**Nocardia asteroides** as a cause of pneumonia in a buffalo calf

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**Summary**

Pulmonary nocardiosis has been described in a four-month-old male buffalo calf (*Bubalus bubalis*) with a history of respiratory distress lasting ten days. Microscopic examination of tissue imprints from the infected lungs revealed numerous Gram-positive, acid-fast, fine, branched, filamentous organisms which were morphologically identical with those of *Nocardia* spp. Cultures of pneumonic lung specimens on plain Sabouraud medium yielded pure growth of *Nocardia asteroides* at 37°C. Lungs from ten healthy buffalo gave negative results for bacteria, suggesting that *N. asteroides* does not occur as a commensal in the pulmonary tissues. Epidemiological investigation established the source of infection in the immediate environment of the animal. It is emphasised that *N. asteroides* may be considered to be a respiratory pathogen and should be included in differential diagnosis of pulmonary diseases.

**Keywords**


**Introduction**

*Nocardia asteroides*, the chief causative agent of human and animal nocardiosis, is an aerobic actinomycete which may act as a primary and opportunistic pathogen (6, 12, 17). The other species of the genus *Nocardia*, such as *N. brasiliensis* and *N. otitidiscaviarum* (*caviae*), are also implicated in the aetiology of the disease (1, 8). The infection is world-wide in distribution and has been reported from many countries, including India (5, 11, 20). However, there seems to be very little information on the nocardial infection of buffaloes (21). The purpose of the present investigation is to record a natural fatal case of pneumonia due to *N. asteroides* in a debilitated young buffalo calf from Gujarat, a western State of India.

**Materials and methods**

A nondescript four-year-old male buffalo calf (*Bubalus bubalis*), belonging to a landless farm worker from Anand, was the subject of this investigation. The animal appeared to be debilitated, dull and anorexic and was reluctant to stand. In addition, the calf also showed the following symptoms: a mucoid nasal discharge, respiratory difficulty and occasional coughing over the previous ten days. The owner said that the calf had had diarrhoea for approximately 16 days. Although the aetiology of the diarrhoea was not ascertained, the animal was treated orally with albendazole at a dose of 2 ml/kg of body weight. The animal died 13 hours after arrival at the veterinary hospital before chemotherapy could be given. An autopsy was performed and pieces of the affected lungs collected aseptically onto a sterilised Petri dish were submitted to the Veterinary Public Health Laboratory in Anand for diagnosis. Wet mounts and impression smears prepared from the purulent exudate of the lung nodules were examined microscopically using potassium hydroxide (KOH), PHOL (14), Gram, modified Kinyon’s acid-fast and Giemsa techniques. The newly discovered PHOL stain contained 3 ml of glycerol, 0.3 ml of a 3% aqueous solution of methylene blue and 5 ml of a 4% aqueous solution of 35% formaldehyde. Small portions of pneumonic lung tissue were directly inoculated onto the duplicate slants of plain Sabouraud dextrose agar, nutrient agar, brain heart infusion agar, Sabouraud dextrose agar with antibiotic (chloramphenicol, 0.1 mg/ml) and sunflower seed agar (Pal’s medium) (10). All were incubated at 37°C, except the latter medium which was kept at 30°C, and were examined daily for up to two weeks for microbial growth. The isolate was identified on the basis of growth, and cultural, morphological and biochemical characteristics (2).

Ten lungs obtained from healthy buffaloes (six adult females and four young males), which were slaughtered for human
consumption at a private local abattoir in Anand, were investigated microbiologically to discover whether \textit{N. asteroides} could form part of the normal respiratory flora.

In order to determine the source of infection, an epidemiological investigation was conducted. In all, eight environmental samples (two paddy husks, three soil samples and three wheat straw samples) were collected in clean dry polythene bags and processed for \textit{N. asteroides} (9).

**Results**

At presentation, the calf had a rectal temperature of 40°C, a respiratory rate of 46 per minute and a pulse rate of 97 per minute. The owner gave some traditional remedies, but no antibacterial antibiotics were administered to the animal. The calf died after eleven days of respiratory illness. At autopsy, both the lungs showed multiple millet- to pea-sized greyish nodules. However, no macroscopic lesions could be observed in other internal organs.

Microscopic examination of the wet mounts, following KOH and PHOL staining, and tissue imprints following Gram, modified Kinyon's stain, acid-fast and Giemsa staining, failed to indicate the presence of \textit{Aspergillus}, \textit{Candida}, \textit{Cryptococcus}, \textit{Zygomycete}, \textit{Mycobacterium} (acid-fast bacilli) or \textit{Chlamydia}. However, such examination did reveal many delicate, Gram-positive, acid-fast, branched, filamentous bacteria, about 1 µm in diameter, which were morphologically consistent with \textit{Nocardia} (Fig. 1).

\textit{Nocardia asteroides} was the only organism which grew on slants of plain Sabouraud dextrose agar, producing small, raised, granular, light-orange-coloured colonies after 10 days of incubation at 37°C. In the PHOL stain, many thin, branched filaments could be seen along with the coccoid and bacillary forms of \textit{Nocardiida}. No other micro-organism could be recovered from the pneumonic lungs on the various cultural media. The isolate did not use casein, xanthine and tyrosine, but did hydrolyse urea.

All ten lungs from the apparently healthy buffaloes gave consistently negative results for \textit{N. asteroides} (Table I).

**Table I**

<table>
<thead>
<tr>
<th>Source of specimen</th>
<th>Number of specimens examined</th>
<th>Number of specimens giving positive results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Pneumonic lungs</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Environmental specimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Paddy husks</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>3</td>
</tr>
</tbody>
</table>

Of the eight environmental samples investigated, bacteria could be recovered only from two soil specimens obtained from the unsanitary calf-pen (Table I).

**Discussion**

Naturally occurring nocardiosis has been encountered in antelope, birds, cats, cattle, chinchillas, deer, dogs, fish, goats, horses, marine mammals, monkeys, pigs and sheep, as well as in humans (4, 12, 20, 21, 22). As the clinical and autopsy findings do not suggest nocardiosis, the diagnosis must be confirmed by isolation of the bacteria and direct demonstration of such bacteria in the clinical material. The identification of \textit{N. asteroides} in the infected lung tissues, both by culture and by direct microscopy, conclusively established its aetiological role in the pneumonia of the buffalo calf.

Failure to recover \textit{N. asteroides} from ten lungs of apparently healthy buffaloes indicates that bacteria do not occur as a commensal in the respiratory tract of buffaloo. Therefore, isolation of \textit{N. asteroides} from clinical samples should be viewed prudently from a diagnostic point of view.

Epidemiological investigation established the presence of \textit{N. asteroides} in the soil of the unhygienic animal shed where the calf was kept. This observation suggested that the animal acquired the infection by inhaling a large number of infectious organisms, which were present in the contaminated environment. Similar epidemiological studies conducted earlier by other workers reported the recovery of \textit{N. asteroides} from the dust and air in a hospital where an outbreak of
Nocardiosis had occurred (7, 19). The increasing number of pulmonary infections due to *N. asteroides* indicates that the soil serves as a reservoir of the organism and the principal source of infection (18).

In India, nocardial infection has been reported from birds, cattle, dogs, goats, sheep and from humans (3, 11, 13, 15, 16). The demonstration of *N. asteroides* in the pneumonic lung of a buffalo calf is indeed an important finding. It is, therefore, advised that nocardiosis should be differentiated from other respiratory diseases, particularly from tuberculosis and pulmonary mycoses, by employing standard microbiological techniques.

Acknowledgements

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**Nocardia asteroides, agent de la pneumonie chez un bufflon**

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**Résumé**

La nocardiose pulmonaire a été décrite chez un bufflon (*Bubalus bubalis*) âgé de quatre mois qui présentait depuis dix jours des troubles respiratoires. L'examen microscopique des tissus prélevés des poumons infectés a révélé la présence de nombreux micro-organismes positifs à la coloration de Gram, acido-résistants, fins, ramifiés et filamentueux, morphologiquement identiques à *Nocardia* spp. La mise en culture de prélèvements de poumons infectés en milieu de Sabouraud simple a mis en évidence le développement de *Nocardia asteroides* à 37 °C. La bactérie n'a pas été retrouvée dans les poumons de dix buffles sains, ce qui montre que *N. asteroides* n'est pas une bactérie saprophyte des tissus pulmonaires. Une étude épidémiologique a établi que la source d'infection se situait dans l'environnement immédiat de l'animal. L'auteur en conclut que *N. asteroides* peut être considéré comme un agent pathogène des voies respiratoires et doit être inclus dans le diagnostic différentiel des maladies pulmonaires.

**Mots-clés**

Buffles – *Nocardia asteroides* – Nocardiose – Pneumonie – Poumon pneumonique.

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**Nocardia asteroides como agente de neumonía en un joven búfalo**

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**Resumen**

Tras describirse un caso de nocardiosis pulmonar en un búfalo (*Bubalus bubalis*) de cuatro meses de edad con un cuadro de problemas respiratorios durante diez días, el análisis microscópico de extensiones tisulares de los pulmones infectados reveló la presencia de abundantes microorganismos Gram-positivos, ácidoresistentes, finos, ramificados y filamentosos, morfológicamente idénticos a *Nocardia* spp. El cultivo a 37°C de muestras de pulmón neumónico en medio sencillo de Sabouraud deparó el crecimiento de colonias puras de *Nocardia*...
asteroides. Los pulmones de diez búfalos sanos arrojaron resultados negativos para esas bacterias, signo indicativo de que *N. asteroides* no está presente como comensal en los tejidos pulmonares. La investigación epidemiológica estableció que la fuente de contaminación se encontraba en el entorno inmediato del animal. Se concluye que *N. asteroides* debería ser considerado un patógeno respiratorio e incluido como tal en las pruebas de diagnóstico diferencial de las enfermedades pulmonares.

Palabras clave

References