⁽¹⁾ and Pier⁽²⁾ have described cases of bovine mastitis due to *Nocardia* spp.

A review of the literature has not revealed *N. brasiliensis* as a cause of disease in Veterinary Medicine previous to this report. The organism was first isolated from a case of mycetoma of the leg and, in the past, has been regarded by some workers as identical to *Nocardia asteroides*.⁽³⁾

CASE REPORT

Milk samples from an aging Holstein

cow were presented to the laboratory with a history of chronic mastitis in the two hind quarters for several years.

Streptococcus agalactiae was the pathogen recovered; a marked increase in leucocytes was noted on the milk smears from the affected quarters (15 + per field). A diagnosis of mastitis due to *Streptococcus agalactiae* was made and treatment with Penicillin infusion of the quarters was instituted.

Eight months later samples were again submitted from this cow. Further history was obtained. The cow apparently showed thick milk from time to time and had been treated with every commercial Mastitis preparation to no avail. The condition would show periods of resolution lasting 1 - 2 weeks, and then would be detectable on a strip-cup again. At no time was much swelling of the affected quarters noticed.

A *Staphylococcus* was recovered from the hind quarters along with unusual chalky colonies. The latter colonies were folded and tenacious; a particular earthy odour was noticeable. Smears were made and stained with Gram's and Acid-fast stain.

The organism was gram-positive and acid-fast. Numerous branching filaments were seen; there were also shorter rod and coccoid forms present.

The organism was tentatively identified as belonging to the *Actinomyces* group and a culture was sent to the Department of Bacteriology, McGill University for positive identification.

DESCRIPTION OF THE ORGANISM

The organism was grown for 24 hours in aerobic incubation at 37° C. On blood agar medium the colonies were small and minute, irregular in shape and size, chalky white coloured, and slightly raised. No hemolysis was produced and a fine aerial mycelium was present around the colonies after 48 hours. On Malt-extract agar the colonies were larger,

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2-3 mm. diameter, and a yellow to orange pigmentation appeared after 48-72 hours. When incubated longer they became chalky, folded and tenacious.

On Sabouraud Glucose Agar, the colonies were of the same size as previously described, folded with an ochraceous pigmentation. The culture on Dorset and Lowenstein Media appeared, and remained for a longer time, smooth and waxy with a yellow to salmon pigmentation and without aerial mycelium.

Growth was obtained on Czapek's paraffin medium in 72 hours. The reactions on differential media were as follows: —

Carbohydrates: negative; Indol production: negative;

H₂S production: negative; Gelatin: liquefied. Litmus-milk: slightly alkaline after three weeks; Milk: coagulated; Nitrates were not produced. On Dorset and Loeffler Media: no liquefaction or peptonization.

Stained by Gram's method, the organism was Gram positive, appearing as fine branching elements, short rods and coccoid forms. With Ziehl Neelsen's Method, the organism was acid fast using 2% H₂SO₄, and partially acid alcohol fast. Lesions could not be produced in animals, guinea pigs and mice, (5) (6) neither with the pure culture nor with the culture and addition of 5% hog gastric Mucin.

Differential diagnosis had to be made with *Streptomyces* genus. The presence of branching filaments, bacillary and coccoid forms,⁽⁴⁾ the acid fastness and ability to grow on Czapek's paraffin medium in aerobic conditions, placed this organism in the genus *Nocardia*. From the reaction obtained on differential media, this strain was classified as *Nocardia braziliensis* (5) (7).

A diagnosis of chronic mastitis due to *Nocardia braziliensis* was made. Due to the duration of infection it was felt that there would be no response to treatment; slaughter was advised and carried out.

Post-Mortem Examination

The udder was obtained for pathological study.

There was no clinical evidence of swelling or nodules in the affected quarters on external palpation. On gross examination there was miliary abscessation in the dorsoposterior regions of the left and right hind quarters. A thick yellow-white exudate was present.

The right and left supermammary lymph nodes were enlarged. The cut surface was quite wet.

Microscopic sections revealed extensive foci of chronic granulomatous inflammation with suppuration. Chronic inflammatory cells, fibroblasts, and giant cells were present. Due to the extensive suppuration a large number of polymorphonuclear leucocytes and macrophages were seen.

Nocardia braziliensis was recovered in pure culture from these abscesses.

The method of isolating the organism from udder tissue was as follows: Samples of pus and abscess walls were obtained and minced with sterile scissors. This material was then ground in a sterile mortar with sterile aluminum oxide. Sterile beef-heart-brain infusion broth was added, enough to give a 20% suspension. This suspension was then used to inoculate blood agar plates. The plates were incubated at 37° C. for 48 hours.

During life the organism was cultured from milk on three successive occasions. Material was also obtained during necropsy examination of the udder from the discharge and walls of numerous small abscesses scattered through the secretory tissue. Smears from milk and pus contained numerous acid-fast bacilli showing branching and fragmentation of mycelia.

DISCUSSION

The species *Nocardia* is found naturally occurring in soil and on soil contaminated objects. It is not a naturally occurring organism in the bovine udder,

although it may appear in milk due to contamination after milking.

It would be difficult to assess the role of the organism if it were recovered solely from milk samples received at the laboratory. Most of the samples we receive are taken by dairy producers and are often contaminated.

Pier et al⁽²⁾ report that *N. asteroides* infection results in a diffuse firmness in the affected gland or else the development of discrete nodules. In their experimental cases milk from affected quarters showed only the presence of inflammation and infection. The case reported by Barnum,⁽¹⁾ similar to our case, did not show clinically detectable udder lesions.

The only apparent solution to diagnosis, is to base such a diagnosis on the recovery of the organism on successive occasions from samples obtained under the most aseptic procedures possible.

How does the organism enter the udder? We are only able to offer suggestions here. Inadequate care in the administration of antibiotics may introduce soil-borne organisms into the udder. The organism may also enter following a teat injury which destroys the protective sphincters. Improper washing of the udder before milking may enable the organism to enter immediately after the milking machine is removed.

Once mastitis due to *Nocardia* has been diagnosed a further problem arises. The mastitis treatments marketed to date have no effect on organisms safely protected by fibrous tissue. The body in attempting to protect itself also prevents us from helping it.

Streptodornase-streptokinase combinations have proven valuable as an adjunct to treatment of chronic inflammatory conditions. It may be that these physiological currettes plus an effective *in vitro* antibiotic would be of some value in treatment of chronic *Nocardia* mastitis.

In a valuable cow a single affected quarter could be dried up providing the

organism did not penetrate to the intact quarters.

Surgical removal of affected quarters may be of benefit in a valuable breeding animal; it would be an economical impossibility in the average farm herd.

A severe chronic case with extensive fibrosis of the gland cannot be salvaged except by slaughter.

The prognosis in all cases of chronic mastitis should be guarded. In chronic mastitis due to *Nocardia* the prognosis should be even more bleak. In the case reported here treatment was of no avail and the cow was eventually slaughtered for meat.

SUMMARY

A case of chronic bovine mastitis due to *Nocardia braziliensis* has been presented. The organism was refractive to treatment and the cow was slaughtered.

Necropsy examination of the udder revealed chronic granulomatous inflammation. This organism, due to the fact that it is soil-borne, may be introduced into the udder by improper milking procedures or careless medication and results in an incurable mastitis.

RESUME

Cet article porte sur un cas de mammite chronique due à *Nocardia braziliensis* chez la vache. L'agent étiologique s'étant montré réfractaire au traitement, l'animal fut abattu.

L'examen postmortem de la mammelle révéla une inflammation granulomateuse chronique. Le microbe que l'on trouve dans le sol peut être introduit dans la mammelle à la faveur de mauvais procédés de traite ou de négligences au cours d'une médication et il en résulte une mammite incurable.

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Brucellosis in Man

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With more widespread pasteurization of milk and other dairy products, brucellosis in the United States has become almost entirely an occupational disease affecting persons intimately exposed to infected animals or their tissues. In the last 10 years the number of cases reported annually has dropped considerably; nevertheless, if one excepts salmonellosis deriving from animal sources, brucellosis in the United States still has a higher reported incidence than any other disease of animals transmitted to man.

While great strides have been made in the last few years in the control of bovine brucellosis, and the incidence of the infection in cattle has been greatly reduced, the decrease in notified incidence in man cannot be clearly related to the success of the control program. Swine continue to be an important source of infection for man.

Despite the decrease in the number of reported cases, the problem of establishing the diagnosis of the disease in man remains an important one. Isolation of the organism from the patient is the only proof of diagnosis. A significant titer in the standard seroagglutination test, or a rising titer, provides presumptive confirmation of a clinical diagnosis. In chronic brucellosis a fluctuating titer is suggestive of active disease. The widespread and often indiscriminate use of the broad-spectrum antibiotics makes cultural proof more difficult, and greater dependence must be placed upon the agglutination test. The skin test has no value as a diagnostic aid.

The current therapy of choice consists of a combination of dihydrostreptomycin and tetracycline, with or without the addition of sulfadiazine or triple sulfonamide. As all presently available drugs primarily suppress the infection, treatment must be continued for a period of time sufficient to allow the body to dispose of the infection. Bed rest is essential. Local lesions may require longer medical treatment or definitive surgical treatment.

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