نتائج موجبة لاختبار الروزبنكال في الخيول

نبيه محمد عطا، محمد جويد علوان، سعدي أحمد غناوي السامرائي، شاكر محمود مرهد، حيدر بديع عبد الجبار
كلية الطب البيطري - جامعة بغداد

ملاحظات قصيرة

لوحظت حالات متفرقة في مجموعة من الخيول شملت ناسور الحارك، التهاب المفاصل وتورم كيس الصفن. سحب عينات دم من بعض هذه الحالات ومن خيول تبدو سليمة لقياس خضاب الدم وأجراء اختبار الـ (Rose Bengal test). وتم تجربة نموذج من السائل في كيس الصفن. أظهرت الفحوصات أن مستوى خضاب الدم كان ضمن الحدود الطبيعية (11.6-16.2) غم/100مل. وان خمسة خيول من عشرة (50%) أظهرت نتيجة موجبة لاختبار الروزبنكال وكانت من الجنسين وبأعمار مختلفة، ولم نتنصر على الخيول التي أظهرت ناسور أو تورم. ولم تظهر جراثيم من السائل المصلي الراقي والحاري على كريات دم حمراء وخلايا قيحية والمسحوب من كيس الصفن لأحد الخيول.
NATURAL NOCARDIOSIS IN CATTLE:
A SURVEY AND PATHOLOGIGIAL STUDY

M.J. ALWAN
Department of Vet. Pathology and poultry, College of Vet. Medicine, University of Baghdad, Iraq.

SUMMARY
This work was done to investigate Nocardiosis in cattle in order to evaluate the incidence of nocardial mastitis in Al-dejiala station, bacteriological examination was conducted on 90 milk samples obtained from mastitic cows Nocardia asteroides was found in 25 out of 90 (27.77% mastitic cows. This M.O was recorered in pure culture in 5 cases and as mixed with other microorganisms in 20 cases. The gross examination of the affected udders showed multiple nodular lesions in firm fibrotic udders, some of them formed draining sinus which exuded pus to the exterior multifocal nodules of varying size were seen in the lungs. Microscopic examination of the affected
udders revealed pyogranulomatous lesions in different areas of the glandular parenchyma multiple classical granulomatous lesions were also noticed in the lung tissues.

**INTRODUCTION**

Since the early isolate of *Nocardia asteroides* in 1888 by Nocard, many naturally occurring condition have been recorded in different animal species, such as dog, sheep, horse and wild animals (1,2,3)

in cattle, the disease was shown in three main form, skin farcy, mastitis and pulmonary form Bovine skin farcy was frequently observed in Africa. Awad9 (4) described the disease an external form of farcy in sudoness cattle characterized by chronic subcutaneous abscesses. The internal form of skin farcy may or may not be accompanied by the external form and is characterized by a Localized or diffuse pyogranulomatous lesion of the internalorgana and tissues (5).

In the pulmonary form, the lungs are involved primary or secondary to skin farcy or mastitis (6,7). Dissemination of the lesions were recoginzed internal organs (8) Nocardial mastitis have been reported as sporadic cases or in outbreaks over the world (9)%.

Hibbs et al ., (10)
reported outbreaks of nocardial mastitis in 3300 dairy cows where 450 cows died and further 500 were culled. In this work, the author aimed to evaluate the occurrence of nocardial mastitis in dairy cattle and to describe the pathological changes accompanied.

**MATERIALS AND METHODS**

This study was carried out on cattle at the AL-Dejiala station. Bacteriological examination was conducted on 90 milk samples obtained from mastitic cows. Five mastitic cows which were positive for nocardial examination were slaughtered. Post-mortem examination were carried out specimens from different organs were fixed in 10% normal buffer formalin for histopathological examination. Milk, pus and tissue samples were collected aseptically for bacteriological examination.

**Bacterial examination**

Soon after collection, samples were cultured into brain heart infusion agar, containing 7% sheep RBC and blood agar. They were incubated at 37°C for three days. Suspected colonies were subjected to gram and acid fast stains.
followed by necessary confirming biochemical and physiological examination chosen according to Alwan (4) and Collins and Anne (II).

Animals inoculation

In order to determine the pathogenicity of the isolates, five guinea pigs were used. They were inoculated intrapulmonary with $1 \times 10^9$ CFU of *N. asteroides*, taken from a suspension of 3 days old culture in sterile saline. Thermodurability of the isolated M.O was checked by incubating at 50°C for 1 hour.

**RESULTS**

*N. asteroides* was found in 25 out of 90 (27.77%) mastitic cows as well as milk tank. Isolation of this M.O. was found in pure culture in 5 cows. In other remaining samples M.O including candida spp, streptococeus spp, staphylococcus spp, klebseila and Bacillus spp were also isolated. *N. asteroides* were also isolated from the lung and supramammary lymph nodes of the necropsied cows.

Colonies of *N. asteroides* were seen on blood agar and brain heart infusion agar after 72 hrs of aerobic inoculation colonies of *N. asteroides* were seen on blood agar and brain heart infusion agar after 72 hrs of aerobic inoculation at 37°C, They were opaque, chalky
white firmly adherent to the medium, and not haemolytic (Fig:1). In 72 hours of incubation, the colour of colony was turned in to yellowish- orage. In broth, a pellicle of waxy growth was noticed after 48 hours of incubation. The M.O. were partially or completely acid fast. Some beaded appearance due to irregular staining. These M.O. remained alive more than 8 hours at 50°C. The isolates were fermented glucose, producing acid and gas, mannitole, sucrose and fructose were not fermented. They reduced nitrate to nitrite, produced urease and hydrolysed aseculine but not casein (Table:1). The inoculated guinea pigs died within 5-6 days post infect infection. N.asteroides was isolated from all internal organs. All infected animals showed massive pmns and mnsinfiltration in the examined organs.

Pathological changes

At early stage, the infected udders were swollen and painful but later on, they become firm and fibritic with palpable nodular lesions. Some of then ruptered or formed draining sinus which exuded whitish pus to the exterior (Fig:2) in addition marked thick walled abscesses were observed. One or more quarter of the udder were involved. In the lung, necropsy findings revealed firm yellowish-white multifocal nodules of
varying size ranging from 1-1.5 cm in diameter. They are uniformly scattered throughout the parenchyma with their cut section revealed dry purulent center. The supramammary lymph nodes were enlarged and edematous mediasinal and bronchial L.N. were also showed the same lesions Histopathological examination of mammary glands showed massive inflammatory cells infiltrated the interacinar tissuc which lead to induce pressure atrophy the adjacent acini and lactiferous duct which showed in addition of epithelial sloughing. Multiple classical granulomatous lesions were also noticed in different areas of the udder. They were characterized by purulent necrotic center surrounded by a zone of epithelioid cells, mononuclear and langhans, giant cells and outer zone of fibrous connective tissuc (Fig:3). The skin at the sinus tract area showed cellulitis. The adjacent udder skin was acanthotic and hyperkeratotic (Fig:4). Microscopic examination of lung revealed pyogranulomatousmatous lesions identical to those described in the mammary glands. The interalveolar and interlobular septa were thickened due to mononuclecar cells and neutrophils infiltration as well as fibroblast proliferation (Fig:5).
DISCUSSION

The results showed that among 90 mastitic cows in the AL-dejiala station, 25 (27.77%) were shedding *N. asteroides* in their milk. Such high incidence was also reported by Argente *et al.* (12) who found a total number of 227 cows in Briton, 41 cases of nocardial mastitis. Tarable *et al.*, (13) have also reported the existence of *N. asteroides* in the milk of 22 out of 25 (84%) mastitic cows in Argentina.

The significantly high percentage of nocardial isolates observed in this study indicates that nocardiosis is not rare in dairy herds in Iraq. The affected cows showed drop in milk yield with loss condition. This eventually should lead to culling of affected. On the other hand, the management of such cows was costly due to continuous treatment with no promising responses. *N. asteroides* has been identified as a problem of economic significance in certain dairy herds (Blood *et al.*, (14).

The isolation of *N. asteroides* in pure culture from 5 cases is a strong evidence to be an important independent cause of bovine mastitis. Previous reports stated that the isolation of this M.O in pure and mixed from mastitic cows (15). The existence of mastitis in
outbreak and the occurrence of the disease lactating heifers might be attributed to the improper hygienic measures employed in AL- dejiala station. The existence of the M.O. in soil and possible in tick facilitates its transmission and infection (16). Although the initial infection might occur during the dry period where a focus of infection remain clinically unrecognized till parturation as the cow an start to lactate and udder is full with milk, the Nocardial foci probably become disrupted and the M.O. spreads through the lacteferous tree resulting in massive infection. The existence of this organism in milk tank may indicate improper, non hygienic handling of the milk or that might be due to bacteremia. pulmonary lesions might be due to hematogenous metastasis (17).
Table 1: Some biochemical and physiological characteristic of *N. asteroides* isolated

<table>
<thead>
<tr>
<th>From mastitic cows</th>
<th>1- Gram stain Reaction</th>
<th>2- Acid fast staining</th>
<th>3- Growth at 50</th>
<th>4- Guinea pig inoculation</th>
<th>5- Fermentation of Mannitole</th>
<th>6- Aesculine Hydrolysis</th>
<th>7- Nitrate reduction</th>
<th>8- Urease activity</th>
<th>9- REC hemolysis</th>
<th>10- Gelation liquefaction</th>
<th>11- Casein hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±</td>
<td>Partial</td>
<td>±</td>
<td>Died after 5-6 days</td>
<td>–</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6- Aesculine Hydrolysis</td>
<td></td>
<td>7- Nitrate reduction</td>
<td></td>
<td>8- Urease activity</td>
<td>+</td>
<td>9- REC hemolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10- Gelation liquefaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11- Casein hydrolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

160
Fig. i: colonies of N. asteroides after 72 hr of incubation on blood agar. Notice opaqu, chalky white, non hemolytic firmly adherent colonies.
Fig. 2: Udder of mastitic cow, swollen with sinus tract.
Fig. 3: Udder of mastitic cow. Pyogranulomatous lesion with purulent necrotic surrounded by epitheloid cells, mononuclear cells, giant cells and enveloped by fibrous connective tissues (H.E. 100X)
Fig. 4: Skin of mastitic udder. Notice cellulitis adjacent to the sinus tract. (H.E.100X).
Fig. 5: Bovine nocardiosis. Lung showing fibrotic thickening of the interalveolar septa due to fibrous connective tissue proliferation and mononuclear cells infiltration
(H. & E. 100X).

165
References


الخلاصة

مرض داء النوكارديا في الأبقار

مسح ميداني ودراسة التغيرات المرضية

محمد جويد علوان
فرع الأمراض والدائم / كلية الطب البيطري
جامعة بغداد – العراق

Nocardia استهدفت هذه الدراسة بحث إصابة الأبقار بجراثيم ولمعرفة مدى انتشار ووبائية المرض في الأبقار الحليب من asteroids محطة الدجيل من خلال فحص (90) نموذج حليب جمتعت من حيوانات مصابة بالتهاب الضرع، أوضحت الدراسة وجود داء النوكارديا بشكل وبائي في محطة أبقار الدجيل حيث عزلت جرثومة من (20) حالة وشكلت نسبة الإصابة 72 و 27%. لقد تعززت هذه النتائج من خلال ظهور عزلات نتية للجرثومة من (5) حيوانات إضافة إلى وجودها مع عزلات جرثومية أخرى من (20) حالة. أظهر الفحص المجهي وجود الورم الحبيبي الفيتي في ضرع الحيوانات المصابة بالتهاب الضرع إضافة إلى وجودها في رئات نفس الحيوانات.